Pediatric Rheumatology 2013, Volume 11 Suppl 1
http://www.ped-rheum.com/supplement/11/S1

PEDIATRIC RHEUMATOLOGY

MEETING ABSTRACTS Open Access

7th Congress of International Society of Systemic Auto-Inflammatory Diseases (ISSAID)

Lausanne, Switerland. 22-26 May 2013

Published: 08 November 2013

These abstracts are available online at http://www.ped-rheum.com/supplement/11/S1

MEETING ABSTRACT

A1

OR2-001 – The possible role of pyrin on cell migration

B Peynircioğlu[1*], ZY Akkaya[1], C Guler[1], A Cetinkaya[1,2], ZE Taskiran[1,2], E Yilmaz[1]
[1]Medical Biology, Hacettepe University, Ankara, Turkey; [2]Medical Genetics, Hacettepe University, Ankara, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A1

Introduction: MEFV which encodes pyrin, cause familial Mediterranean fever (FMF), the most common auto-inflammatory disease. The pyrin protein appears to be a regulator of inflammation, but its exact role on inflammatory pathways is still controversial. Several pyrin-interacting proteins have been identified, each of which are related to inflammation through regulation of cell death, cytokine secretion, and cytoskeletal signaling. It has been documented that in migrating human monocytes, pyrin protein is dramatically polarized at the leading edge, where it co-localizes with polymerizing actin. Thus, we hypothesized that pyrin may have a key role in cell migration through its interaction with well-known regulators of inflammatory cell migration.
Objectives: In this study we aimed to examine more closely the distribution of pyrin and possible pyrin-related proteins that has cytoskeletal functions through actin machinery such as; LSP1(Leukocyte-specific protein 1), PSTPIP2 (Proline-serine-threonine phosphatase interacting protein 2), LPXN(Leupaxin), DAAM1(Dishevelled associated activator of morphogenesis 1, WDR1(WD repeat containing protein 1), and WIPF3 (WAS/WASL-interacting protein family member 3) in migrating cells.
Methods: Firstly; wound healing assay was used to show the effect of pyrin in transiently transfected HeLa cells and Boyden chamber assays were usedin order to quantitate this effect in transiently transfected COS-7 cells migrating against an insulin gradient. Then the distribution and expression of pyrin and actin related proteins were analyzed during cell migration process using a functional HL-60 cell migration assay. qRT-PCR was performed in order to analyze the expression profile of actin related proteins during cell migration. Then IF (immunofluorescence) staining technique was used to demonstrate the cellular distribution of these proteins.
Results: A significant relationship between wild type pyrin overexpression and increased cell migration was shown both in Boyden chamber and wound healing assays. Preliminary results related with HL-60 cell migration assay showed that expression levels of LSP1, LPXN and WDR1 change during migration but not significantly; while PSTPIP2, DAAM and WIPF3 have a very low basal expression level in these cells. Interestingly; IF co-stainings showed that pyrin and these proteins co-localize at the leading edge of the migrating cells, where actin polymerization occurs.
Conclusion: These studies described here provide a new insight to the potential role of pyrin protein in the process of cell migration. We have demonstrated that the regulation of pyrin and its interacting partners during cell migration occurs post-translationally rather than transcriptional level. Further studies on; i) RNA interference mechanism targeting MEFV expression and ii) LPS treatment during cell migration are underway and may lead to understand the exact role of pyrin in inflammatory cells and result in novel treatment options for inflammatory diseases in general.
This study was supported by The Technical and Scientific Research Council of Turkey (TUBITAK) Project Number: TUBITAK 1001-SBAG-111S507.
Disclosure of interest: None declared.

A2

OR2-002 – The risk of FMF in MEFV heterozygotes

I Jéru[1,2,3*], V Hentgen[4], E Cochet[3], P Duquesnoy[1], G Le Borgne[1,2], E Grimprel[2,5], K Stankovic Stojanovic[6], S Karabina[1,2], G Grateau[2,6], S Amselem[1,2,3]
[1]UMR_S933, INSERM, France; [2]Université Pierre et Marie Curie-Paris6, France; [3]Service de Génétique, APHP, Hôpital Trousseau, Paris, France; [4]Centre de Référence des Maladies AutoInflammatoires, Centre Hospitalier de Versailles, Le Chesnay, France; [5]Service de Pédiatrie Générale, APHP, Hôpital Trousseau, France; [6]Service de Médecine Interne, APHP, Hôpital Tenon, Paris, France

Pediatric Rheumatology 2013, **11(Suppl 1):**A2

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder due to *MEFV* mutations and one of the most frequent Mediterranean genetic diseases. The observation of many heterozygous patients in whom a second mutated allele was excluded led to propose that heterozygosity could be causal; however, this might often be coincidental due to the very high rate of mutations in Mediterranean populations.
Objectives: To better delineate the pathogenicity of heterozygosity in order to help genetic counselling and better manage the disease.
Methods: Complementary statistical approaches were used: estimation of FMF prevalence at population levels, genotype comparison in siblings from 63 familial forms, and genotype study in 557 patients from four Mediterranean populations.
Results: At population level, we did not observe any contribution of heterozygosity to the disease prevalence. In affected siblings of patients carrying two *MEFV* mutations, 92% carry two mutated alleles whereas 4% are heterozygous with typical FMF diagnosis. We also demonstrated statistically that patients are more prone to be heterozygous than healthy individuals, as shown by the higher ratio heterozygous carriers/non carriers in patients ($p<10^{-7}$ - $p<0.003$). The risk for heterozygotes to develop FMF was estimated between $2.1x10^{-3}$ and $5.8x10^{-3}$ and the relative risk, as compared to individuals carrying no *MEFV* mutation, between 6.3 and 8.1.
Conclusion: This is the first statistical demonstration that heterozygosity is not responsible for classical Mendelian FMF, but constitutes a susceptibility

factor for clinically-similar complex conditions. We also provide a first estimate of the risk for heterozygotes to develop FMF.
Disclosure of interest: None declared.

A3

OR3-001 – RIP2 kinase is activated in Blau Syndrome and IBD

KP Foley[1*], B Desai[1], A Vossenkämper[2], MA Reilly[1], P Biancheri[2], L Wang[1], DB Lipshutz[1], J Connor[1], M Miller[1], PA Haile[1], LN Casillas[1], BJ Votta[1], PJ Gough[1], TT MacDonald[2], CH Wouters[3], CD Rosé[4], J Bertin[1]
[1]Pattern Recognition Receptor Discovery Performance Unit, GlaxoSmithKline, Collegeville, United States; [2]Barts and The London School of Medicine and Dentistry, London, United Kingdom; [3]University of Leuven, Leuven, Belgium; [4]Alfred I. duPont Hospital for Children, Wilmington, United States
Pediatric Rheumatology 2013, **11(Suppl 1):**A3

Introduction: Blau Syndrome (Blau) is a granulomatous auto-inflammatory disease caused by mutations in NOD2 that have been proposed to result in phosphorylation of RIP2 kinase and the production of pro-inflammatory cytokines. Such monogenic diseases can bring to light pathways that are also likely to be involved in more genetically complex diseases. For example, increased RIP2 phosphorylation has been observed in inflammatory bowel disease (IBD), although the role of RIP2 in IBD has not been determined. We are developing a first-in-class, highly potent and selective inhibitor of RIP2 kinase, which may provide therapeutic benefit in both Blau and IBD.
Objectives: To explore the role of RIP2 in disease, we examined its phosphorylation state in peripheral blood mononuclear cells (PBMCs) and synovial fluid (SF) cells isolated from a cohort of Blau patients. We also assessed the ability of a RIP2 inhibitor to block inflammatory cytokine production in *ex vivo*-cultured mucosal biopsies from IBD patients.
Methods: Phospho-Serine176-RIP2 (pRIP2) levels were measured in PBMCs from 5 Blau patients and 4 normal healthy volunteers (NHVs) and SF cells from the inflamed knee joints of 1 Blau patient by immunoblotting with a novel monoclonal antibody. Inflamed biopsies from 28 IBD patients were cultured *ex vivo* in serum-free media for 18 hrs in the presence or absence of RIP2 inhibitor GSK'214, and culture supernatants were assayed for TNF-α, IL-1β and IL-6 by ELISA.
Results: pRIP2 levels were found to be elevated by an average of 8-fold in PBMCs isolated from Blau patients relative to NHVs. SF cells from a Blau patient also expressed 240-fold higher levels of pRIP2 than NHV PBMCs. Studies are underway to assess the effect of RIP2 inhibition on pRIP2 in cultured PBMCs from Blau patients.
Treatment with RIP2 inhibitor GSK'214 resulted in dose-dependent inhibition of pRIP2 in *ex vivo*-cultured IBD biopsies. GSK'214 also inhibited spontaneous production of TNF-α, IL-1β and IL-6 in these cultures, with efficacy equivalent to that of prednisolone or dexamethasone. Similar results were observed with biopsies from both Crohn's disease and ulcerative colitis patients.
Conclusion: Studies in transfected cells have suggested that Blau NOD2 mutations act in a gain-of-function manner, however, direct evidence for the activation of RIP2 in patients has been lacking. We have for the first time shown that increased phosphorylation of RIP2 on Serine176, a well-established phosphorylation site associated with kinase activation, occurs in primary Blau patient cells, confirming the dominant gain-of-function nature of these mutations. Prompted by reports of increased pRIP2 in mucosal tissues from IBD patients, we have also shown that inhibition of RIP2 activation suppresses the spontaneous production of inflammatory cytokines by *ex vivo*-cultured IBD biopsies. Our results suggest that both Blau and IBD patients may benefit from therapeutic inhibition of RIP2.
Disclosure of interest: K. Foley Employee of: GlaxoSmithKline, B. Desai Employee of: GlaxoSmithKline, A. Vossenkämper Grant / Research Support from: GlaxoSmithKline, M. Reilly Employee of: GlaxoSmithKline, P. Biancheri: None Declared, L. Wang Employee of: GlaxoSmithKline, D. Lipshutz Employee of: GlaxoSmithKline, J. Connor Employee of: GlaxoSmithKline, M. Miller Employee of: GlaxoSmithKline, P. Haile Employee of: GlaxoSmithKline, L. Casillas Employee of: GlaxoSmithKline, B. Votta Employee of: GlaxoSmithKline, P. Gough Employee of: GlaxoSmithKline, T. MacDonald Grant / Research Support from: GlaxoSmithKline, C. Wouters Grant / Research Support from: GlaxoSmithKline, C. Rosé Grant / Research Support from: GlaxoSmithKline, J. Bertin Employee of: GlaxoSmithKline.

A4

OR3-002 – Blau Syndrome cohort study: ocular outcome

CD Rose[1*], R Cimaz[2], C Thomee[3], R Khubchandani[4], G Espada[5], R Russo[5], M Harjacek[6], B Bader-Meunier[7], P Brissaud[8], N Wulffraat[9], S Vastert[9], R Merino[10], A Naranjo-Hernandez[11], S Oliveira Knupp[12], F Mackenson[13], J Arostegui[14], J Anton Lopez[14], J Fernandez-Martin[15], C Wouters[16]
[1]Nemours AI Dupont Hospital for Children, Wilmington, United States; [2]Università degli Studi di Firenze, Florence, Italy; [3]Centre Hospitalier de Luxembourg, Luxembourg; [4]Jaslok, Breach Candy and Saifee Hospitals, Mumbai, India; [5]Universidad Nacional de Buenos Aires, Buenos Alres, Argentina; [6]University of Zagreb, Zagreb, Croatia; [7]Hôpital Necker, Paris, France; [8]None, Neuilly/Seine, France; [9]UMC Utrecht, Utrecht, Netherlands; [10]Hospital La Paz, Madrid, Spain; [11]Hospital Dr. Negrin, Gran Canarias, Spain; [12]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [13]Universitäts Klinikum Heidelberg, Heidelberg, Germany; [14]Universidad de Barcelona, Barcelona, Spain; [15]Hospital do Meixoeiro., Vigo, Spain; [16]Katholieke Universiteit Leuven, Leuven, Belgium
Pediatric Rheumatology 2013, **11(Suppl 1):**A4

Introduction: BS is an autosomal dominant monogenic granulomatous disease due to gain of function mutations at or near the NACHT domain of NOD2. It is characterized by a triad of granulomatous polyarthritis, uveitis and rash. Retrospective work by our group showed a life time risk of ocular involvement of 60% with significant morbidity and poor visual outcome. Prospective studies on natural history of visual outcome are not available. In view of current lack of effective therapies, research on relevant pathways downstream NOD2 is essential and may lead to appropriate targeted drug development.
Objectives: To study prospectively in detail the phenotype of ocular involvement and visual outcome in the context of a prospective cohort study on BS. Secondary goals: investigate possible biomarkers of disease activity and explore relevant pathways and candidates for therapeutic targeting.
Methods: Participating centers of an ongoing international registry were invited to enroll patients with NOD2 mutation. IRB approval was obtained. This 3 year prospective study consists of one baseline and 3 yearly assessments with a standardized clinical evaluation, functional assessment, visual analogue scales, a comprehensive ophthalmologic assessment and blood sampling for fundamental in vitro research. Coded data are kept in a secured database at the coordinating center.
Results: We are reporting baseline ophthalmologic evaluation of the first 23 patients, virtually a cross section of ocular status along disease course. Ages were 0-54 years. 50% 0-15. More than half had substitutions R334W or R334Q. 19/23 have ocular involvement. Onset of eye disease was 67 months (6-264), 30 months after the onset of arthritis. Uveitis never preceded joint disease, was bilateral in 90% and "pan" in 15/19. Despite intense therapy there was evidence of active disease (+ cells and/or flare for anterior segment or macular edema) at the time of evaluation in 15/19 patients, (anterior in 8, posterior in 4 and global in 3).For severity assessment in bilateral disease we used the worse eye. Disease was mild if no local complications (except cataracts), moderate when complicated and severe if there was visual loss (WHO). Accordingly 2 were mild, 6 moderate and 11 severe. Corrected visual acuity (10=100%) was poor, with an average of 7.1 for the right eye and 6.7 for the left.
Conclusion: Eye involvement in Blau disease is common, severe, requires intense therapy and lends significant impact on morbidity. This first prospective cohort multicenter study of Blau syndrome shows that development of effective therapies with bioactivity in ocular tissue is critical.
Disclosure of interest: None declared.

A5

P01-001 – Musculoskeletal sonography in FMF patients

O Karadag[*], S Yilmaz, V Yazisiz, M Cinar, H Erdem, S Pay, A Dinc
Division of Rheumatology, Gulhane School of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A5

Introduction: Familial Mediterranean fever (FMF) is an autoinflammatory disorder characterized by recurrent febrile polyserositis and musculoskeletal findings. Chronic arthritis and/or accompanying seronegative spondyloarthropathy have been reported in FMF. But little is known about musculoskeletal changes during attack free period.

Objectives: This study is aimed to investigate the musculoskeletal sonographic changes during attack free period.
Methods: Totally 29 consecutive FMF male patients at attack free period and 17 male controls were included into the study. Physical examination (PE) was performed to detect Achilles enthesitis and/or retrocalcaneal bursitis, knee arthritis. US of the lower extremity were performed bilaterally. Grey-scale (GS) and power Doppler (PD) scores on a 0–2 semi-quantitative scale (0: no, 1: mild, 2: moderate, 3: marked).
Results: Mean ages of patients and controls were 24.3 ±4.9, 25.8± 5.5 years (p=0.338). Fourteen (48.2%) of FMF patients had reported arthralgia and/or arthritis during attacks. In 7 of 15 patients who had not reported joint problems, a sonographic pathology was found (4 of them was mild effusion at knee and 3 of them was retrocalcaneal bursitis and medial tenosynovitis. No PDS was found in knee or ankle joint. But there was no pathology in controls.
Conclusion: Even though none of the FMF patients had chronic arthritis, an increased rate of mild effusion and tenosynovitis was found. This finding might reflect an increased rate of musculoskeletal finding compared to healthy controls.
Disclosure of interest: None declared.

A6

P01-002 – Comparison between different colchicines responders

ZB Özçakar[1*], AH Elhan[2], F Yalcinkaya[1]
[1]Ankara University, Pediatric Rheumatology, Turkey; [2]Biostatistics, Ankara University, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A6

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive disease, characterized by recurrent, self-limited attacks of fever with serositis involving the peritoneum, pleura and joints; and colchicine is its universal treatment.
Objectives: To explore whether the demographic and clinical features of FMF patients with different colchicine response vary or not.
Methods: Files of patients who had been seen in our department (during routine follow-up visits) between January 2009 and January 2013 were retrospectively evaluated.
Results: The study group comprised 221 FMF patients (116F, 105M) with a mean age of 12.7±5.3 years. Mean duration of colchicine use was 58.9 ±45.3 months. Patients were divided into two groups according to their colchicine response; Group I (n=131) included patients with no attacks after colchicine and Group II (n=90) patients with partial or no response to colchicine. Mean age, sex, age at disease onset, age at colchicine onset, family history of FMF, attack frequency, attack duration, clinical features during attacks, duration of colchicine use and M694V carriage were similar between the groups. Final colchicine doses, disease severity scores, acute phase reactant levels (during attack free period) were significantly higher in Group II when compared with those of Group I ($p<0.5$).
Conclusion: Colchicine response seems to be related with disease severity scores and acute phase reactant levels (during attack free periods) in FMF patients.
Disclosure of interest: None declared.

A7

P01-003 – Bleeding disorder in FMF

O Karadag[1*], T Akin Telli[1], N Sayinalp[2], I Ertenli[1]
[1]Division of Rheumatology, Ankara, Turkey; [2]Division of Haematology, Ankara, Turkey, Hacettepe University Faculty of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A7

Introduction: The most serious complication in Familial Mediterranean fever (FMF) is the development of amyloidosis, which usually determines the prognosis. Amyloid deposition can be systemic or organ-specific. The clinical features of amyloidosis are dependent on the organs involved, type of amyloidosis, *rate* of *amyloid deposition and amount of amyloid fibrils. Organ dysfunction can cause life-threatening bleeding.* Amyloid deposition is the main cause of abnormal bleeding but also coagulation factor deficiencies, hyperfibrinolysis, platelet dysfunction and amyloid angiopathy with increased fragility of blood vessels can be regarded as other important pathogenetic factors. Herein a case of FMF amyloidosis with splenomegaly, refractory cytopenia and bleeding disorder is presented.
Case report: Thirty-one year old male patient was admitted with complaints of upper gastrointestinal (GI) bleeding and hematuria. *In his past medical history, he had recurrent attacks of abdominal pain and fever since early childhood and 9 years ago a renal biopsy* was *performed* to evaluate proteinuria and AA amyloidosis was detected. He had been diagnosed as FMF and colchicine treatment was begun. On physical examination, *ecimotic* skin *lesions* on lumbar area and legs were found. The abdominal examination revealed painless massive splenomegaly palpable up to the pelvis. Also physical examination revealed markedly thickened skin in each ear in the area of concha and amyloid nodules of ear. Laboratory results were as follows: Hemoglobin level 8.8 g/dL, white blood cell count:9000/ mm3, platelet count 41000/ mm3, INR level 1.7. Peripheral blood smear examination showed *poikilocytosis*, *acanthocytosis*, neutrophils %61, *lymphocytes* %38, platelet count compatible with thrombocytopenia. LDH level was normal and coombs tests were negative. *Beta-2 microglobulin level was too high.*(13417 ng/ml). Bone marrow aspiration and biopsy revealed normocellularity of the marrow and *deposition* of *amyloid in* the walls of the blood *vessels. All coagulation factor levels* were decreased in plasma. *As the reason of bicytopenia peripheral degradation and splenic sequestration were evaluated primarily. Amyloidosis caused platelet aggregation defect. Because of the high bleeding risk, he* underwent radiotherapy *instead of splenectomy for hypersplenism. Despite* splenic irradiation *thrombocytopenia didn't improve, radiation therapy didn't shrink* the spleen. Genetic mutation analysis showed homozygous M694V alleles on MEFV gene. Red blood cells, platelets, fresh frozen plasma and fibrinogen were transfused in order to improve *bleeding diathesis. On follow-up* the nosocomial infections led to exitus through a septic shock.
Discussion: In Familial Mediterranean fever (FMF), amyloid deposition in any other organ except kidney can also cause morbidity and mortality. Amyloid deposition should be kept in mind in differential diagnosis of FMF patients with refractory cytopenia and bleeding disorder.
Disclosure of interest: None declared.

A8

P01-004 – MEFV genes and FMF

F Salehzadeh[1,2*], M Jafariasl[2], S Jahangiri[1], S Hosseiniasl[3]
[1]Pediatric Rheumatology, Bouali Hospital, Iran, Islamic Republic Of; [2]Pediatric Rheumatology, ARUMS, Iran, Islamic Republic Of; [3]Genetic, Emam Khomeini hospital, Ardabil, Iran, Islamic Republic Of
Pediatric Rheumatology 2013, **11(Suppl 1):**A8

Introduction: Familial Mediterranean Fever (FMF) is a hereditary autoinflammatory disease with autosomal recessive inheritance pattern often seen in the Turks, Arabs, Armenians and Jews people characterised by recurrent episoded of fever and polyserositis and rash. Recently the definitive diagnosis of FMF determines by MEFV gene analysis.
Objectives: In this study we analysed twelve MEFV gene mutations inmore than two hundred FMF patients who had Mediterranean fever diagnosis on the basis of clinical Tel – Hashomer criteria.
Methods: In northwest of IRAN, 216 patients with FMF diagnosis based on Tel-Hashomer criteria, referred to the genetic laboratory to12 common MEFV genes analysis. P369S, F479L, M680I(G/C), M680I(G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H, E148Q mutations were analysed by using amplification refractory system for 11 of those and the PCR was performed for E148Q.
Results: Among Of these FMF patients, no mutation was detected in 51 (23/62%) patients and 165 (76/38%) patients had one or two mutation. 33 patients (15/28%) homozygous, 86 patients (39/81%) were compound heterozygous, 46 patients (21/29%) were heterozygous. The most common mutation, were M694V (23/61%) V726A (11/11%) and E148Q (9/95%) respectively.
Conclusion: Common 12 MEFV genes analysis could not detect 50% of our patient who had FMF on the basis of Tel – Hashomer clinical criteria. Therefore it needs more genes analysis in genotyping studies, we conclude that clinical criteria is still the best way in diagnosis of FMF.
Disclosure of interest: None declared.

A9

P01-005 – Idiopathic uveitis and FMF

F Salehzadeh[1,2*], O Yasrebi[2], S Jahangiri[1], S Hosseiniasl[3]
[1]Pediatric Rheumatology, Bouali Hospital, Iran, Islamic Republic Of; [2]Pediatric Rheumatology, Arums, Iran, Islamic Republic Of; [3]Genetic, Emam Khomeini hospital, Ardabil, Iran, Islamic Republic Of
Pediatric Rheumatology 2013, **11(Suppl 1):**A9

Introduction: Familial Mediterranean fever (FMF) is an auto inflammatory disease characterized by attacks of fever and polyserositis. FMF is often associated with other autoimmune diseases such as rheumatoid arthritis, PAN, Behcet.
Objectives: Uveitis is an inflammatory process of eyes caused by underlying infectious and inflammatory disorders. This study investigates the probable relationship between Idiopathic Uveitis and familial Mediterranean fever.
Methods: Patients with idiopathic uveitis that didn't have Infectious and inflammatory causes referred to the genetic laboratory to12 common MEFV genes analysis. P369S, F479L, M680I(G/C), M680I(G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H, E148Q mutations were analysed by using amplification refractory system for 11 of those and the PCR was performed for E148Q.
Results: 12 patients with idiopathic uveitis were enrolled in this study. 10 of them were female and 2 were male. The youngest patient was a 7-year-old child and the oldest was 57. The most common complaints of patients was blurred vision and then eye redness. One patient was heterozygous for Wt/R761H in the MEFV genetic analyses. Genetic analysis of 12 most common MEFV mutations in the patients with idiopathic uveitis didn't have any positive results.
Conclusion: According to the analysis of 12 most common MEFV gene mutations, FMF is not an underlying cause of idiopathic uveitis. On the other hand uveitis merely could not be the first presentation of FMF.
Disclosure of interest: None declared.

A10

P01-006 – MEFV mutation detection in Arabic patients

R Taha[1], S Ayesh[2], M Kambouris[1], H El-Shanti[1*]
[1]Shafallah Medical Genetics Center, Doha, Qatar; [2]Gene Medical Labs, Gaza, Palestinian Territory, Occupied
Pediatric Rheumatology 2013, **11(Suppl 1):**A10

Introduction: Autoinflammatory diseases are a group of disorders characterized by seemingly unprovoked inflammation in the absence of high-titer autoantibodies or antigen-specific T-cells. Familial Mediterranean fever (FMF) is the archetypal hereditary periodic fever syndrome and autoinflammatory disorder. It is characterized by recurrent self-limiting episodes of fever and painful polyserositis. FMF is an autosomal recessive disorder, with considerable prevalence in specific ethnic groups, namely, non-Ashkenazi Jews, Armenians, Turks and Arabs and the FMF carrier rate can be as high as one in four. The gene responsible for FMF, *MEFV*, is located on the short arm of human chromosome 16, and was independently identified by two positional cloning consortia. Mutations, as well as, polymorphisms in *MEFV* are continuously identified. In Arabic FMF patients the spectrum and distribution of *MEFV* mutations are distinctive and the portion of unidentified mutations is undoubtedly the highest amongst the groups commonly affected by FMF. The comprehensive identification of *MEFV* mutant alleles among FMF patients is needed for the efficient examination of specific genotype – phenotype correlation patterns and for the development of molecular tools to support the clinical diagnosis.
Objectives: To identify *MEFV* mutations in a cohort of Arabic patients using a comprehensive approach that could identify coding and non-coding variations, large duplications or deletions, as well as intronic variations.
Methods: We obtained 100 patients of Palestinian origin with clear FMF symptomatology consistent with the clinical diagnostic criteria and for whom only one mutation has been identified. We applied a comprehensive mutation analysis approach that involves sequencing of exons and splice sites, sequencing putative regulatory regions, Multiplex Ligation-dependent Probe Amplification (MLPA) technique to detect large deletions or duplications, and sequencing of the entire genomic sequence of MEFV.
Results: We did not identify any mutations by sequencing *MEFV* exons and splice sites, as well as putative regulatory regions. Similarly, MLPA did not reveal any large deletions or duplications within the genomic sequence of MEFV. The sequencing of the entire genomic sequence identified 20 different rare intronic variants that were each identified in 1-3 affected individuals only, and were not identified in about 700 ethnically matched control chromosomes. The biological significance of these variations could not be determined.
Conclusion: It has been suggested that the reduced identification of *MEFV* mutant alleles in Arabic patients is due to the lack of application of a comprehensive mutation detection methodology. However, the current study negates that hypothesis. We hypothesize that the current sequencing technology produces a preferential amplification of one allele over the other due to extensive polymorphism within the genomic sequence. To examine this hypothesis we developed a parallel sequencing approach for the genomic *MEFV* sequence. At this stage of the study, we could not exclude the effect of modifier genes or any other loci that influence the clinical picture of FMF in Arabic populations.
Disclosure of interest: None declared.

A11

P01-007 – Evaluation of potential risk factors of Amyloidosis

T Kasifoglu[*], S Bilge, E Gonullu, F Bekdemir, C Korkmaz
Department Of Internal Medicine Division Of Rheumatology, Eskisehir Osmangazi University Medical Faculty, Eskisehir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A11

Introduction: Familial Mediterranean Fever (FMF) is a genetic disease which is frequently seen in Middle East Region. The most common reason of morbidity and mortality is the end stage renal failure due to amyloidosis.
Objectives: The potential risk factors of amyloidosis are known as ethnic origin (Jewish, Armenian, Turkish, and North African origin), non-usage of colchicines, and family history. Various investigators suggest that M694V mutation, especially homozygote pattern, is a risk factor for amyloidosis. In literatures reported from Turkey, it is stated that there is only a limited association. In this study, we aimed to investigate the affects of MEFV mutations and other potential risk factors on amyloidosis.
Methods: The findings of 396 FMF patients of our clinic was retrospectively evaluated such as amyloidosis, MEFV mutations, gender, clinical features, family history, ages at initial symptoms, ages at diagnosis, the duration of colchicin usage. Amyloidosis was diagnosed by rectal or renal biopsies of patients with proteinuria over than 500 mg/day.
Results: Renal amyloidosis was diagnosed at 8.3% (33) patients. There was no difference between patients with amyloidosis and without amyloidosis in terms of clinical features, family history, ages at initial symptoms, ages at diagnosis, the duration of colchicin usage. MEFV mutation was studied in 159 of 363 patients without amyloidosis and in 27 of 33 patients with amyloidosis. The mutations detected in patients with amyloidosis were M694V homozygote, M694V heterozygote, and compound heterozygote of M694V/V726A, M694V/M680I, V726A/M680I or V726A/R761H. The most common mutation was M694V homozygote of patients with and without amyloidosis (59.3% versus 32%). The frequency of M694V homozygote of patients with amyloidosis was statistically high from the frequency of patients without amyloidosis ($p<0.001$). But there was no difference in terms of other mutations.
Conclusion: It is shown that, the only most common risk factor of Turkish patients is the M694V homozygote gene mutation in our study. But amyloidosis is not seen in all patients with M694V homozygote mutation. Therefore, large series are needed to evaluate the other potential risk factors. Close monitoring could be necessary for patients with M694V homozygote mutation.
Disclosure of interest: None declared.

A12

P01-008 – FMF genotype-phenotype correlations in Germany

M Jeske[1], P Lohse[2], T Kallinich[3], T Berger[4], C Rietschel[5], D Holzinger[6], C Kamlah[7], P Lankisch[8], R Berendes[9], G Dückers[10], G Horneff[11], E Lilienthal[12], JP Haas[13], A Giese[14], F Dressler[15], J Berrang[16], C Pütter[17], L Braunewell[1], U Neudorf[1*], T Niehues[10], E Lainka[1]
[1]Children's Hospital, University Duisburg-Essen, Essen, Germany; [2]Labor Blessing und Partner, Singen, Germany; [3]Charité Berlin, Children's hospital, Berlin, Germany; [4]Children's Hospital, University Witten/Herdecke, Datteln, Germany; [5]Department of Pediatric Rheumatology, Clementine Hospital,

Frankfurt, Germany; [6]Children's Hospital, University Münster, Münster, Germany; [7]Children's Hospital, UKE Hamburg, Hamburg, Germany; [8]Pediatric Rheumatology, University Düsseldorf, Düsseldorf, Germany; [9]Pediatric Rheumatology, St. Marien's Children's Hospital, Landshut, Germany; [10]Pediatric Immunology and Rheumatology, HELIOS Children's hospital, Krefeld, Germany; [11]Centre of Pediatric Rheumatology, Asklepios Clinic, St. Augustin, Germany; [12]Children's Hospital, Ruhr-University , Bochum, Germany; [13]German Center for Pediatric Rheumatology, Children's Hospital, Garmisch-Partenkirchen, Germany; [14]Marienhospital Herne, Ruhr-University Bochum, Herne, Germany; [15]Pediatric Rheumatology Clinic, Hannover Medical School, Hannover, Germany; [16]Pediatric Rheumatology, Westfaelisches Kinderzentrum, Dortmund, Germany; [17]Institute for Medical Informatics, Biometry and Epidemiology, University Hospital, Essen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A12

Introduction: Familial Mediterranean fever (FMF) is one the most common autoinflammatory disease (AID). Pathogenomic relevant mutations in the MEFV gene show autosomal recessive inheritance, but co-dominant mutations have been described.
Objectives: We aimed to evaluate correlations between ethnic origin, phenotype and genotype for FMF patients in the German AID-Net-registry.
Methods: We used two common scoring systems modified for children (Mor et al., Pras et al.) to assess disease severity in 243 FMF patients of the AID-Net-registry. For the four most frequent mutations, we tested for a correlation of the genotype with the phenotype, mean CRP and ethnic origin, respectively. Furthermore, we evaluated the applicability of the two severity scores for children.
Results: Among the 243 patients, we detected a total of 433 pyrin mutations and 22 different sequence variants, including one new mutation (p.Gly488Asp). The four most frequent alterations were p.Met694Val (55%, n=238), p.Met680Ile (12%, n=52), p.Val726Ala (10%, n=44) and p.Glu148Gln (8%, n=34). Ethnic origin could be determined in 224 cases; the prevailing ancestry was Turkish (83%, n=185), 8% (n=18) were Lebanese. P.Met694Val in homozygous form (n=74; 30.5%) was correlated with a more severe disease activity, based on the score by Mor, as well as with a higher mean CRP (74 mg/l, n=60, 31 mg/l, n=59) compared to patients without this mutation (p=0.01 and p<0.01, respectively). The score suggested by Pras did not yield a significant genotype-phenotype correlation; indeed, the two scoring systems were inconsistent with each other (κ<0.07). Although a typical distribution of mutations in different ethnic populations was obvious, this trend was not statistically significant, probably due to the divergent number of cases.
Conclusion: The homozygous p.Met694Val substitution was associated with a more severe disease activity. There was no origin-genotype correlation in this FMF population. The well-known severity scores for children (Mor, Pras) are inconsistent.
Disclosure of interest: M. Jeske: None Declared, P. Lohse: None Declared, T. Kallinich: None Declared, T. Berger: None Declared, C. Rietschel: None Declared, D. Holzinger : None Declared, C. Kamlah: None Declared, P. Lankisch: None Declared, R. Berendes: None Declared, G. Dückers: None Declared, G. Horneff Consultant for: financial support for clinical trials from Abbott, Pfizer and Roche, E. Lilienthal: None Declared, J. Haas Consultant for: Pfizer, Roche and Novartis, A. Giese: None Declared, F. Dressler: None Declared, J. Berrang: None Declared, C. Pütter: None Declared, L. Braunewell: None Declared, U. Neudorf: None Declared, T. Niehues: None Declared, E. Lainka: None Declared.

A13

P01-009 – 2 years of colchicine IV in intractable FMF

L Kaly*, N Boulman, D Rimar, G Slobodin, I Rosner, M Rozenbaum
Rheumatology, Bnai Zion medical Center-Haifa Israel, Haifa, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A13

Introduction: Oral colchicine therapy has been shown effective both in controlling the symptoms and preventing the development of amyloidosis in FMF. However, 5 to 10% of FMF patients are resistant to this conventional therapy.
Objectives: The objective of this study is to evaluate the efficacy and safety of weekly intravenous colchicine (IVC) adjunct therapy for more than one year.
Methods: We retrospectively reviewed records of our day-hospitalization from 2007 to 2012 for patients with FMF unresponsive to oral treatment who were treated with supplemental 1mg IVC (120 min infusion) once a week. We tabulated records of clinical events: fever, number of attacks per month and CRP levels before and after at least one year of this treatment. Adverse events were also recorded, and compared with a matched control population of FMF patients only treated with oral colchicine.
Results: Twelve patients were identified. Two with poor compliance were excluded. Mean age was 39; 70% male. Mean daily dose of oral colchicine was 2.1mg. Mean duration of treatment with IVC was 2.1 years. The number of attacks per month decreased by 68% (4 versus 1.3, before and after respectively, p< 0.008). One patient exhibited an adverse event: deep vein thrombosis of lower extremity.
Conclusion: Although IVC is a controversial option as treatment for oral-colchicine- non-responsive FMF patients, our study provides evidence of efficacy and relative safety for this modality as long term treatment ,in young FMF patients without comorbidities treated in an academic center, experimented with the use of IVC
Disclosure of interest: None declared.

A14

P01-010 – Anti-TNF agents in intractable FMF: four cases

L Kaly*, D Rimar, G Slobodin, N Jiries, I Rosner, M Rozenbaum
rheumatology, Bnai Zion medical Center-Haifa Israel, Haifa, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A14

Introduction: Familial Mediterranean Fever (FMF) is an autoinflammatory disease characterized by recurrent attacks of fever and serositis. A relation between FMF and Ankylosing Spondylitis (AS) has been suggested in small cohort studies, although there is no consensus regarding the role of HLA B27. Colchicine, the mainstay treatment in FMF, does not improve the axial or peripheral symptoms due to spondylarthropathy. There are controversial data about the efficacy of Tumor Necrosis Factor Alpha (TNF α) blockade in FMF patients [1].
Case Report: We report our experience in 4 patients with intractable FMF treated with oral colchicine and supplemental weekly IV colchicine [2], that were treated with TNF α blockade for symptomatic axial spondylarthropathy. One 26 years old man with MEFV mutations V726A and E148Q, negative for HLAB27, with concomitant ulcerative colitis was treated with infliximab and then with adalimumab; and 3 women (42, 48 and 55 years old), two of them treated with Infliximab and one treated with adalimumab. Two of the women were homozygous for the M694V mutation. All developed severe to moderate adverse events: exacerbation of FMF in 2 of them, myositis and ulcerative colitis exacerbation in the male patient, and staphylococcus aureus sepsis in another patient. Three of them had to stop the TNF α blockade treatment. One patient developed psoriatic rash, with no need to stop the treatment.
Discussion: In our limited experience, TNF α blockade in patients with both intractable FMF and AS is not very effective and may be associated with severe adverse events. Little is known about the possible interaction between intravenous colchicine and anti-TNF treatment.
Disclosure of interest: None declared.
References

1. Bilgen SA, Kilic L, Akdogan A, Kiraz S, Kalyoncu U, Karadag O, Ertenli I, Dogan I, Calguneri M: **Effects of anti-tumor necrosis factors agents for familial Mediterranean fever patients with chronic arthritis and/ or sacroiliitis who were resistant to colchicine treatment.** *J clin Rheumatol* 2011, **7**:358-62.
2. Rozenbaum M, Boulman N, Feld J, Avshovich N, Petrovich S, Elias M, Slobodin G, Rosner I: **Intravenous colchicine treatment for six months: adjunctive therapy in Familial Mediterranean fever (FMF) unresponsive to oral colchicine.** *Clin Exp Rheumatol* 2009, **27(2 suppl 53)**:S 105.

A15

P01-011 – Colchicine compliance and amyloidosis

H Ozdogan*, S Ugurlu, G Hatemi
Department of Internal Medicine, Division of Rheumatology, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A15

Objectives: To assess colchicine compliance in patients with familial Mediterranean fever and amyloidosis prior to the development of amyloidosis.

Methods: Twenty-six patients with FMF amyloidosis were questionned for disease onset, date of diagnosis for FMF and amyloidosis, delay in diagnosis, colchicine dose, response, compliance, disease manifestations, family history, and associated diseases.
Results: In 14 of the 26 patients, FMF and amyloidosis were diagnosed at the same time with a mean delay in diagnosis of 22 ±9.2 years. In the remaining 12, there was a mean delay of 9.6±8 years from the onset to the diagnosis of FMF and 23±9.6 years from the onset to the diagnosis of amyloidosis. These patients were on colchicine for a mean of 13±7.6 years after the diagnosis of FMF. Eight were non-compliant, however 4 were compliant and recieved 1.5 mg/day of colchicine for a mean of 7.5 years (range 4-12 years) before the development of amyloidosis. One of these 4 compliant patients stopped colchicine 1 year prior to the diagnosis of amyloidosis after 12 years of treatment. Response to Colchicine was reported in 3 patients. History of amyloidosis was present in one and history of FMF in 3 of the 4 compliant patients. None had an associated disease. Two were homozygous and one was heterozygous for M694V.
Conclusion: This retrospective data may indicate that in a proportion of patients with FMF who had recieved a proper dose of Colchicine can still develop amyloidosis. This observation deserves to be tested in a larger group of patients with FMF amyloidosis.
Disclosure of interest: None declared.

A16

P01-012 – Evaluation of autonomic function in FMF

N Aktay Ayaz[1*], N Melikoğlu Kiplapinar[2], G Keskindemirci[1], G Aydogan[1], O Isal Tosun[2], A Guzeltas[2], E Odemis[2]
[1]Pediatric Rheumatology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Turney; [2]Pediatric Cardiology, Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Center, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A16

Introduction: Familial Mediterranean fever (FMF) is characterized by acute and recurrent attacks of fever and polyserositis. It is associated with conduction disturbances and rhythm abnormalities. Heart rate variability (HRV) is the term used to indicate the fluctuation in cardiac frequency over time. HRV analysis is used to evaluate the condition of the autonomous nervous system, which regulates general cardiac condition and cardiac activity. Significant correlations between cardiovascular mortality and the autonomous nervous system have been evidenced in the last twenty years. In adult FMF patients abnormal heart rate variability (HRV) parameters were found, suggesting to autonomic dysfunction.
Objectives: To assess cardiac autonomic functions in FMF patients during childhood period.
Methods: A prospective randomized clinical trial was performed by a tertiary referral pediatric cardiology and a pediatric rheumatology center. The study group consisted of 53 patients with FMF (28 female, 25 male) that were followed-up by the pediatric rheumatology out-patient clinic. They were all under colchicine treatment. The control group was chosen from age and sex matched 44 healthy children (21 female, 23 male). All participants underwent 24-hour Holter rhythm monitoring (CardioNavigator Plus Impresario Medical Spider view, 3.07.0158, Delmar Reynolds; Paris, France). The HRV parameters were evaluated in both groups.
Results: The mean age of the study group was 11.6±3.5 years and the control group was 10.4±3.4 years. Height and weight of the study group were 143.2±19 cm and 37.9± 11.7 kg respectively. The control group's height and weight were 143.6±18.1 cm and 38.5±14.1 kg respectively. The mean duration of colchicine treatment was 43.4±41.5 months. The time-domain analysis of HRV revealed similar values of mean "standard deviation of all NN intervals" (SDNN;152.3±46.2 vs 143.13±41.99 msec, p=0.423), "SD of the 5 min mean RR intervals" (SDANN;131.3±36.3 vs 128.6 ±36.5 msec, p=0.451), "root square of successive differences in RR interval" (RMSSD; 70.8±53.5 vs 69±33.6 msec, p=0.481), and "proportion of differences in successive NN intervals >50 ms" (PNN50; 21.2±14 vs 21.3±12.1%, p=0.524), "triangular interpolation of NN interval histogram" (TINN; 623±219 vs 615±170 msec, p=0.451) and "HRV index" (20.8±6.8 vs 20.3±5.2 msec, p=0.633) in both groups. Frequency domain analysis revealed similar values of high frequency (HF; 48.2±13.9 vs 46.3±14.8, p=0.451), low frequency (LF; 42.5±12.7 vs 44±15.3, p=0.451) and LF/HF (1.08±0.84 vs 1.31±1.5, p=0.542) components in both groups.
Conclusion: Autonomic nervous system has an important role in the supervision of cardiac functions. In adult patients with uncomplicated FMF there are two published studies about the autonomic dysfunction, one revealing autonomic indices abnormalities and the other with similar normal autonomic function compared to healthy subjects. As being the first study concerning the autonomic function in children with FMF, we had found no significant differrences between both groups. This may be atrributed to the shorter duration and uncomplicated course of disease in children with FMF.
Disclosure of interest: None declared.

A17

P01-013 – Cochlear involvement in FMF

N Aktay Ayaz[1*], G Keskindemirci[1], A Batıoglu[2], G Aydogan[1], E Aldemir[1], Z Donmez[2], O Yigit[2], A Akçay[3], S Ozen[4]
[1]Pediatric Rheumatology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Turkey; [2]Otolaryngology Head and Neck Surgery, Istanbul Education and Research Hospital, Turkey; [3]Pediatric ematology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Istanbul; [4]Pediatric Rheumatology, Hacettepe University Medical Faculty, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A17

Introduction: FMF is a monogenic autoinflammatory disease with recurring episodes of fever and serositis attacks. FMF is associated with mutations in pyrin. On the other hand mutations in a molecule in the same pathway, cryopyrin, is characterized by inflammatory features involving the inner ear as well. A study has suggested the involvement of cochlea in Behçet disease, which is a polygenic autoinflamatory disease.
Objectives: To evaluate the cochlear function of children with the diagnosis of FMF prospectively.
Methods: Children included to the study were diagnosed as FMF according to previously suggested criteria. Forty-three children with FMF and 20 controls were enrolled to the study. Demographic data and MEFV mutation analysis were recorded. Patients with any middle and external ear pathology were excluded from the study. After otoscopic inspection, audiometric examinations were carried out including otoacustic emission testing by distortion products (DP) and signal noise ratio (SNR) testing with 1000, 1400, 2000, 2800 and 4000 Hz and audiometric evalution including pure tone average (PTA) measurements with high frequency levels that were 8000, 10000, 12500, 16000 Hz. The results of cochlear function evaluations of the patients and controls were analysed.
Results: The patient group included 43 children (27 female and 16 male patient) with mean age 11.9 (range 26 months-18 years) and the control group was age and sex matched. PTA levels were normal in both FMF patients and the control group. However, hearing levels at the frequency of 10000 Hz was found to be significantly higher in the FMF group ($p<0.05$). In otoacoustic emission evalution, SNR of the FMF group was lower in frequency at 1000 Hz ($p<0,05$).
Conclusion: Even though hearing function was normal there were a number of abnormalities especially at higher frequencies like 10000 Hz. Our results need to be confirmed in larger groups. Further studies are needed to understand whether these subtle changes are significant and whether they are due to subclinical inflammation of FMF.
Disclosure of interest: None declared.

A18

P01-014 – Subclinical atherosclerosis in FMF

S Ugurlu[1], SN Karaca[2], Y Demirel[2], E Seyahi[1*]
[1]Department of Internal Medicine, Division of Rheumatology, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey; [2]Department of Family Mediciney, Medical Faculty, University of Cumhuriyet, Sivas, Turkey, Sivas, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A18

Introduction: Whether atherosclerosis is increased or not in Familial Mediterranean Fever (FMF) is a much debated issue. Carotid artery intima media thickness (IMT) a surrogate marker for subclinical atherosclerosis is found to be increased in a number of studies [1-5], however this contrasts with the lack of increased frequency of atherosclerotic plaques [1-5]. Also, population surveys do not indicate an increased prevalence of

ischaemic heart disease in FMF patients. In the current study, we hypothesized that FMF patients with no apparent atherosclerotic risk factor would have no increase in the carotid artery IMT.
Objectives: To investigate markers of carotid atherosclerosis and oxidized low density lipoprotein (oxLDL) levels in patients with FMF who have no risk factors for cardiovascular disease.
Methods: We studied 44 patients (25 F/19 M; mean age: 33.5±7.5) with FMF in attack free period and gender and age matched 44 healthy subjects (25 F/19 M; mean age: 33.4±7.0). Exclusion criteria were clinical coronary artery disease, chronic renal disease, diabetes mellitus, hypertension, history of myocardial infarction, angina pectoris or cerebrovascular disease, dyslipidemia, metabolic syndrome or active infection. Those who were in postmenopausal status and using antilipid drugs were also excluded. We measured carotid artery IMT and investigated presence or absence of atherosclerotic plaques in the carotids, using Doppler ultrasound. We also assessed serum lipid and OxLDL levels.
Results: Mean disease duration of the FMF patients was 20±9 years. The mean carotid IMT (C-IMT) did not differ between patients and controls (0.52±0.10 mm vs 0.53±0.06 mm, respectively, P=0.709). None of the patients or controls had atherosclerotic plaques. Total and LDL cholesterol levels were significantly lower among patients compared to controls (total cholesterol: 157.07±34.18 vs 181.05±36.79, respectively, P=0.002; LDL cholesterol: 100.48±30.13 vs 126.25±34.05, respectively, P=0.001). Whereas OxLDL levels were significantly higher in FMF patients (337.48±438.56 ng/dl) when compared to controls (156.19±383.24 ng/dl), (P=0.044). There was no correlation between CIMT and OxLDL levels among both patients (r= -0.156, p=0.324) and controls (r=-0.196, p=0.246).
Conclusion: Our study supports further evidence for no increased atherosclerosis in FMF. As previously shown patients with FMF have low cholesterol levels when compared to healthy controls [1,2]. On the other hand, increased OxLDL levels could be associated with increased sub-clinic inflammation.
Disclosure of interest: None declared.
References

1. Ugurlu S, Seyahi E, Cetinkaya F, *et al*: *Rheumatology (Oxford)* 2009, **48**:911-5.
2. Akdogan A, Calguneri M, Yavuz B, *et al*: *J Am Coll Cardiol* 2006, **48**:2351-3.
3. Peru H, Altun B, Doğan M, *et al*: *Clin Rheumatol* 2008, **27**:689-94.
4. Sari I, Karaoglu O, Can G, *et al*: *Clin Rheumatol* 2007, **26**:1467-73.
5. Bilginer Y, Ozaltin F, Basaran C, *et al*: *Rheumatol Int* 2008, **28**:1211-16.

A19

P01-015 – Effect of Cochicine on cholesterol in FMF and BS

S Ugurlu[1], E Seyahi[1*], I Hanci[1], SM Pehlevan[2], H Ozdogan[1], H Yazici[1]
[1]Department of Internal Medicine, Division of Rheumatology, Cerrahpasa Medical Faculty, University of Istanbul, Turkey; [2]Department of Internal Medicine, Division of Rheumatology, University of Fatih, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A19

Introduction: We and others have previously shown that patients with Familial Mediterranean Fever (FMF) had low cholesterol levels when compared to healthy controls [1,2]. This was initially brought up by Ozkan E [3]. The causes of this abnormality are not understood. It could be due to an inherent effect of FMF or due to a lipid lowering effect of colchicine, as the patients in these studies were all regular users. Additionally, earlier studies had suggested that colchicine may have hypocholesterolemic effect.
Objectives: We conducted a 12 week study to determine whether colchicine would decrease serum lipid levels in patients with FMF and Behçet's syndrome (BS). Lipid levels were measured in each patient before and after colchicine use.
Methods: Blood cholesterol and triglycerides levels were measured in 24 patients with FMF (11 M, 13 F) and 16 (8 M, 8 F) patients with BS who were registered at the outpatient clinic of Cerrahpasa Medical Faculty. All patients were naive to colchicine or immunosuppressive treatment or any other lipid lowering drugs at study entry. Blood cholesterol and triglycerides levels were measured again after 12 weeks of colchicine 1.5 mg daily. Colchicine was withdrawn in one patient with FMF because of liver toxicity and in another because of nausea. Two patients with FMF did not use colchicine and another with FMF was lost to follow-up. Colchicine was switched to azathioprine in 1 patient with BS because of active disease. Only patients who completed 12 weeks period were analyzed.
Results: There were 19 (8 M, 11 F) patients with FMF and 15 (7 M, 8 F) patients with BS who completed the 12 week period. Patients with FMF were (mean age: 33.8±14.1 years) significantly younger than BS patients (mean age: 36.5±9.5) (P = 0.001). Colchicine did not change cholesterol and triglycerides levels in patients with FMF (T.Cholesterol: 169±77 vs 181±48 mg/dl, P = 0.58, Triglycerides: 122±82 vs128±70 mg/dl, P= 0.75, LDL:120±44 vs 112±40, P=0.35, HDL: 42±13 vs 47±11 mg/dl, P=0.1, before and after colchicine use, respectively). This was also true for BS patients (T.cholesterol:181±51 vs 172±44 mg/dl, P=0.53, triglycerides:112±63 vs 107 ±52 mg/dl, P=0.18, LDL:115±38 vs 106±40 mg/dl, P = 0.85, HDL:48±9 vs 48.3 ±9.9 mg/dl, P= 0.3).
Conclusion: This study provided no evidence that colchicine use affects lipid levels in patients with FMF and BS.
Disclosure of interest: None declared.
References

1. Ugurlu S, Seyahi E, Cetinkaya F, Ozbakir F, Balci H, Ozdogan H: **Intima-media thickening in patients with familial Mediterranean fever.** *Rheumatology (Oxford)* 2009, **48(8)**:911-5.
2. Akdogan A, Calguneri M, Yavuz B, *et al*: **Are familial Mediterranean fever (FMF) patients at increased risk for atherosclerosis? Impaired endothelial function and increased intima media thickness are found in FMF.** *J Am Coll Cardiol* 2006, **48**:2351-3.
3. Ozkan E, Okur O, Ekmekci A, Ozcan R, Tag T: **A new approach to the treatment of periodic fever.** *Med Bull Istanbul* 1972, **5**:44-9.

A20

P01-016 – Decreased vitamin D levels in FMF patients

H Onur[1], H Aral[2], V Arıca[3], G Bercem[1], M Usta[4], Ö Kasapçopur[5*]
[1]Department of Pediatrics, Istanbul Training and Research Hospital, Istanbul, Turkey; [2]Department of Biochemistry, Istanbul Training and Research Hospital, Istanbul, Turkey; [3]Department of Pediatrics, Mustafa Kemal University Medical Faculty, Hatay, Turkey; [4]Department of Biochemistry, Giresun University Medical Faculty, Giresun, Turkey; [5]Pediatric Rheumatology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A20

Introduction: Familial Mediterranean fever (FMF) patients suffer from recurrent self-limited inflammatory febrile attacks, abdominal, chest or joint pain. It is still unknown what triggers or ends these periodical attacks. Vitamin D, in addition phospho-calcium metabolism, has immunomodulation effects as pleotropic hormone. Vitamin D status has been linked to the occurrence and severity of autoimmune and inflammatory diseases.
Objectives: The aim of this study was to determine whether vitamin D deficiency is present in patients with Familial Mediterranean fever (FMF) compared with healthy child individuals.
Methods: The study group was comprised of 126 patients diagnosed with FMF (female/male (n):66/60); and 50 healthy control (female/male (n):25/25).Serum baseline 25-hydroxy vitamin D levels were measured. The FMF patients has divided into four groups according to mutation analysis.
Results: Vitamin D levels in FMF patients and healthy controls were 24,47± 8,48 and 28,70±11,70 ng/ml respectively. FMF patients had significantly decreased vitamin D levels compared with healthy controls (p<0,01). The study has shown plasma vitamin D level was similar in FMF patients with different MEFV gene mutation groups (P>0.05).The groups has been comprised as M694V/M694V(n=26), M694V/Other(n=38), Other/Other(n=46), Negative(n=16). The increase in age was significantly correlated with the decrease in vitamin D levels (r:-0.327 p<0.0001). Plasma vitamin D levels has not shown significance between patients with joint symptom(n:62) and without joint symptom (n:64) and has been detected as 23.72 ± 7.93, 25.20 ±8.99 ng/ml respectively.(p =0.328).
Conclusion: The etiology of recurrent attacks of serositis in familial Mediterranean fever (FMF) is not completely understood. Uncontrolled clinical case series have reported that factors associated with emotional, physiological, or physical stress precede and might trigger the attacks. In conclusion it is thought that vitamin D deficiency in pediatric FMF patients may have provide basis the attacks. Vitamin D level should be carefully examined and nutritional supplementation should be needed in FMF patients. Further studies with larger patient populations need to hold to investigate the vitamin D deficiency in patients with FMF.
Disclosure of interest: None declared.

A21

P01-017 – FMF presention with features of malignancy

N Aktay Ayaz[1*], G Keskindemirci[1], D Tugcu[2], A Akçay[3], E Aldemir[1], G Aydogan[1]
[1]Pediatric Rheumatology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey; [2]Pediatric Oncology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey; [3]Pediatric Hematology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A21

Introduction: Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by periodic attacks of fever and serositis caused by mutations in FMF gene (MEFV). Splenomegaly and lymphadenopathy has been reported in FMF. The abdominal lymphadenopahy was reported in the mesentery during laparotomies for acute abdominal attacks of FMF.
Case Report: A 14 year-old girl was admitted to the hospital with the complaints of fever, fatique and weight loss of 12 kg in 2 months duration. She had been prescribed different antibitiocis for fever. Her laboratory work-up was as follows; Hb. 7.4 gr/dl, Hct 23%, wbc 6000/mm^3, Plt 231000/mm^3, ESR: 112 mm/hr, C-reactive protein: 52 mg/l, differential count and bone marrow aspiration was normal. Vitamin B12 level was low. Autoantibodies and microbiological work-up were unremarkable. She had hyperglobulinemia. Abdominal ultrasound revealed mild hepatosplenomegaly, but this was not noticed at physical examination. Pericardial effusion of 7 mm was present at echocardiography. Abdominal MRI revealed lymphadenopathy at paraaortic region and and splenic hilus. Positron emission tomography was performed and increased fdg involvement at paraaortic, splenic and hepatic region, hypermetabolism at malignancy level and hypermetabolism in the spleen were detected. With the possible diagnosis of lymphoproliferative disease involving the spleen, an excisional biopsy was planned. During evaluation, the patient developed arthritis at her wrists. Due to the presence of fever, pericardial effusion, splenomegaly, arthritis and high inflammatory markers; MEFV mutation analysis was done. But in a month time, she lost 5 more kg, so a laparatomy and excisional biopsy was performed. Histopathology revealed only reactive lymphadenopathy without any malignant infiltration. She was found to be homozygous for M694V mutation. Colchicine treatment was introduced and nearly in a month time her ESR level decreased to 50 mm/hr and she had started to gain weight. In the next month's visit all of her complaints were gone and ESR had become normal. She is still under colchicine treatment without any complication for 3 months.
Discussion: This is a very interesting FMF case presenting with the symptoms of malignancy and we were obliged to have a biopsy in order to exclude malignancy. In the literature there are few reports about such severe cases involving abdominal lymph nodes. This case is presented due to its unusual severe presentation and excellent response in 2 months time to colchicine.
Disclosure of interest: None declared.

A22

P01-018 – An earliest diagnosis of FMF

N Aktay Ayaz*, E Aldemir, G Keskindemirci, C Aydogmus, G Aydogan, S Kavuncuoglu
Pediatric Rheumatology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Istanbul, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A22

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive disease, mainly affecting Jews, Armenians, Turks, Arabs and other groups living around Mediterranean basin. Major symptoms of disease are recurrent periodic fever accompanied by serositis. The disease is usually diagnosed at ages less than 20 years. Onset of the disease at older age can rarely occur. Symptoms related to FMF are noted when children become more verbal, usually after 2 years of age. Mutation analysis supports diagnostic evaluation.
Case report: Here, we are reporting the youngest FMF patient, that were internalized after birth as sepsis. Physicians were unable to discharge her from the hospital due to high acute phase response, that was dedicated to meningitis, urinary tract infection, sepsis and so on. Her metabolic screenings were done and were found to be negative. She was consulted to pediatric rheumatology for the high acute phase response and fever. With a detailed history and evaluation, it was learned that her mother had recurrent swelling of her ankle joints. Mutation analysis was performed and two homozygous mutations (M694V andR202Q) were identified. She was diagnosed as FMF at 3 months of age and colchicine was started with a dose of 0.25 mg/day. She responded to colchicine both clinically and in laboratory basis. Her uncontrolled acute phase response declined gradually.
Discussion: This case was reported to point out the importance of early remembrance of possible autoinflammatory diseases even at very early ages especially at endemic countries.
Disclosure of interest: None declared

References

1. Padeh Shai, Livneh Avi, Pras Elon, Shinar Yael, Lidar Merav, Feld Olga, Berkun Yackov: **Familial Mediterranean Fever in the First Two Years of Life: A Unique Phenotype of Disease in Evolution.** *The Journal of Pediatrics* 2010, **156(6)**:985-9.
2. Tunca M, Akar S, Onen F, Ozdogan H, Kasapcopur , Yalcınkaya F, *et al*: **Familial Mediterranean Fever (FMF) in Turkey: results of a nationwide multicenter study.** *Medicine* 2005, **84(1)**:1-11.
3. Yalcinkaya F, Ozcakar ZB, Tanyildiz M, Elhan AH: **Familial Mediterranean Fever in Small Children in Turkey.** *Clin Exp Rheumatol* 2011, **29(4 Suppl 67)**:S87-90.

A23

P01-019 – Anti-CCP antibodies are not associated with FMF

H Onur[1], H Aral[2], V Arıca[3], G Bercem[1], M Usta[4], Ö Kasapçopur[5*]
[1]Department of Pediatrics, Istanbul Training and Research Hospital, Istanbul, Turkey; [2]Department of Biochemistry, Istanbul Training and Research Hospital, Istanbul, Turkey; [3]Department of Pediatrics, Mustafa Kemal University Medical Faculty, Hatay, Turkey; [4]Department of Biochemistry, Giresun University Medical Faculty, Giresun, Turkey; [5]Pediatric Rheumatology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A23

Introduction: *Familial Mediterranean fever* (*FMF*) is an autosomal recessive disease that is prevalent among eastern Mediterranean populations, mainly non-Ashkenazi Jews, Armenians, Turks, and Arabs. Arthritis seen in FMF patients is generally acute monoarthritis which predominantly affecting the lower limbs, and it occurs during attack periods and also is a common clinical manifestation in patients with FMF alike Rheumatoid arthritis (RA). *Anti-cyclic citrullinated peptide* (*anti-CCP*) *antibodies* testing is useful in the diagnosis of Rheumatoid arthritis with high specificity. The citrulline residues are essential part of the antigenic determinants recognized by the RA antibodies.
Objectives: The aim of the study was to show the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies in child individuals diagnosed with Familial Mediterranean Fever (FMF) .
Methods: The study group was comprised of one hundred and twenty six patients diagnosed with FMF (female/male (n):66/60); and fifthy healthy control (female/male(n):25/25) . Clinical and laboratory assessments of the FMF patients were performed during attack-free periods. Erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), fibrinogen and anti-CCP antibody levels were measured.
Results: Anti-CCP results were negative in healthy controls and also in all FMF patients. There was not a significant difference in anti-CCP between the patient and the control groups. The patient individuals were divided into four groups according to genetic mutation analysis. The groups has been comprised as M694V/M694V(n=26), M694V/Other(n=38), Other/Other (n=46),Negative(n=16). No significant difference detected between four mutation groups and anti-CCP levels. Our study has shown moderate positive correlations between age (r_s= 0.271; p= 0.0020), duration of illness (r_s= 0.331; p<0.0001), colchicinetherapy (r_s= 0.259; p= 0.004) and anti-CCP levels. Also poor positive correlations between fibrinogen and anti ccp levels was detected (r_s= 0.192; p= 0.0330). Anti-CCP levels has not shown significance between patients with or without arthritis(p=0.148).
Conclusion: In conclusion, no published data in children establish anti-CCP values in patients with FMF compared with healthy controls. Our data show that anti-CCP antibodies are not associated with FMF. Anti-CCP does not have a priority for identifying FMF arthritis from the other inflammatory arthritis.
Disclosure of interest: None declared.

A24

P01-020 – Starting time of inflammatory attacks in patients

F Berktaş[1], N Alpay[2], B Toz[3], OK Bakkaloğlu[3], B Erer[2*], A Gül[2]
[1]Medicine and Life Sciences, Faculty of Health, Maastricht University, Maastricht, Netherlands; [2]Department of Internal Medicine, Division of Rheumatology, İstanbul University, İstanbul, Turkey; [3]Department of Internal Medicine, İstanbul Medical Faculty, İstanbul University, İstanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A24

Introduction: Familial Mediterranean fever (FMF), the most common of the hereditary autoinflammatory diseases, is characterized by recurrent self-limiting attacks of fever and/or serositis accompanied with acute phase response. Recurrence of attacks does not show a clear periodicity, and its timing is usually unpredictable. Little is known about the factors triggering or precipitating the attacks, and some patients describe physical or emotional exertion, menstrual cycle and dietary changes as possible triggers of the attacks. In other hereditary autoinflammatory disorders, a diurnal variation for the attack was observed with a tendency to experience attacks during evening or night. Recent data suggest that expression of some genes may show a circadian rhythm and affect the immune system, especially innate immune response.
Objectives: In this study, we aim to collect data retrospectively from FMF patients about the starting time of their attacks.
Methods: As a pilot study, we did a questionnaire based survey in 113 consecutive adult FMF patients. All patients fulfilled the Tel-Hashomer criteria for the diagnosis of FMF, and experienced attack(s) during the last year. All patients were interviewed directly or by telephone contacts to answer the questionnaire items about their attacks. The list of questions included usual start time of attacks during the day, their attack frequency, severity and possible triggers (such as sleeplessness, hunger, tiredness, stress, diet, medications, other diseases, menstruation and cold exposure) of the attacks during the past year.
Results: All patients (n=113) agreed to participate in the study and provided answers to the questions. Their mean age was 34.6 and sixty-two (55%) were female. The most commonly reported attack triggering factors were emotional stress (59%), menstruation (53% of female patients), tiredness (49%), followed by dietary changes (22%), cold exposure (15%), sleeplessness (10%) and hunger (4%). Only 9 patients (8%) had an occupation with night shifts. In all group, 76% of the patients provided a definite answer to the question about starting time of attacks. The majority reported that of their attacks started in the evening (41%), and less frequently in the morning (19%), at night (11%) and in the afternoon (4%). There was no correlation between the start time of the attacks and triggering factors or night shifts.
Conclusion: This questionnaire-based retrospective survey suggests that starting time of FMF attacks have a tendency for evening. This information may provide some clues about circadian changes affecting inflammation and attack tendency, after confirmation with prospective data collection.
Disclosure of interest: None declared.

A25

P01-021 – Macrophage migration inhibitory factory in FMF

I Sari[1*], Y Savran[2], DL Kozaci[3], N Gunay[4], F Onen[1], S Akar[1]
[1]Rheumatology, Dokuz Eylul University School of Medicine, izmir, Turkey; [2]Internal Medicine, Dokuz Eylul University School of Medicine, izmir, Turkey; [3]Biochemistry, Adnan Menderes University School of Medicine, Aydin, Turkey; [4]Biochemistry, Adnan Menderes University School of Medicine, izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A25

Introduction: Familial Mediterranean fever (FMF) is an autoinflammatory disorder characterized by recurrent, inflammatory, self-limited episodes of fever and serositis. Neutrophils are one of the key players in the pathophysiology of FMF. Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine involved in several inflammatory processes including innate and adaptive immune responses . In addition, MIF has been shown to regulate trafficking of inflammatory cells includingneutrophis to the sites of inflammation. Because its association with innate immunity, leukocyte trafficking, and inflammation MIF may be considered as an attractive cytokine in the pathogenesis of FMF.
Objectives: In this study we aimed to investigate MIF levels and its relationship with M694V mutations in patients with FMF.
Methods: Fifty one unrelated attack free FMF patients (14 M and 27 F, 32.8±8.7 years) and 30 healthy controls (16 M and 14 F, 32.7±7 years) were included in the study. Serum MIF were studied and allele frequency of M694V was calculated.
Results: Age, sex distribution, waist circumference, body mass index, smoking status and serum lipids were not different between the patient and control groups ($P > 0.05$). On the other hand, the levels of CRP, ESR, and MIF were significantly higher in FMF patients compared to those of controls ($P < 0.05$; 4.7±7.1 vs. 1.8±2 mg/L, 15.8±17 vs. 8.3± 5.2 mm/h, and 30.1±18.8 vs. 9±4.4 ng/mL respectively). Comparison of patients with and without M694V mutation revealed that MIF levels were not different between the groups. Regression analysis showed that none of the variables including disease duration, CRP, ESR, and BMI were predicting MIF concentrations ($P > 0.05$).
Conclusion: In this study we showed that: (1) MIF concentrations were significantly higher in attack-free FMF patients compared to healthy subjects; (2) increased MIF levels were independent from the inflammatory activity as assessed by ESR and CRP and (3) M694V mutations had no impact on MIF concentrations.
Disclosure of interest: None declared.

A26

P01-022 – MEFV gene mutations registered to infevers

A Berdeli*, S Nalbantoglu, D Tigli, I Demirel, M Atan
Molecular Medicine Laboratory, EGE UNIVERSITY, Izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A26

Introduction: Familial Mediterranean Fever (FMF) is the most common hereditary autoinflammatory disorder characterized by fever and abdominal pain. 16p13.3 chromosomally located MEFV gene has been responsible for disease outcome and its protein product, Pyrin, is the key regulator protein of inflammasome complex which leads to IL-1B production and inflammation.
Objectives: Here, we aimed to identify responsible MEFV gene mutations in clinically prediagnosed FMF patients and link to typical phenotype.
Methods: Bidirectional DNA Sequencing analysis of MEFV gene in all coding exons and exon-intron boundaries was performed in Turkish patients clinically pre-diagnosed as FMF consulted in Ege University School of Medicine between years 2009-2013 (n=8000) and in healthy control group individuals (n=250). For patients who were mutation negative in screened exons, exons 1, 4, 6, 7, 8, and 9 were also analysed.
Results: 14 novel missense and nonsense mutations were investigated and registered to INFEVERS (http://fmf.igh.cnrs.fr/ISSAID/infevers) p.R151S (c.453G>C); p.S154P (c.460T>C); p.S166L(c.497C>T); p.S179N (c.536G>A); p.R241K (c.722G>A), p. P350R(c.1049C>G), p.E456D (c.1368A>C) ; p.Y471X (c.1413C>A); p.R501C (c.1501C>T); p.S503C (c.1508C>G); p.I506V (c.1516A>G), p.K695N (c.2085G>C); p.L709R (c.2126T>G) and p. I729V (c.2185A>G). In phenotypic correlation, p.Arg241Lys and p.Ser166Leu mutations were linked to recurrent fever; and p.Ile506Val and p.Leu709Arg missense mutations were seen as atypical FMF phenotype while the remaining ones were correlated well with FMF clinical implications.
Conclusion: Identification of responsible mutations has great importance in disease maintanence, follow-up and proper treatment. It is recommended to prevent overlook uncommon pathogenic mutations in routine techniques via whole gene mutation analysis.
Disclosure of interest: None declared.

A27

P01-023 – Leukocytoclastic vasculitis in a patient with FMF

A Taylan
Romatoloji, TEPECIK EĞİTİM VE ARAŞTIRMA HASTANESİ, Izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A27

Introduction: A patient with familial Mediterranean fever (FMF) in addition to undiagnosed Anklosing Spondylitis (AS) and also having cutaneous leukocytoclastic vasculitis (LV) is presented. Coexistence of FMF with inflammatory disorders including spondyloarthritis and various systemic

vasculitides has been reported before. Meanwhile this is the first reported case of FMF, AS, and LV present together.

Case report: A 35 year old Caucasian man with known diagnosis of FMF was admitted for his cutaneous eruption of both lower limbs. He also had swelling and pain in his right wrist and left knee. Apart from colchicine pills, the patient had no history of recent drug exposure and also infectious symptoms like fever, abdominal pain, and diarrhea,but complaining of inflammatory back pain without diagnosis for 10 years. On examination, there were macular, cutaneous eruptions on both lower legs. In addition to arthritis, the patient also features of AS, such as painful restriction of spinal movements (modified schober 3cm).The remaining of the physical examination was normal.

Laboratory tests revealed hemoglobin of 9.3 g/dL.ESR and CRP were 59 mm/h, 7.27 mg/dL respectively. Radiographs of sacroiliac joint were compatible with bilateral grade IV sacroiliitis. A skin biopsy from tibia showed fibrin deposits, nuclear debris, endothelial swelling and neutrophils disrupting a capillary wall. Immune fluorescence staining was clear for immune deposit. Serum IgA level was 617mg/dl (82-453) and HLA-B27 antigen was positive. ANA, ANCA, and cryoglobulins were undetectable; complement levels and urine examination were in normal limit. Genetic analysis was consistent with compound heterozygote mutation (R202Q/R726A) at MEFV gene.

Treatment with corticosteroid, sulfosalazine, asemetazin, colchicin was commenced. Almost 10 days after treatment the skin eruptions was fade; arthritis and back pain regressed.

Discussion: Besides arthritis, several spondyloarthropathic features like sacroiliitis and enthesopathy can be seen in the course of FMF. Sacroiliitis with apophyseal joint involvement without vertebral squaring and bamboo spine characterize FMF-related sera negative spondyloarthropathy, while anterior radiologic involvement of the spine tent to show concomitant presence of AS with FMF. Only apophyseal joints involvement of this case without anterior vertebral column involvement suggests that the lesion of this case was FMF-related.A skin biopsy showing no immune deposit, several urine examination without microscopic hematuria or proteinuria together with lack of GI symptoms and preceding infection all rules out IgA nephropathy and Henoch Schönlein Purpura .Some patients who have taken drugs may develop drug related LV. In this case, absences of history of recent exposure to drugs also discard this possibility Several vasculitic disorders including HSP, PAN, and Protracted febrile myalgia was reported to be associated with FMF . Among the MEFV gene, M694V mutations through high IL-1β activity cause a severe form of FMF. To our knowledge, cutaneous vasculitis with immune complex nephritis in a FMF patient was reported by Schlesinger et al [1]. In our case, MEFV mutation and HLA-B27 might have acted as a genetic susceptibility factor for LV development.

Disclosure of interest: None declared.

Reference

1. Schlesinger M, *et al*: A case of familial Mediterranean fever with cutaneous vasculitis and immune complex nephritis. *Am J Clin Pathol* 1983, **80(4)**:511-4.

A28

P01-024 – Vascular risk assessment and MMP-3 gene in FMF

S Mir[1], B Sozeri[2*], K Ozdemir[1], A Berdeli[3]
[1]peadiatric nephrology, Ege University, Izmir, Turkey; [2]pediatric rheumatology, Ege University, Izmir, Turkey; [3]molecular genetic, Ege University, Izmir, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A28

Introduction: The patients characterized with chronic subclinical inflammation even during attack-free periods, are now considered to have an increased risk of atherosclerotic complications as well as other autoinflammatory disease. Damage to the arterial wall due to atherosclerosis causes increased arterial stiffness. Pulse wave velocity (PWV), a noninvasive measure of arterial stiffness, is accepted to be an indicator of subclinical atherosclerosis. Cardiovascular disease included various risk markers; blood biomarkers and genetic markers.

Matrix metalloproteinases (MMPs) are closely related proteinases that together are able to degrade all macromolecules of the extracellular matrix. MMPs are potentially implicated in atherogenesis, progression of atherosclerosis. The gene encoding MMP-3 is polymorphic and an insertion (6A)/deletion (5A) polymorphism (5A/6A polymorphism) in the MMP-3 gene may have functional significance in the regulation of its expression. The 5A allele was associated with higher and the 6A allele with lower transcriptional activity. Up to date, the 6A/6A and 5A/6A genotypes were associated with coronary artery disease and carotid atherosclerosis in adults.

Objectives: We aimed to evaluate the effect of inflammation and the strength of association MMP-3 promoter low- and high-activity genotypes on the increased risk of subclinical atherosclerosis in FMF patients.

Methods: Forty-seven patients (M/F =21/26) with FMF, and 50 age- and sex-matched controls were recruited. We measured lipid profile (LDL, total cholesterol and lipoprotein a level) and acute phase reactants (APRs) (white blood cells, erythrocyte sedimentation rate, high sensitive C-Reactive Protein and Serum Amyloid A) in attack free period of all patients. Aortic PWV was determined by using an automatic device (Vicorder, Germany) that allowed on-line pulse wave recording and automatic calculation of the PWV. The 5A/6A polymorphism was typed by RFLP-PCR.

Results: The mean APRs values were not found statistically significant in patients than control. The distribution of the genotypes of the 5A/6A polymorphism in both control and study patients did not differ significantly (40%,32.8% , respectively p>0.05) from those predicted by the Hardy-Weinberg distribution.

The PWV was slightly higher in patients with FMF than in control subjects (P=0.05). Fifteen patients (32%) have PWV values above the average. These patients have also high SAA and lipoprotein-a levels in attack free period. A significant correlation between PWV and lipoprotein a (P<0.001, r=0.67), and SAA level (P<0.001, r=0.52) was found in patients with FMF. There was no detected hypertension. There were no significant differences (p>0.05) in genotype distributions (hyperlipidemia and arterial stiffness index) and allele frequencies between subgroups.

Conclusion: The results showed that arterial stiffness is correlated with hyperlipidemia and subclinical inflammation in FMF patients. But, the 5A/6A polymorphism of MMP-3 gene may not be linked with appearance and/or progression of arterial stiffness in FMF patients. Our suggestion is that SAA levels as well as the use of therapy monitoring can be predict in cardiovascular disease in patients with FMF.

Disclosure of interest: None declared.

A29

P01-025 – Decreased vitamin D levels in children with FMF

B Makay[1*], A Anık[2], G Çatlı[2], A Abacı[2], T Küme[3], E Böber[2], E Ünsal[1]
[1]PEDIATRIC RHEUMATOLOGY, DOKUZ EYLÜL UNIVERSITY HOSPITAL, İzmir, Turkey; [2]PEDIATRIC ENDOCRINOLOGY, DOKUZ EYLÜL UNIVERSITY HOSPITAL, İzmir, Turkey; [3]BIOCHEMISTRY, DOKUZ EYLÜL UNIVERSITY HOSPITAL, İzmir, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A29

Introduction: Several recent studies have reported a link between vitamin D deficiency and certain chronic inflammatory disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Behçet's disease. These recent findings have led to greater emphasis on treatment of vitamin D deficiency and vitamin D supplementation in rheumatological diseases. To our knowledge, vitamin D levels have not been previously investigated in children with FMF disease.

Objectives: To determine the frequency of vitamin D deficiency in children with familial Mediterranean fever (FMF) and to investigate the factors associated with low vitamin D status.

Methods: Forty-four patients with FMF and 39 age- and sex-matched healthy controls were enrolled in this study. Demographic data, disease duration, time to delay for diagnosis, FMF symptoms, disease severity score, MEFV mutation, dose and duration of colchicine therapy and compliance to treatment were recorded for each patient. Serum 25- hydroxyvitamin D levels were measured by original commercial kit based on Chemiluminescent Microparticle İmmunoassay (CMIA) principle.

Results: The serum 25- hydroxyvitamin D levels were significantly lower in FMF patients than the healthy controls (12.9 ± 3.6 and 16.3 ± 5.5, respectively, p=0.001). The vitamin D level was similar in patients homozygous for M694V and other genotypes (11.8 ± 3.7 and 13.2 ± 3.6, respectively, p=0.21). There was a significant negative correlation between the duration and cumulative dose of colchicine use and vitamin D levels (r=-0.410, p=0.006 and r=-443, p=0.004, respectively). There was no correlation between vitamin D levels and C-reactive protein, white blood cell count, disease duration, disease severity score or age of the patient.

Conclusion: The results of this study suggest that serum 25- hydroxyvitamin D levels are decreased in children with FMF. Duration of colchicine use and cumulative colchicine dose appear to effect vitamin D levels negatively.

Disclosure of interest: None declared.

A30

P01-026 – A case of FMF and hereditary coproporphyria

A Ganesha*, S Savic
Clinical Immunology and Allergy, St James's University Hospital, Leeds, United Kingdom
Pediatric Rheumatology 2013, **11(Suppl 1):**A30

Introduction: We report a unique case in a 17 year old male patient of Algerian origin with two rare genetic conditions with overlapping clinical symptoms. Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by sporadic, paroxysmal attacks of fever and serositis. Hereditary coproporphyria (HCP) is one of the type of acute hepatic porphyria resulting in neurovisceral symptoms caused by deficient activity of mitochondrial enzyme coproporphyrinogen oxidase. Both are considered rare differential diagnosis for acute abdominal pain.
Case report: 17 year old boy of Algerian origin presented with long history of recurrent episodes of fever, abdominal pain since infancy. He experiencied 3-4 attacks per year each lasting typically for 2-3 days. There was no family history.
Patient was referred simultaneously to immunology and metabolic medicine for further assessment. Differential diagnoses considered at the time included: periodic fever syndromes, hereditary angioedema, vasculitis and porphyria.
FBC results over the year showed intermittent leucocytosis during acute attacks with elevated C-reactive protein (CRP) and plasma viscosity (PV). Serum amyloid A (SAA) was not measured. Investigations during quiescent phase showed normal levels of SAA but slightly elevated CRP 13.4 mg/l (ref <10) and neutrophilia of 9.9 x10^9/l (ref 2.00-7.50).
Genetic investigations for periodic fever syndromes confirmed two pathogenic MEFV gene mutation on sequencing Exon 2 and 10 at p. (Met694Ile(;)Glu148Gln), supporting diagnoses of FMF. Sequencing for MVK and TNFRSF1A gene were negative.
Investigations undertaken by metabolic medicine specialists revealed urine coproporphyrin III at 42.75nmol/mmol cret (1.2-24.8) with porphrin/creat Ratio of 54.6 nmol/mmol (Ref <28). Faecal porphrin were 1639nmol/g dry weight (ref <130) with faecal coproporphyrin III: I ratio at 19.51 (Ref <2). The results confirmed diagnosis of HCP. Genetic tests are awaited for the patient. He has management plan for hereditary coproporphyria.
Patient was commenced on colchicine at the dose of 500 micrograms twice daily. No further episodes of abdominal pain have been reported in the last 9 months since prophylaxis starting prophylaxis with colchicine.
Discussion: FMF and HCP are both recognised as rare causes of unexplained acute abdominal pain associated with fever. However there are several additional features which would favour FMF over HCP. The patient's ethic origin is more suggestive of FMF. The majority of HPC was reported in the North European ancestry. The onset of HCP also tends to be in puberty whilst FMF usually presents in childhood with initial attacks before the age of 10 in 65% of cases. Low grade persistent inflammatory response is a feature of FMF and not necessarily seen HCP. Finally apparent response to colchicine would further support diagnosis of FMF.
Disclosure of interest: None declared.
References

1. Tunca M, *et al*: Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine (Baltimore)* 2005, **84(1)**:1.
2. With TK, *et al*: Hereditary coproporphyria and variegate porphyria in Denmark. *Dan Med Bull* 1983, **30(2)**:106.

A31

P01-027 – Normal HRV in colchicine-resistant FMF patients

U Nussinovitch[1*], A Livneh[2]
[1]Israel Naval Medical Institute, Haifa, Israel; [2]The Heller Institute of Medical Research, and Department of Medicine F, Sheba Medical Center, Tel Hashomer, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A31

Introduction: The relationship between autonomic nervous system (ANS) dysfunction and familial Mediterranean fever (FMF) is controversial. Heart rate variability (HRV) is a powerful, simple and reliable technique for the evaluation of ANS dysfunction. Recently, we reported on normal HRV parameters, suggestive of normal ANS function, in patients with uncomplicated FMF. Also, we reported on an association between decreased HRV parameters (suggestive of ANS dysfunction), and amyloidosis of FMF, particularly at a progressive stage.
Objectives: The aim of the current study was to evaluate whether FMF patients, who do not respond to colchicine treatment, and thereby endure persistent inflammation, have abnormal ANS function, using the HRV tool.
Methods: Twenty-four FMF patients suffering from recurrent FMF attacks despite treatment with a maximal colchicine dose, were selected for the study. Electrocardiogram was measured under strict conditions and HRV parameters were calculated. Results were compared with age- and sex-matched unaffected controls.
Results: No statistically significant difference was found between the groups in any determined HRV parameter: maximal RR, minimal RR and average RR intervals, standard deviation of RR interval, square root of the mean squared differences of successive RR intervals, HRV triangular index, NN50, pNN50, and power spectral analysis. Nevertheless, a statistically non-significant trend towards lower HRV parameters was observed in the colchicine non-responders group.
Conclusion: Although a small difference in HRV parameters cannot be entirely excluded in the current study design, FMF patients, in whom colchicine did not provide adequate symptomatic relief and who did not develop amyloidosis, seem to have HRV parameters similar to those of healthy subjects, suggestive of normal ANS function.
Disclosure of interest: None declared.

A32

P01-028 – MEFV mutation in Morrocan child wuth familial Mediterranean fever

B chkirate*, A Dibi, A Bentahila, service de Pédiatrie 4, Hôpital d'Enfants, CHU, IbnSina, Rabat, Maroc
Faculte de medecine, Rabat, Morocco
Pediatric Rheumatology 2013, **11(Suppl 1):**A32

Introduction: Familial Mediterranean Fever (FMF) is an autosomal recessive inherited disease mostly wide spread in the Mediterranean basin. It is manifested by a fever associated with paroxystic painful attacks. The prognosis is determined by the occurrence of renal amyloidosis. The purpose of our work is to establish a genotype- phenotype correlation between the MEFV gene mutation and the expression of the FMF in 10 Moroccan children.
Case report: Material and methods: It's a retrospective study of children responding to the FMF Yalcinkaya criteria screened at infantile hospital of Rabat. The genetic study was conducted at the hygienic national institute of Rabat.
Results: There are 6 boys and 4 girls at the average of 10 years old. The consanguinity was found in 2 cases. Similar familial cases were found in 3 cases. Fever and abdominal pain were present in all cases. Articular pains in 60% of cases and muscular ones in 30%. An inflammatory syndrome was found in all cases. The renal tests were normal in all cases. The genetic study revealed the presence of the MEFV gene mutation in 5 cases (50%): M694I in 2 cases, M694V in 1 case, M694V/M694I at a composite state in 1 case and M680I in 1 case. All the patients received colchicine. The evolution was favorable in 9 cases. The biotherapy was done to one patient because of the persistence of clinical symptomatology.
Discussion: The "pathogene" effect of MEFV gene mutations is too variable. The foundering mutations M694V, M694I and M680I, which are very frequent in the populations at risk of FMF are also those linked to the most severe phenotypes. Nevertheless, variable penetrance and expression of FMF could be explained by the type and number of mutation but also by other modifiers genes and/or environmental factors.
Disclosure of interest: None declared.

A33

P01-029 – Microscopic hematuria in FMF

L Grabowitz[1,2], OL Kukuy[3*], M Lidar[1,2,4], I Ben Zvi[2,4], O Feld[2,4], Y Kesel[4], A Livneh[1,2,4]
[1]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; [2]Heller Institute of Medical Research, Israel; [3]Institute of Nephrology and Hypertension, Sheba Medical Center Tel HaShomer, Ramat Gan, Israel; [4]Medicine F, Sheba Medical Center Tel HaShomer, Ramat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A33

Introduction: Hematuria is a recognized feature of familial Mediterranean fever (FMF), but its prevalence and clinical, genetic and demographic correlates are not known.
Objectives: To study the rate and features of microscopic hematuria in FMF.
Methods: We studied consecutive FMF patients, who came for a pre-scheduled follow up visit in the FMF clinic for the presence of microscopic hematuria, defined as ≥5 RBC/HPF or ≥25RBC/μl in urine analysis performed during remission, recorded at least once in the 3 previous clinic visits. Exclusions were known kidney, urinary tract, prostate or gynecologic diseases, bleeding or thrombotic diatheses, pregnancy or menstruation, intensive physical activity and anticoagulant/platelet treatments. Patients presenting with hematuria were compared to patients without hematuria for various clinical, genetic and demographic parameters, using a questionnaire, patient files, and an interview.
Results: The frequency of microscopic hematuria among FMF patients was found to be 17% (30/173), not conspicuously higher than in the general population (1-16%). Hematuria was associated with higher levels of acute phase reactants during the attack-free phase, and higher rates of a history of vasculitides: protracted febrile myalgia and Henoch Schonlein Purpura. There were no differences in the distribution of severity scores among patients of the hematuria and control groups. The rate of homozygosity to M694V and the rate of 2 affected MEFV alleles was similar to that of the control group.
Conclusion: This study could not confirm the notion that microscopic hematuria is more common in FMF. However, its occurrence may reflect an active disease and renal vascular inflammation.
Disclosure of interest: None declared.

A34

P01-030 – Proteinuria in FMF – prediction of nephropathy type

OL Kukuy[1*], A Livneh[2,3], A Ben-David[1], J Kopolovic[4], A Volkov[5], Y Shinar[3], E Holtzman[1,2], D Dinour[1,2], I Ben-Zvi[2,6]
[1]Institute of Nephrology and Hypertension, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel; [4]Department of Pathology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [5]Department of Pathology, Sheba Medical Center, Tel Hashomer, Israel; [6]Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A34

Introduction: Reactive (AA) amyloidosis may complicate Familial Mediterranean fever (FMF), the prototype of autoinflammatory diseases. Thus, proteinuria in FMF is commonly viewed as resulting from amyloidosis and kidney biopsy is deemed superfluous. However, nephropathy other than amyloidosis has been described in FMF, but its rate and distinctive characteristics are unknown.
Objectives: To determine the rate and underlying pathology of FMF related non-amyloidotic proteinuria and compare its clinical course, demographic and genetic features to those of FMF-amyloid nephropathy.
Methods: This study is a retrospective analysis of data from all FMF patients, undergoing kidney biopsy for proteinuria above 0.5 gram/24 hrs, during 10 years (2001- 2011). Clinical, laboratory, genetic and pathology data were abstracted from patient files. Biopsies were viewed by an experienced pathologist, as necessary.
Results: From 27 patients referred to kidney biopsy, only 16 (59.3%) were diagnosed with amyloid kidney disease (AKD), 11 were diagnosed with another nephropathy. The AKD and non amyloid kidney disease (NAKD) groups were comparable on most variables, but showed distinct characteristics with regard to the range of proteinuria (6.46 ±4.3 g vs. 2.4±1.7 g, p= 0.0136), rate of severe FMF (14 vs. 5 patients, p= 0.03) and rate of development of end stage renal disease (75% vs. 27.2%, p=0.02) respectively.
Conclusion: NAKD is common in FMF and is featured with milder course and better prognosis. Contrary to common practice, it is highly suggested to obtain kidney biopsy from patients with FMF and proteinuria more than 0.5 gr/24 hrs.
Disclosure of interest: None declared.

A35

P01-031 – Anakinra for colchicine resistant FMF

I Ben-Zvi[1*], OL Kukuy[1], M Lidar[1], O Feld[1], O Perski[1], S Kivity[1], P Langevitz[1], B Pistrom[2], A Livneh[1]
[1]Heller Institute of Medical Research, Sheba Medical Center, Ramat-Gan, Israel; [2]Swedish Orphan Biovitrum, (SOBI), Stockholm, Sweden
Pediatric Rheumatology 2013, **11(Suppl 1):**A35

Introduction: About 10-20% of familial Mediterranean fever (FMF) patients do not achieve complete remission, due to colchicine resistance or sensitivity. Disease control in these patients is still an unmet challenge. Case reports and a small case control study, suggest a role for interleukin 1 beta blockage, particularly anakinra, in the management of this limitation of colchicines.
Objectives: To embark on a study, evaluating the efficacy and safety of anakinra in the treatment of colchicine refractory FMF.
Methods: We plan to include patients, agreeing with clinical and genetic diagnosis of FMF, who suffer from FMF attacks, at least once per month, in one of the sites commonly involved by FMF (Chest, abdomen, lower extremity large joints, and skin), despite treatment with colchicine 2 mg/day or less (in case of colchicine intolerance). Involvement with other diseases relevant (vasculitis, spondyloarthropathy, Behcet's disease, etc.), or irrelevant (rheumatoid arthritis, SLE, etc.), to FMF, or possible non compliance, will serve as exclusion criteria. The study is planned to continue for 4 months per patient, in which patients will receive anakinra (s.c. 100 mg/day, 25 patients), or control drug (anakinra vehicle, same volume, same package, 25 patients). Randomization will be sequential for a predetermined order of the interventional drug (anakinra or vehicle), for which the study team will be blinded. Analysis of the results will be performed by an external company. Anakinra effect will be compared to placebo effect by computing the reduction of number of attacks per each patient. Secondary outcome include reduction of severity of attacks.
Results: No results are yet available. The study is an investigator initiative project, with sponsorship of the manufacturing drug company. Measurements were taken to avoid any bias in the performance of the study or interpretation of the results. It is expected that the whole study will continue 2 years.
Conclusion: A favorable anakinra effect in the prevention of FMF attacks in colchicine failure, supported by ample case reports is expected to be confirmed by the present controlled double blinded study by mid 2015.
Disclosure of interest: None declared.

A36

P01-032 – Characterization of genetic-negative FMF

I Ben-Zvi[1,2*], C Herskovizh[2], Y Kassel[3], A Livneh[1,2]
[1]Heller Institute of Medical Research, Departemnt of Medicine F, SHEBA MEDICAL CENTER, Ramat-Gan, Tel Aviv, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]Departemnt of Medicine F, SHEBA MEDICAL CENTER, Ramat-Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A36

Introduction: Up to 20 percent of FMF cohorts consist of patients fulfilling the diagnostic criteria of familial Mediterranean fever, yet carry no MEFV mutations (genetic-negative). The phenotype of these patients has been poorly characterized.
Objectives: To define clinical and demographic parameters of genetic negative FMF.
Methods: In this observational comparative study, 47 sequential genetic negative FMF patients and 78 sequential genetic positive (for at least one allele) FMF control patients were compared using a comprehensive questionnaire completed at the time of their routine clinic visit, using direct questioning and patients' files. The definition of FMF was based on our clinical tool, widely accepted for FMF diagnosis. Absence of the 5 most common MEFV mutations in routine genetic testing of FMF was considered genetic negative FMF. Disease severity was determined by Mor criteria.
Results: The mutation-negative and mutation positive cohorts differed respectively on the age of disease onset (19.6 vs. 10.1 years, p<0.001), family history of FMF (44% vs. 76.9%, p<0.001), rate of severe disease (23.4% vs. 64.1%, p<0.001), and rate of erysipelas-like erythema which was

higher in the control group (p=0.024). There was a trend for diagnosis delay (9.95 years vs. 6.68 (p=0.08). There were no significant differences in gender and in a wide array of clinical manifestations. The average dose of colchicine, the response to treatment and the rate of chronic manifestation of FMF were also comparable between the two patient groups.
Conclusion: The FMF specific phenotype manifested in mutation-negative FMF, together with low prevalence of family history, suggest the occurrence of a de-novo genetic event downstream the MEFV related pathway.
Disclosure of interest: None declared.

A37

P01-033 – Co-occurance of Crohn's disease and FMF

A Epstein[1], I Ben-Zvi[1,2*], Y Shinar[3], M Lidar[1,2], S Ben-Horin[2,4], A Livneh[1,2]
[1]Heller Institute of Medical Research, Departemnt of Medicine F, SHEBA MEDICAL CENTER, Ramat-Gan, Tel Aviv, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Isreal; [3]Heller Institute of Medical Research, Isreal; [4]Gastroenterology Unit, SHEBA MEDICAL CENTER, Ramat-Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A37

Introduction: There is an increased prevalence of Crohn's disease (CD) in familial Mediterranean fever (FMF). Previous studies found that neither MEFV, nor NOD2/CARD15 may serve as susceptible genes, leading to FMF-CD comorbidity. In addition to NOD2/CARD15 polymorphism, ATG16L1 and IL-23R gene SNPs were also found to predispose to Crohn's disease (CD). The role of these genes in the occurrence of FMF-CD is currently unknown.
Objectives: To determine the role of polymorphism in NOD2, ATG16L1 and IL-23R genes in FMF-CD, and characterize the clinical correlates of this association.
Methods: To enrich for CD associated genes with possible effect on the occurrence of FMF-CD, we identified all patients with FMF-CD in our computerized registry of approximately 12,000 FMF patients. All patients were tested for MEFV, NOD2, ATG16L1 and IL-23R relevant gene mutations and completed a questionnaire, detailing the phenotype of their disease. CD diagnosis was established by typical clinical, radiological and endoscopic findings, while a diagnosis of FMF was determined based on our established set of criteria.
Results: Nineteen patients with FMF-CD were identified. Of them, 17 consented to participate in this study (8 females, 9 males). All patients were of North-African origin. Ten patients (58%) were carriers of the MEFV M694V mutation (5 homozygous). Eight patients (47%) needed biological treatment to control their CD. Two patients (11.7%) had amyloidosis with chronic renal failure. When compared to published patients with CD alone, the FMF-CD group had comparable rate of Gly908Arg NOD2 mutation (19% vs. 9.8%, P=0.2), Thr300Ala ATG16L1 mutation (78% vs. 58%, p=0.14) but significantly increased rate of the rs1004819 polymorphism in IL-23R (61% vs. 38%, p=0.006).
Conclusion: The rs1004819 IL-23R polymorphism predisposes for the occurrence of FMF-CD. In patients with this comorbidity, the CD appears to be more severe.
Disclosure of interest: None declared.

A38

P01-034 – Cancer in FMF: a population based study Israel

R Brenner[1], S Kivity[2,3], Y Shinar[4], I Iiphshitz[5], E Ben-Chetrit[6], A Livneh[2,4,7], B Zvi[8*]
[1]Cancer Unit, Edith Wolfson Medical Center, Holon, Tel Aviv, Israel; [2]Rheumatology Unit , Zavladovich center of autoimmune disease, Israel; [3]Department of Medicine A and C, Sheba Medical Center, Ramat-Gan, Israel; [4]Heller Institute of Medical Research, Sheba Medical Center, Ramat-Gan, Israel; [5]Israeli National Cancer Registry, Ministry of Health, Israel; [6]Department of Medicine A, Hadassah-Hebrew University and Medical Center, Jerusalem, Israel; [7]Department of Medicine F, Sheba Medical Center, Ramat-Gan, Israel; [8]Rheumatology Unit at Zavladovich center of autoimmune disease, Heller Institute of Medical Research , Department of Medicine F. , Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A38

Introduction: Recurrent or persistent inflammation, featuring familial Mediterranean fever (FMF), may induce, promote, or influence susceptibility to carcinogenesis. However, the association between FMF and malignancy was rarely described before.
Objectives: To assess the prevalence of malignancy in FMF.
Methods: Demographic data of FMF patients, followed in the national FMF center at Sheba medical center (n=8352) and Hadassah Medical Center (n=1083) were obtained from FMF patient hospital registries. The prevalence of cancer in the general population, and in the study registries was attained from the cancer registry of Israel and analyzed according to age, origin, and cancer type. The Standardized **incidence** rates (SIR) of the different cancers in FMF patients were calculated and compared to the cancer SIR of the parallel Israeli ethnic population.
Results: Of 9435 FMF patients (4696 men, 4739 female), 363 developed cancer during the years 1970- 2011. FMF female patients developed significantly more lymphoma (Hodgkin and non-Hodgkin) and in-situ cervical cancer than the matched general population, SIR 2.07 (95% CI 1.12-2.99) and 1.86 (95% CI 1.20-2.51), respectively. In contrast, male and female with FMF had a lower gastrointestinal cancer incidence, SIR 0.68 (95% CI 0.42-0.95) and 0.63 (95% CI 0.36-0.90).
Conclusion: The risk for lymphoma and in-situ cervical cancer is increased about twice in FMF. Understanding the underlying mechanism (inflammation? Colchicine? Genetic predisposition?) may improve patient prognosis.
Disclosure of interest: None declared.

A39

P01-035 – Long-term IV colchicine in oral colchicines failure

O Feld[1], I Ben Zvi[1], OL Kukuy[2], A Livneh[3,4*]
[1]Medicine F, SHEBA MEDICAL CENTER, Rammat Gan, Israel; [2]Nephrology and Hypertension Institute, SHEBA MEDICAL CENTER, Rammat Gan, Israel; [3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [4]Heller Institute for Medical Research, SHEBA MEDICAL CENTER, Rammat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A39

Introduction: Various levels of oral colchicine resistance is still an unmet challenge of FMF treatment. IV colchicine was shown to be effective and safe answer, but its long term outcomes are not known.
Objectives: To determine long term effectiveness and safety of IV colchicine treatment in oral colchicine refractory or intolarant FMF patients.
Methods: Included were all patients, experiencing ≥1 attack per month, or intolerant to adequate dose of oral colchicin, for which they receive IV colchicine (pharmacy preparation under government control) for at least 1 year. Retrieval of data was based on patient interviews, files and a detailed questionnaire, focusing on clinical, demographic and genetic data. Effect of colchicine was determined, by computing attack rate, duration and intensity, the later with 1-10 scale.
Results: Ten of 11 identified patients, on long term IV colchicine treatment, consented to partake. Treatment lasted 3.6 ±2.7 (1- 10) years. More than 50% reduction in the rate of abdominal, chest, joint and skin attacks was noted by 8 of 10 patients. The intensity of the attacks dropped by a mean of 50±22% and the duration by 40±20%. In 4 patients the favorable effect has decreased partially, but in only one treatment was stopped for this reason. Treatment was terminated in another 2 for loss of venous access (1) and for paresthesis. Adverse effects included diarrhea (1 patient), vomiting (2), injection site pain (3), headache (1), muscle pain (1), injection site phlebitis (1) and arm paresthesis (1).
Conclusion: Long term parenteral colchicine treatment proved effective and safe. Downloading I.V. colchicine off the shelves due to intoxication associated with uncontrolled and unjustified use for back pain, had serious negative impact on our armamentarium for oral colchicine nonresponsiveness and intolerance.
Disclosure of interest: None declared.

A40

P01-036 – Systemic amyloidosis presenting with amyloidoma

H Nalcacioglu[1], G Genc[1], S Ayyildiz[2], M Kefeli[3], O Aydin[3], M Elli[4], M Ceyhan[5], O Ozkaya[1*]
[1]Pediatric Nephrology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey; [2]Pediatric Surgery, Ondokuz Mayis University Faculty of

Medicine, Samsun, Turkey; [3]Pathology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey; [4]Pediatric Oncology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey; [5]Radiology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A40

Introduction: Amyloidosis is a heterogeneous group of disorders characterized by extracellular deposition of unique protein fibrils. The least common presentation of an amyloid deposition is as a discrete mass called amyloidoma or amyloid tumor. It has been reported in many anatomic site including the respiratory, genitourinary, and gastrointestinal tracts, as well as internal viscera, the central nervous system, skin, breast, and soft tissues. We report a case of a soft tissue amyloidoma in the abdomen of an 16-year-old girl diagnosed with systemic amyloidosis.
Case report: A 16-year-old girl was admitted to the hospital with the complaint of abdominal pain and artralgia for 4 months. She was referred to our hospital with a pre- diagnosis of a retroperitoneal mass documented with an abdominal ultrasonography and tomography. Her physical examination was normal except pretibial edema. Proteinuria, hypoalbuminemia, hypertriglyceridemia and nephrotic range proteinuria was found in laboratory examination. She underwent a surgery for complete resection of the lesion and routine histopathological examination with Congo red and crystal violet dyes verified the diagnosis of an amyloidoma. Immunohistochemical study for AA protein is positive. Nephrotic syndrome was diagnosed and renal biopsy was compatible with AA amyloidosis. A search for systemic disease was performed. Further investigations, for the etiology of the systemic amyloidosis;only heterozygous V726A was detected. Since the other causes of secondary amyloidosis were ruled out, the diagnosis of familial Mediterenean fever was made and treatment with colchicine and anakinra (1mg/kg/day sc) were started. After 3 months of the anakinra treatment, laboratory findings returned to normal and excessive proteinuria disappeared.
Discussion: Amyloidoma is an unusual cause of soft tissue mass in the abdomen however a systematic approach incorporating clinical, radiological and pathological assessments will lead one to reach the diagnosis. Anakinra treatment is effective in the treatment of kidney and GIS amyloidosis.
Disclosure of interest: None declared.
References

1. Biewend M, Menke DM, Calamia KT: **The spectrum of localized amyloidosis: a case series of 20 patients and review of the literature.** *Amyloid* 2006, **13**:135-142.
2. Lavatelli F, Perlman DH, McComb ME, *et al*: **A proteomic approach to the study of systemic amyloidoses.** *Amyloid* 2006, **13(Suppl 1)**:13A.
3. Soriano A, Verecchia E, Afeltra A, Landolfi R, Manna R: **IL- 1B Biological Treatment of Familial Mediterranean Fever.** *Clin Rev Allergy Immunol* 2013.

A41

P01-037 – Genetic analysis practice prior to FMF diagnosis

Y Shinar[1*], Y Berkun[2], A Livneh[1,3], S Padeh[4]
[1]Heller Institute of Medical Research, SHEBA MEDICAL CENTER, Tel Hashomer, Israel; [2]Department of Pediatrics, Hadassah Hebrew University Medical Centre, Jerusalem, Israel; [3]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; [4]Department of Pediatrics, SHEBA MEDICAL CENTER, Tel Hashomer, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A41

Introduction: Despite accepted clinical diagnostic criteria overruling both negative and positive genetic results, Mediterranean fever gene (*MEFV*) testing for familial Mediterranean fever (FMF) is regular practice.
Objectives: This study aimed at identifying the benefits and impact of this practice.
Methods: Previously diagnosed pediatric patients (N=681), at a tertiary pediatric FMF clinic, were stratified according to the availability of genetic results at colchicine prescription, and according to their phenotype, defined as typical or atypical at colchicine prescription. Subgroups were compared with respect to their genetic features.
Results: At colchicine prescription, genetic results were not available for 229/681 patients (34%). A typical phenotype was significantly more common in this subgroup than in patients with genetic testing at prescription (212/229, 92%, vs. 260/452, 58%, OR=9.2 95% CI 5.4-15.6, p=0.0001). Of note, despite the high frequency of typical phenotype in this group, the rate of 2 pathogenic variants of *MEFV* was higher (61.5% vs. 49.3%, p=0.002), the rate of genetic negative FMF was lower (7% vs. 17.4%, p<0.0001), and the rate of p.M694V homozygosity tended to be higher (31% % vs. 25, a trend) in those with gene analysis available at prescription. When focusing on typical presentation alone (n=472), the distinction between the groups increased, as in the subgroup with typical phenotype plus genetic analysis prior to prescription the rates of 2 pathogenic variants **and** homozygosity to M694V were higher than in typical phenotype without genetic testing (60.7% vs. 50%, p=0.02, and 37% vs. 27%, p= 0.037 respectively).
Conclusion: It appears that in real life most FMF patients awaited genetic testing before colchicine prescription, with particular predilection to atypical patients. This practice results in a better match between clinical and genetic diagnosis of FMF. Of interest, an overall similar distribution of *MEFV* genotypes in typical and atypical patients suggests substantial modulation of the *MEFV* genotype–phenotype correlation in atypical patients, by yet unknown factors.
Disclosure of interest: None declared.

A42

P01-038 – QT and JT dispersion in children with FMF

K Fidanci[1], A Kilic[1], M Gulgun[1], C Acikel[2], G Basbozkurt[2], E Demirkaya[3*], F Gok[3],
FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology (FAVOR)
[1]Pediatric Cardiology, Gulhane Military Medical Faculty, Ankara, Turkey; [2]FMF Arthritis Vasculitis and Orphan Disease Research Center, Gulhane Military Medical Faculty, Ankara, Turkey; [3]Pediatric Rheumatology, Gulhane Military Medical Faculty, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A42

Introduction: Familial Mediterranean Fever (FMF) is an autoimmune, autosomal recessive inherited disorder, and characterized by recurrent episodes of peritonitis, plöritis and arthritis. Patients with inflammatory disease are at increased risk of cardiovascular complications due to rhythm disorders. QT and JT dispersions are simple and non-invasive arrhythmogenic markers and can be used to assess the homogeneity of cardiac repolarization.
Objectives: The aim of this study was to determine the risk of cardiac arrhythmias in patients with FMF by evaluating QT and JT dispersion.
Methods: A total of 48 FMF patients who are in the attack-free period and use regular colchicine therapy (26 male, 22 female, 11.10 ± 3.42 years) and 31 healthy children (17 males, 14 females, 9.61 ± 2.83 years) were included in the study. The study group and the control group were evaluated with a standard 12-lead electrocardiography (ECG). QT, JT and RR distances were measured in both groups. The corrected QT (QTc) and corrected JT (JTc) were calculated. QT dispersion (QTcd) and JTd dispersion (JTcd) were determined.
Results: There was no statistically significant difference was found between the study and control groups in terms of RR, QT, QT, QTcd, JT, JTc, JTd and JTcd measurements. QTc value is found to be higher in patients with FMF than the control group (412.15±21.45–393.58±35.18, t=2916, p=0.005), although the difference was statistically significant, the value is within normal limits (below 0.44).
Conclusion: QTc value indicates the increased ventricular sensitivity and is an important marker of cardiovascular mortality. It has an important effect on sudden cardiac death and arrhythmia. In the ligths of these results, electrocardiographic monitoring may be useful in patients with FMF.
Disclosure of interest: None declared.

A43

P01-039 – Autonomic functions in children with FMF

K Fidanci[1], M Gulgun[1], A Kilic[1], C Acikel[2], E Demirkaya[3*], S Ozen[4], F Gok[3],
FMF Arthritis Vasculitis and Orphan Disease Research in Pediatric Rheumatology (FAVOR)
[1]Pediatric Cardiology, Hacettepe University, School of Medicine, Ankara, Turkey; [2]FMF Arthritis Vasculitis and Orphan Disease Research Center, Hacettepe University, School of Medicine, Ankara, Turkey; [3]Pediatric Rheumatology, Gulhane Military Medical Faculty, Hacettepe University,

School of Medicine, Ankara, Turkey; [4]Pediatric Rheumatology, Hacettepe University, School of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A43

Introduction: Familial Mediterranean Fever (FMF) is an autoinflammatory disorder characterized by recurrent fever associated with inflammation of serous membranes. There is no study reporting the assessment of autonomic functions by using heart rate variability (HRV) in children with FMF. HRV is a practical and reliable method for evaluation of autonomic functions. HRV studies have pointed to the presence of autonomic dysfunctions in many autoinflammatory disorders, possible contributing factors to ventricular tachyarrhythmias and sudden cardiac death in these patients.
Objectives: In this study, we investigated possible alterations in cardiac autonomic functions and other probable cardiac effects in children with FMF by HRV analyses and conventional echocardiography.
Methods: In each patient, it was performed twelve lead electrocardiography (ECG) at 25 mm/s (paper speed), 24 h ambulatory electrocardiographic monitorization (AECG), and transthoracic echocardiography by a Siemens Acuson Sequoia C256 cardiac ultrasonographic scanner, with 2.5- to 3.5-MHz transducers.
Results: Seventy FMF patients and 50 healthy controls were enrolled in the study. It was noted that SDNN (standard deviation of all NN intervals) value was lower in patients with FMF as compared to the control group. Frequency-dependent HRV parameters were similar in both groups. There was no difference in patient and control groups in terms of conventional echocardiographic parameters.
Conclusion: Studies with larger cohorts and more comprehensive methods are required to assess the presence and consequences of possible autonomic dysfunction in children with FMF.
Disclosure of interest: None declared.

A44

P01-041 – Patient management and rare FMF symptoms

MM Moradian[1*], H Hayrapetyan[2], G Amaryan[3], A Yeghiazaryan[2], T Sarkisian[2]
[1]Department of Molecular Genetics and Diagnostics, Morava Inc, Los-Angeles, United States, Yerevan, Armenia; [2]CENTER OF MEDICAL GENETICS AND PRIMARY HEALTH CARE, Yerevan, Armenia; [3]Arabkir Medical Center, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A44

Introduction: Analysis of various symptoms from 20000 FMF patients indicates that several issues, including the clinical manifestation in a variety of combinations and the genotype penetrance, make FMF diagnosis and management challenging. Severe phenotypes with development of serositis, ELE, splenomegaly, and vasculitis are associated with high penetrance mutations of exon 10, mainly M694V allele.
Objectives: Several forms of arthritis were associated with FMF and the life-threatening complications such as adhesive intestinal obstruction were present in some patients as the first and only manifestation of FMF. Family studies revealed the personalized nature of FMF symptoms and treatment based on genotype and other environmental factors. A significant number of patients have to endure pain for a long time before being properly diagnosed or treated. A major cause for such complexity could be the presence of less common FMF symptoms such as fibromyalgia or multiple sclerosis.
Methods: Phenotype-genotype correlation was performed after molecular-genetic testing of FMF patients and their family members.
Results: FMF patient management may seem straightforward due to general response to colchicine therapy yet many issues including but not limited to misdiagnosis, delayed diagnosis, and renal amyloidosis could potentially complicate the patient management. We have observed a limited response to colchicine at the nephrotic stage of renal amyloidosis in FMF patients homozygous for M694V mutation. Almost all such patients have SAA1 a/a genotype suggesting that homozygous M694V patients could benefit from SAA1 genotyping for colchicine dosage adjustment and management of renal amyloidosis. FMF patients with other genotypes had a good chance to ameliorate the nephrotic syndrome and to maintain renal function. Presence of only one symptom in FMF has been a major factor for misdiagnosis and delay in treatment further emphasizing that mutations in MEFV gene could result in various forms and combinations of symptoms in different individuals. Diagnosis of member of family with FMF while his father and sister had the same genotype and no symptoms also points to a personalized development and progression of the disease. Therefore each case should be discussed in details using genotype and symptoms correlation and treated accordingly.
Conclusion: In our experience prevention of the attacks has been a useful tool in patient management. Abrupt and extreme changes in weather patterns, strenuous activity or exercise, anxiety and stress, and even diet have triggered FMF attacks. These conditions either cause additional inflammation or lower/distort the effectiveness of Pyrin in patients with affected genotypes. Although colchicine therapy remains the dominant treatment for FMF and largely prevents the development of renal amyloidosis, its effect in some cases remains controversial. Of course for genotypes with potential for amyloidosis analysis of SAA1 gene could provide preventive values. Once the diagnosis is final immediate initiation of colchicine therapy could prevent renal complications yet emphasis on identifying environmental and/or social factors that trigger FMF attacks could reduce their frequency and facilitate patient management.
Disclosure of interest: None declared.

A45

P01-042 – Joint involvement in Armenian children with FMF

G Amaryan[1*], G Khloyan[2], R Davtyan[2], T Sarkissian[3], A Tadevosyan[4]
[1]National Pediatric Centre for Familial Mediterranean Fever of "Arabkir" Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan State Medical University, Armenia; [2]National Pediatric Centre for Familial Mediterranean Fever of "Arabkir" Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan State Medical University, Armenia; [3]Centre of Medical Genetics and Primary Health Care, Yerevan, Armenia; [4]Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A45

Introduction: Familial Mediterranean Fever (FMF) as an ethnic disease is wide-spread in Armenia. In most cases FMF manifests in childhood. Joint involvement is the third major FMF manifestation. It usually presents as acute recurrent arthritis (ARA), arthralgia, more rare - chronic arthritis (Juvenile Idiopathic Arthritis, JIA), which are important to better learn more about the overlap between the FMF and JIA.
Objectives: to investigate clinical and genetic characteristics of the joint manifestations in Armenian children with FMF.
Methods: A group of 715 children with FMF was observed at the National Pediatric Centre for FMF. There were 438 boys and 277 girls, aged from 3 months to 17 years (mean age 8.64±0.17). The diagnosis of FMF was confirmed according to the generally recognized Tel-Hashomer criteria, the "Guidelines for the genetic diagnosis of hereditary recurrent fevers"(2011) and molecular-genetic detection of 12 *MEFV* mutations common for Armenians. The findings were processed with the use of standard statistical Epi-Info 2000 Program. For comparison of two nominal variables in table "two by two" Yaet's corrected for continuity chi-square test was used, significance level $p<0.05$.
Results: Joint involvement were observed in 56.4% FMF patients and manifested mainly as ARA in 30.5%, arthralgia in 21.2% and chronic arthritis in 4.7%, who also qualify for diagnosis of JIA. The risk of development of both ARA and JIA was associated with high penetrance M694V mutation, mainly M694V homozygous and M694V heterozygous genotypes. M694V heterozygous genotype was noticed significantly more frequently among FMF patients with spondylloarthropathy in compare to those without it. The probability of the development of JIA was significantly high also in heterozygotes without M694V mutation in comparison with compound-heterozygotes.
Conclusion: The frequency of joint involvement among Armenian children with FMF (56.4%),especially in combination with JIA (4.7%), were more frequent than expected. The carriers of a single M694V mutation had more frequent arthritis, in particular JIA, spondylloarthropathy. In patients with M694V mutation chronic arthritis may be the first, early and only manifestation of FMF. We suppose, that Armenian children with all types of arthritis should be investigated for FMF. The presence of M694V mutation, in both heterozygous and homozygous genotypes, could be considered as a risk factor for arthritis, causing atypical (in heterozygotes) or severe (in homozygotes) course of FMF.
Disclosure of interest: None declared.

A46

P01-043 – Comparative characteristic of FMF and FMF with HSP

H Sargsyan[1*], P Ghazaryan[2]
[1]Yerevan State Medical University, Yerevan, Armenia; [2]Haematological Centre, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A46

Introduction: According the literature data about Familial Mediterranean fever (FMF), with the combination of Henoch- Sheilen purpura (HSP), as well as the results of the work done by the former of our studies we attempt to identify and compare membranes aspects of pathogeneses of FMF and combination of FMF with HSP.
Objectives: The aim of our study is to identify speed of lipid peroxidation (LPO) and the role of degradation of membrane phospholipids (PL) of FMF and combination of FMF with HSP.
Methods: Clinical studies conducted in 61 non complicated of amyloidosis FMF children in the Republican FMF Children Center, Center "Arabkir". The age of the patients varies from 5-15. Three patients of FMF are accompanied with HSP. We are selected as a control group of 11 healthy people in practice.Biochemical studies carried out in Hematological Center of Armenia. In erythrocytes of membrane we determine the following index: phospholipase A_2 activity, intensity of LPO, as well as the level of citotoxic - Lysophatidylcholines(LysoPCH). The activity of phospholipase A_2 and LPO was determined by the spektrofotometrik methods. The fractions of separate PL in erythrocyte membranes were carried out by thin-layer chromatography methods.
Results: The results of our research showed that FMF is followed by rising the activity of phospholipase A_2 (approximately two times, P<0,001), with sharp increase of citotoxic- LysoPCH (P<0,001) and intensity of LPO (about 3 times, P <0,001).It is noteworthy, that in the second group of our patients (FMF with HSP) these indices will be raised. Therefore, the mentioned follows that the second group of patients treatment is more difficult and requires a special, single-minded approach.
Conclusion: The results in our experience are discussed to find possible ways of increasing efficiency of treatment FMF with HSP.
Disclosure of interest: None declared.

A47

P01-044 – Uncommon manifestations of familial Mediterranean

A Beglaryan[1*], K Ginosyan[2], A Ayvazyan[2]
[1]Rheumatology, Center of family planning and sexual health, Yerevan, Armenia; [2]Internal Diseases, Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A47

Introduction: Familial Mediterranean fever (FMF) is the most common autoinflammatory disease characterized by recurrent self-limited attacks of fever accompanying with peritonitis, pleuritis or arthritis. The testing of MEFV gene expanded the frame of various clinical manifestations of FMF.
Objectives: The aim of this study is to determine systemic uncommon non-classical manifestations of FMF and reveal the possible association with autoimmune diseases.
Methods: We examined 50 patients (27 male, 23 female) with FMF. The mean age of patients was 34.7±12.2. FMF was determined clinically and approved by testing of MEFV gene. To reveal systemic manifestations of FMF investigation of all organ-systems was carried out.
Results: Classical symptoms of FMF: abdominalgy, thoracalgy, arthralgy with recurrent fever and pleuritis, splenomegaly, hepatomegaly were seen in almost all patients. Monoarthritis was met in 36 (72%) and polyarthritis in 8(16%) patients. 40(80%) patients developed spondyloarthrtitis, in 11 cases (22%) unilateral sacroiliitis and in 29(58%) bilateral sacroiliitis was observed. All patients with sacroiliitis fulfilled the classification criteria of the European Spondyloarthropathy Study Group for the diagnosis of seronegative spondyloarthropathy. 10(25%) patients of 40 that having sacroiliitis developed significant limitation of lumbar motion, which was assessed by Schober's test (1-2 cm), had bilateral sacroiliits grade III-IV and fulfilled the modified New York criteria for ankylosing spondylitis. HLA B-27 was examined in 15 patients with symptoms of spondyloarthropathy. In 7 patients it was negative and in 8-positive. In19 (38%) from 50 patients coxarthrosis was revealed and 2 patients underwent total endoprothesis of hips. Skin involvement also was observed during the observation: 4(8%) of them developed erythema similar "butterfly" rash, livedo reticularis and photosensitivity with high titer of circulating immune complexes, ANA and anti-dsDNA antibodies like systemic lupus erythematosus, 1 patient had hemorrhagic rash on legs with developing of hemorrhagic vasculitis. In 1 patient trophic ulcers, miscarriage were developed with high titer of anticardiolopin autoantibodies as in classic antiphospholipid syndrome. Scleroderma-like syndrome was developed in 1 patient with Raynaud's phenomenon and skin induration of wrists and face and pneumofibrosis. Also panniculitis (1 patient), aphthae (2 patients), angiorethinopathy (2 patients), mononeyropathy and polyneyropathy (2 patients respectively), pneumonitis (4 patients), xerophthalmia (1 patient) like Sjogren's syndrome were observed. The prevalent mutation of MEFV gene was M694V- in 38patients (79.1%), from which 8(16.7%) were homozygote and 14(29%) were heterozygote (M694V/N).
Conclusion: FMF may have systemic manifestations of autoimmune diseases. It may be due to vascular involvement especially in accompaning amyloidosis cases. The peculiarity of ankylosing spondylitis-like syndrome in FMF is its independent existence from carrying HLA B-27 antigen.
Disclosure of interest: None declared.

A48

P01-045 – Epilepsy in Armenian children with FMF

N Mkrtchyan[1*], G Amaryan[2], N Aghababyan[3], K Mirzabekyan[4], T Sarkissian[5]
[1]National Pediatric Centre for Familial Mediterranean Fever of Arabkir Joint Medical Centre - Institute of Child and Adolescent Health, Armenia; [2]National Pediatric Centre for Familial Mediterranean Fever of Arabkir Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan State Medical University, Armenia; [3]Arabkir Joint Medical Centre - Insitute of Child and Adolescent Health, Yerevan, Armenia; [4]Arabkir Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan, Armenia; [5]Centre of Medical Genetics and Primary Health Care, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A48

Introduction: FMF is an ethnically restricted disease for ancestors from Mediterranean sea region and it has high prevalence in Armenia as well. Neurologic manifestations in children with FMF are relatively uncommon. Headaches occur frequently in FMF and have mainly migraine-like nature. Aseptic meningitis and convulsive disorders as well have been reported.
Objectives: To study the epilepsy in Armenian children with FMF.
Methods: We observed 2300 patients with FMF (1408 boys and 892 girls; mean age: 8.86±0.29) in the National Pediatric Centre for FMF. Diagnosis of FMF was based on Tel-Hashomer criteria and MEFV genetic analysis. The epilepsy was diagnosed based on clinical manifestations (>2 unprovoked epileptic seizures),neurological history, exam, EEG and MRI. The statistical analysis was performed using Epi-Info 2000 software.
Results: Epilepsy was diagnosed in 12 (0.5%) FMF patients (5 boys and 7 girls; aged from 7 to 18 years). The frequency of the epilepsy was not exceed the average indices for a healthy Armenian population (0.6%). The mean age of FMF manifestation was 3.5 years and the same indices for epilepsy onset made 7.5 years. Family history on FMF and epilepsy was observed in 9 and 6 patients respectively.
FMF with moderate activity was diagnosed in most (8) patients. Four patients with severe course of FMF had acute recurrent arthritis. At that the following mutations of *MEFV* were detected: 694V/M694V (4 patients); M694V/V726A (3 patients) and by each one of M694V/R764H, M694V/E148Q, V726A/M680I, M694V/0, E148Q/0 (in total - 5 patients).High penetrance M694V mutation was determined in 5 patients, mainly with severe homozygous genotype (4 patients). Colchicine therapy was effective for 7 patients. Partial epilepsy with secondary generalized and/or complex partial seizures was diagnosed in 6 FMF patients. Four had primary generalized epilepsy with frequent polymorphic convulsions. Symptomatic epilepsy with polymorphic partial fits had two FMF patients with cerebral palsy. Idiopathic generalized epilepsy with photosensitive absences was in one case. In 7 FMF children epilepsy was diagnosed after manifestation of FMF and they were responsive to antiepileptic treatment. In 5 patients convulsive disorder precede the diagnosis of FMF. In these cases, convulsions were resistant to antiepileptic drugs alone and they subsided only when colchicine was added.
Conclusion: Taking into account, that epilepsy is a genetically determinate disorder and the majority of FMF patients with epilepsy were carriers of M694V mutation and severe homozygous genotype, it is not excluded, that the combination of FMF with certain type of convulsive disorders/epilepsy

(probably based on some vascular lesions) might be possible in the presence of similar environmental and social triggers. However, we suppose that the low frequency of epilepsy (0.5%) among Armenian children with FMF, which does not exceed the frequency for general healthy population, as well as the positive response of these patients to both colchicine and antiepileptic drugs indicate the presence of two separate diseases.
Disclosure of interest: None declared.

A49

P01-046 – Membrane aspects of Hemostasis disorders at ATYPIC

P Ghazaryan*, A Zakharyan, N Mkrtchyan
Yerevan State Medical University after M. Heratsi, Hematology Centre After Prof. R. Yeolyan, MH RA, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A49

Introduction: The increasing trend of atypical form of FMF among the Armenian population is one of the actual problems in the Armenian medicine. Hence, it induced and promoted the necessity of studying molecular mechanisms for correction of disturbed metabolic processes and for elaboration of new methods at pathogenic therapy of FMF. The specific features of the clinical symptoms in atypical forms of FMF give the rise of complications at differential diagnosis.
Objectives: In this research the informativity of clinical-laboratory and biochemical indicators has been studied. Also the relations between individual phospholipids of biomembranes and leuko/erythroid cells during atypical FMF were investigated.
Methods: The intensity of 14C-glycerol and 14C-glucose incorporation was studied in vitro in the contents of individual phospholipids of erythrocytes and lymphocytes membranes at children with atypical FMF. The phospholipids were fractionated by thin layer chromatography.
Results: The substantial increment in the myeloid cell number was observed in all investigated patients. The leuko/erytroid sells relation was 4:1 instead of normal 3:1. The erythroid cell maturation index was low. The number of leukocytes was high in all patients. The basophilic erythronormoblasts predominated over polychromatic and oxyphilic ones. In all patients the expressed thrombocytosis and megakaryocytosis accompanied with active platelet formation were described. It is established that FMF is characterized by a sharp increase of ^{14}C-glucose incorporation rate in the lysophosphatidylcholines (LPC) with simultaneous decrease of rate for incorporation in the contents of phosphatidylcholines (PC) and sphyngomyelines. It is observed an increase of activity of phospholipase A_2 and the reduction of the activity of glycerolkinase and glycerol phosphate dehydrogenase. Also an increase of LPC/PC relation coefficient and decrease of PC/phosphatic acid relation were established.
Conclusion: Apparently the revealed changes are inherent atypical FMF. The membrane aspects of hemostasis disorders mechanisms at atypical FMF are discussed.
Disclosure of interest: None declared.

A50

P01-047 – PH with right-sided heart failure in FMF

AV Sargsyan[1*], MZ Narimanyan[2]
[1]Internal Medicine, Yerevan, Armenia; [2]Family Medicine, Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A50

Introduction: FMF is associated with pulmonary hypertension(PH) due to amyloidosis. However, clinically overt PH with right-sided heart failure remains a rare event limited to few patients with pulmonary amyloidosis secondary to FMF. We report two cases of FMF patients, with and without amyloidosis, who experienced PH with right-sided heart failure. To our knowledge, this is the first case report on cor pulmonale in FMF patient without amyloidosis.
Case report: First: A.R.,72 year old non-smoker male presented with 6-month history of cough, dyspnea and intensive weight loss (8 kg). He had typical FMF abdominal and chest attacks since the age of 28. He took colchicine irregularly,during attacks only. He gave a positive family history. His daughter (40,has had FMF attacks since the age of 35) and cousin brothers were also affected. No history of occupational exposure. Series of X-ray and thoracic CT scan demonstrated unilateral ground-glass opacities in a basal segment of right lung and cardiomegaly. Echocardiography showed hypertrophy of left ventricle, dilated right ventricle, tricuspid regurgitation, and PH, findings compatible with cor pulmonale. Upper GI endoscopy with gastric biopsy revealed atrophic gastritis. Laboratory investigations revealed: CRP 79.2mg/L, SAA 210g/L, creatinin 162.1-189μmol/l and proteinuria 0.19g/daily. Two months later patient died of heart failure. Postmortem examination showed emphysema, lung fibrosis and sclerosis, myocardial hypertrophy and kidney arteriolosclerosis. Daughter's genetic test revealed one mutation in exon 2 in the heterozygous state E148Q/N.
Second: S.H.,47 year old male, a smoker, presented with cough, progressive dyspnea and pedal edema. He had typical FMF abdominal and chest attacks since early childhood. He gave a positive family history. His sister and brother were also affected. He took colchicine irregularly, during attacks only. Genetic test showed compound heterozygosity (M694V M680IG/C). ElectroKG showed atrial fibrillation and low QRS voltage in the limb leads. Doppler ECG demonstrated biventricular wall thickening, dilated right ventricle, diastolic dysfunction, and pulmonary hypertension. Laboratory investigations revealed: creatinin 172.4μmol/l, proteinuria 1.4g/daily. CT demonstrated diffuse interstitial lung infiltrates and bullas (marked air cysts). Eight months later, patient died of heart failure. Postmortem examination showed extensive deposits of amyloid in the kidneys, pulmonary vasculature, alveolar septa, pleura and myocardium.
Discussion: FMF patients with chest attacks may develop PH. If present, PH is a sign of advanced disease, and the survival rate after diagnosis is low. A diagnosis of PH should be considered in patients with and without amyloidosis and unexplained dyspnea or fluid overload. Although pulmonary involvement may occur in FMF, PH with right sided heart failure is considered an infrequent diagnosis and is rarely the cause of death.
Disclosure of interest: A. Sargsyan Consultant for: clinical and lab tests, M. Narimanyan Consultant for: clinical and lab tests.
References

1. Dingli D, Utz PJ, Gertz MA: **PH in patients with amyloidosis.** *Chest* 2001, **120**:1735-1739.
2. Johnson WJ, Lie JT: **PH and FMF: a previously unrecognized association.** *Mayo Clin Proc* 1991, **66**:919-25.
3. Lidar M, Langevitz P, Livneh A, Pras M: **Thoracic and lung involvement in FMF.** *Clin Chest Med* 2002, **23(2)**:505-11.
4. Utz JP, Swensen SJ, Gertz M: **Pulmonary amyloidosis.The Mayo Clinic experience from 1980 to 1993.** *Ann Intern Med* 1996, **124**:407-13.

A51

P01-048 – Systemic onset JIA with coronary artery dilation

N Aktay Ayaz[1*], G Keskindemirci[1], E Aldemir[1], N Melikoğlu Kiplapinar[2], G Aydogan[1]
[1]Pediatric Rheumatology, Istanbul Kanuni Sultan Suleyman Education and Research Hospital, Turkey; [2]Pediatric Cardiology, Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Center, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A51

Introduction: Systemic-onset juvenile idiopathic arthritis (SoJIA) is characterized with arthritis, fever, typical salmon pink rash, generalized lymphadenopathy and hepatosplenomegaly. The incomplete and atypical presentations of Kawasaki disease (KD) put it to the first order in the differential diagnosis.
Case Report: A 2-year old boy had been treated as incomplete KD by 2 gr/kg/day of IVIG and 80 mg/kg/day of aspirin at a general pediatrics clinic. From his previous data it was learned that, fever was present for 24 days and it was peaking two to three times a day. He had had a faint rash, arthralgia and lymphadenopathy during follow-up period. But he had't got conjuctivitis or extremity changes. It was learned that his lips were becoming red when the fever had rised. At that time,he had had very high inflammatory markers. Microbiological work-up had been performed and no etiology to clarify the fever of unknown origin had been found. At his echocardiography, left coronary artery dilatation had been detected and he had been accepted as incomplete KD. But due to unresponsiveness of his complaints and high acute phase response to this treatment protocol, he had been sent to our pediatric rheumatology unit for further diagnostic evaluation. When he was first seen at our outpatient clinic, arthritis of both wrists and knees were noticed. He was internalized and his temperature charts were closely followed. He had two peaks of fever/day with salmon colored rash over his chest. On his physical examination,

cervical lymphadenopathy and hepatosplenomegaly were remarkable. There were no peeling of fingers, conjunctivitis and crackles above lips. His hemoglobin was 9.1 gr/dl, leukocyte count was 7600/µl, platelet count was 582000/µl, erythrocyte sedimentation rate was 84 mm/hr, C-reactive protein was 57 mg/l. At his echocardiography z-scores of LMCA was 3.23, LAD was 4.83, RCA was 2.83. At abdominal ultrasound presence of hepatosplenomegaly was confirmed. Bone marrow aspiration was done and exclusion of infiltrating malignant diseases and macrophage activation syndrome were made. The diagnosis was SoJIA with dilatation of coronary arteries. Oral methotrexate of 15 mg/m^2/week and pulse steroid of three doses of 30 mg/kg/day with an antiaggregating dose of aspirin were commenced. His fever had subsided and laboratory values began to decline. Both pain and limited range of motion of the affected joints had ameliorated. Even though, still with high z-scores, coronary dilatation began to regress at his follow-up echocardiographic evaluations.

Discussion: Incomplete and atypical nature of KS at early infancy put forward a diagnostic dilemma. Coronary artery dilatation (CAD) was once accepted as one of the differentiating features of KD and SoJIA. Disease duration and prolonged inflammation may be a causative factor for CAD in SoJIA. According to a previously published study and as in our case CAD might not be an uncommon feature of SoJIA [1]. Pediatricians as the first evaluating physicians should be aware of the symptoms, signs and possibility of coronary artery involvement in SoJIA.

Disclosure of interest: None declared.

Reference

1. Binstadt Bryce A, *et al*: Coronary Artery Dilation Among Patients Presenting With Systemic Onset Juvenile Idiopathic Arthritis. *Pediatrics* 2005.

A52

P01-049 – Assessment of vascular function in systemic JIA

B Sozeri[2*], K Ozdemir[1], S Mir[1]
[1]peadiatric nephrology, Ege University, Izmir, Turkey; [2]pediatric rheumatology, Ege University, Izmir, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A52

Introduction: An increased incidence of cardiovascular disease has been found in rheumatic disorders. Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children. Prolonged immunological inflammatory process leads in these patients to an early onset of atherosclerosis.

Objectives: We aimed to assess the presence of early vascular dysfunction in patients with systemic onset juvenile idiopathic arthritis (SoJIA) and investigate the role of therapy SoJIA in vascular health.

Methods: Eighteen patients (12 males, 6 females) with diagnosis of SoJIA according to the International League of Associations for Rheumatology criteria were compared to 75 age- and sex-matched controls. No participant was overweight, obese, or had a history of hypertension, dyslipidemia, diabetes mellitus, or cardiovascular disease. Arterial stiffness (As) was evaluated by measurement of carotid-femoral pulse wave velocity (PWV) and augmentation index (AIx) with Vicorder.

Results: The mean age onset of disease was 80,4±28,7 months (range 36-122months). The mean duration of disease and active disease was 79 ± 45 months (range 6–162 months) and 58 ± 49 months (range 1–161 months), respectively.

Patients with SoJIA presented a higher mean PWV and AIx than in controls [(6.16±1.45 m/s vs 5.19±0.63 m/s,P =0.01)and (14.7 ± 8.1%vs 10.4 ± 7.35%, P=0.02)]. Eight (44%) patients with JIA had active disease at study entry. The highest levels of PWV and Aix were found in active patients. Six patients had been macrophage activation syndrome at presentation. In these patients, vascular changes higher than other patients (6.30±0.42 m/s vs 5.17± 0.55 m/s,P =0.01, respectively). The corticosteroid therapy was found associated with higher PWV, (P< 0.05), while there was not different between vascular parameters and used non steroid therapy (NSAIDs, MTX, or anti-TNF agents). We also find statistically significant correlation between PWV and disease duration (p = 0.003, r = 0.45).

Conclusion: Vascular function is impaired in patients with SoJIA at a very young age. Vascular dysfunction may be partly attributed to the effects of disease-related characteristics (inflammation, disease activity, and medications).

Disclosure of interest: None declared.

A53

P01-050 – Anakinra in systemic JIA: single center experience

M Pardeo, A Insalaco*, C Bracaglia, R Nicolai, AE Tozzi, G Prencipe, F De Benedetti
Division of Rheumatology, Department of Pediatric Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy

Pediatric Rheumatology 2013, **11(Suppl 1):**A53

Introduction: Systemic juvenile idiopathic arthritis (sJIA) accounts for 10-20% of all patients with JIA. The clinical features include fever, evanescent rash, arthralgia and arthritis, myalgia, lymphadenopathy, hepatomegaly, splenomegaly and serositis. Interleukin 1 (IL-1) has been shown to be a major mediator of the inflammatory cascade that underlies sJIA (1). Treatment with anakinra, IL-1 receptor antagonist has been reported to be effective in a subset of patients with sJIA (2).

Objectives: To assess anakinra as a therapy for sJIA in a single-center series.

Methods: We reviewed twenty-one consecutive patients with sJIA treated with anakinra for at least 6 months in our institution. The diagnosis of sJIA was established according to the International League of Associations for Rheumatology (ILAR) criteria. We analyzed the effect of Anakinra on fever, rash, number of actives joints, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell count, platelet count and ferritin levels. Clinically inactive disease was defined according to Wallace criteria. Clinical and laboratory data were obtained using a standard data collection form end resulting data were analyzed using Fisher exact test.

Results: At the beginning of treatment mean age (range) was 8.57 (2.15-16.63) years; 19 of 21 patients had fever and median number of active joints was 3 (1-15). After 6 months of treatment 11 patients (52.3%) met the criteria for inactive disease. Among 21 patients 7 (33%) received anakinra in monotherapy and 14 (66.6%) received anakinra with glucocorticoids. There were no statistically significant differences between the two groups for demographic, clinical and laboratory features. Five of 7 pts (71.4%) treated with anakinra alone and 6 of 14 pts (42.9%) treated with anakinra and glucocorticoids met criteria for inactive disease at 6 months (p= 0.361). Among the 21 patients, 10 (47.6%) received anakinra in the first 6 months of disease. There were no statistically significant differences for demographic, clinical and laboratory features among patients who started anakinra in the first 6 months of disease and those that started it after 6 months from onset of disease. At 6 months after initiation of anakinra treatment 8 of 10 patients (80%) who started anakinra during the first 6 months of disease and 4 out of 11 (36.4%) who started anakinra after 6 months of disease reached clinical inactive disease (p=0.08).

Conclusion: In agreement with several observations, anakinra is effective in a significant proportion of patients with sJIA. Our observation, albeit on a small number, show that association with glucocorticoids does not significantly affect outcome at 6 months and suggest, on the other hand, that earlier treatment may be associated with a better outcome.

Disclosure of interest: None declared.

A54

PW01-001 – Pyrin-PSTPIP1 relation during cell migration

ZY Akkaya[1*], B Balci-Peynircioglu[1], A Cetinkaya[1,2], N Purali[3], E Yilmaz[1]
[1]Medical Biology, Hacettepe University, Ankara, Turkey; [2]Medical Genetics, Hacettepe University, Ankara, Turkey; [3]Biophysics, Hacettepe University, Ankara, Turkey

Pediatric Rheumatology 2013, **11(Suppl 1):**A54

Introduction: MEFV (MEditerranien FeVer) gene mutations cause Familial Mediterranean Fever (FMF). This gene encodes a protein termed as Pyrin, which appears to play an important role in the inflammatory pathways. It is far characterized that Pyrin, which is expressed in neutrophils, interacts with PSTPIP1 and actin proteins. In previous studies PSTPIP1 has been shown to interact with cell migration proteins and actin polymerization is a main force driving neutrophil migration. Therefore, we hypothesized that Pyrin can play role in cell migration through the interaction with actin and PSTPIP1.

Objectives: In this study, Pyrin-PSTPIP1 interaction was analyzed during cell migration. Our aim was to investigate whether these two proteins co-localize at the leading edge of the cell, where actin polymerization occurs.

Methods: A cell migration assay was generated using HL-60 cells. First of all, HL-60 cells were differentiated in to neutrophil like cells by using appropriate concentration of DMSO (DiMethyl SulfOxide). To test the efficiency of differentiation, neutrophil cell surface receptor FPR1 (Formyl Peptide Reseptor 1) gene expression levels were measured. Secondly, after differentiation, cells were stimulated for migration using fMLP (N-formyl-Met-Leu-Phe), a well-known chemoattractant of neutrophils. The suitable fMLP concentration was determined by actin immunofluorescence staining. Neutrophil migration was demonstrated by using Live-cell imaging analysis. Lastly, un-differentiated, differentiated and differentiated-stimulated cells were co-stained for Pyrin-Actin, PSTPIP1-Actin and Pyrin-PSTPIP1 in order to test if the proteins localize together at the leading edge of the cell. Slides were analyzed by drawing profile and correlation curves with the help of confocal microscopy.
Results: The suitable concentration of DMSO for the experiment was 1,28% DMSO. In this concentration, FPR1 gene expression showed 165,89 fold increase (p<0,0021). After differentiation, cells showed 90% actin polymerization following 2 hours incubation with 150 nM fMLP. In stimulated cells Pyrin-Polymerized actin, PSTPIP1-Polymerized actin and Pyrin-PSTPIP1 are found to be co-localized.
Conclusion: In differentiated and differentiated-stimulated cells, Pyrin was localized with actin and PSTPIP1 at the leading edge of the migrating cell. Also an interaction between PSTPIP1 and polymerized actin was shown. So far, PSTPIP1 was shown to localize rear of the cell, mostly in uropods. For the first time, PSTPIP1 was found to interact with dynamic actin and Pyrin at the site of polarization. Further studies on the effect of colchicine on this interaction during cell migration are under way. These data may contribute to understand the exact mechanism of cell migration through Pyrin-PSTPIP1 interaction.
Disclosure of interest: None declared.

A55

PW01-002 – Colchicine resistant FMF in Turkish children

FK Kara Eroglu*, Y Bilginer, F Ozaltin, R Topaloglu, S Ozen
Pediatric Rheumatology Department, Hacettepe University Faculty of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A55

Introduction: At least 5% of familial Mediterranean fever(FMF) patients do not respond to colchicine. We present our initial treatment results with colchicine resistant patients.
Case Report: Methods: FMF resistance was defined as having ≥2 attacks in a month and persistently high CRP and SAA levels during the attack free period, in spite of adequate colchine dose. All patients were homozygous or compound heterozygotes for MEFV mutations. All continued colchicine tratment at a mean dose of 0,04±0,01 mg/kg.
Results: Eleven patients with mean age of 12,7±7,7 years (median 14, ranging 1,5-23 years) were studied. These patients were on colchicine treatment for a mean of 5,5±4,2 years. In one patient initially etanercept was used however, this was switched to anakinra since there was no repsonse to anti TNF treatment. A total of 7 patiets were started anakinra, however, 2 had local reactions and 2 was unresponsive; they were switched to canakinumab treatment and they all responded with normal acute phase reactants. At this time a total of 8 patients are now being treated with canakinumab with a mean duration of 10,8 ±6,8 months and 3 patients with anakinra with a mean duration of 19,6 months. One patient who is on anakinra treatment has HIDS mutation as well. There were no side effects.
Discussion: Anti IL1 treatment is beneficial in FMF patients who are resistant to colchicine and can be used safely.
Disclosure of interest: None declared.

A56

PW01-003 – Frequency of MEFV mutations in Turkish population

F Yalcinkaya[1*], A Duzova[2], S Gonen[3], B Ozcakar[1], E Baskin[4], O Ozkaya[5], O Soylemezoglu[3]
[1]Ankara University, Turkey; [2]Hacettepe University, Turkey; [3]Gazi University, Turkey; [4]Baskent University, Ankara, Turkey; [5]Ondokuz Mayis University, Samsun, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A56

Introduction: Data on the epidemiology of familial Mediterranean fever (FMF) and the prevalence of disease causing mutations among different ethnic groups and geographical regions around the world are insufficient. The prevalence of mutations that account for FMF in Turkey has been defined in the past by determining the frequency of MEFV mutations in affected individuals or in hospital-based controls. This study is a population-based study and is, therefore, different from previous patient-based studies.
Objectives: To investigate the prevalence and distribution of MEFV mutations in Turkish population with a nationwide population-based study.
Methods: Subjects were included from 12 statistical regions according to the EuroStat NUTS level 2. The distribution of the study population was parallel with the general Turkish population according to gender, residence, and geographical regions. To date a total of 388 unrelated healthy Turkish participants (M/F:189/199; age:5.3-79.75 years) were tested for 10 mutations in the *MEFV* gene: p.A761H, p.A744S, p.V726A, p.K695R, p.M694V, p.M694I, p.M680I (G®A) in exon 10, p.F479L in exon 5, p.P369S in exon 3, and p.E148Q in exon 2, using pyrosequencing technique.
Results: Our results showed that 62 of 388 participants (16.0%) (95% CI:12.5-20.0) were carriers of MEFV mutations. Seven individuals were compound heterozygous, two homozygous and 53 were heterozygous for the mutations. Mutation frequency was 9.2% (95% CI: 7.22-11.4). The most common mutations in the Turkish general population were p.E148Q, p.M694V and p.P369S and the frequencies were 3.6% (95% CI: 2.4-5.2), 2.6% (95% CI: 1.6-4.0) and 1.0% (95% CI: 0.5-2.0), respectively.
Conclusion: Our study shows a high frequency of carriers and independently confirms that M694V is the second most common mutation in the healthy Turkish population. If all the patients who had two most common mutations present the disease is anticipated the results of our study show that the disease is more common than predicted and one can speculate that patients with mild clinical findings might exist.
Disclosure of interest: None declared.

A57

PW01-004 – The sequence analysis in E148Q homozygous patients

R Topaloglu[1*], C Yildiz[1], E Taskiran[2], E Korkmaz[3], N Besbas[1], S Ozen[1], A Duzova[1], N Akarsu[2], F Ozaltin[1]
[1]Pediatrics, Hacettepe University, Ankara, Turkey; [2]Medical Genetics, Hacettepe University, Ankara, Turkey; [3]Nephrogenetic lab, Hacettepe University, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A57

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive disease associated with a number of mutations of the MEFV gene. To date 246 variants responsible for the disease were identified, one such a variant is E148Q in exon 2. The role of E148Q variant in the development of FMF remains inconclusive. Some authors believe it causes the disease, whereas others favor the concept of a non causative role.
Objectives: The aim of this study was to perform MEFV gene sequence analysis in E148Q homozygous patients in order to detect an associated novel mutation/variant; to determine the frequency of E148Q allele in healthy society, and ultimately, to clarify the controversy about whether E148Q is a disease causing mutation or a benign polymorphism.
Methods: Patients with homozygous E148Q variation previously determined by strip assay were evaluated. FMF clinical forms were filled in for those. All coding exons including exon-intron boundaries of MEFV gene were sequenced using DNA obtained from peripheral blood lymphocytes. E148Q allele frequencies of the parents and that of control group consisting of 100 healthy individuals were determined.
Results: Eighteen E148Q homozygous patients were studied. Age at the onset of disease was 5±3.7 (median 5) years. 44% of the patients were males and 56% were females. Presenting manifestations were abdominal pain (78.5%), fever (71.4%), arthralgia (57.1%), pleuritis (28.5%) and myalgia (7.1%). With colchicine treatment, significant decrement in annual number of attacks, duration of attacks and disease activation scores was observed (p<0.05). There was no difference in terms of achievement of full or partial remission, possessing low or moderate disease activation score, living without attack or with >20 attacks/year between groups carrying associated variants (detected as R314R, P369S, R408Q, E474E, Q476Q, D510D, P588P) and those with no associated variants. In control group

consisting of 100 healthy individuals, the frequency of E148Q allele was found to be 6.5%.
Conclusion: The frequency of E148Q variant in healthy population was found to be 6.5% in contrast to a previous report as 12% in Turkey. The E148Q variant may be related with disease since most of the patients carrying this specific variant were symptomatic (78% of our patients) and responsive to colchicine treatment, and no other variants in any coding region of the gene were detected by direct sequence analysis to be responsible for the phenotype.
Disclosure of interest: None declared.

A58

PW01-005 – Effects of placebo and colchicine on FMF patients

F Özaltın[1], Y Bilginer[1], B Gülhan[1*], I Bajin[1], Ö Erdoğan[2], S Özen[1]
[1]Pediatric Nephrology&Rheumatology, Hacettepe University Faculty of Medicine, Turkey; [2]Pediatric Nephrology&Rheumatology, Sami Ulus Children's Hospital, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A58

Introduction: The diagnosis of Familial Mediterranean fever (FMF) is basically a clinical one although genetic confirmation is of great help to the clinician. A response to colchicine has been suggested as a diagnostic criterion.
Objectives: The placebo effect of a drug has never been assessed on FMF diagnosis. We aimed to assess this effect in children.
Methods: Patients who fulfilled the pediatric criteria for the diagnosis of FMF were included in this study. Demographic and clinical features (attack frequency, features of each attack, etc) were recorded. In first part of the study, patients were randomized in two treatment groups (colchicine and placebo) double blind, with a cross-over study design in 3 months duration.
Results: A total amount of 50 patients (22 girls, 28 boys) were included. The median age of the patients was 8.5 years (2.5-17.5). 78% of the patients suffered from fever attacks suggestive of FMF every 1-4 weeks. The attack interval of the remaining patients was more than one month. At the time of admission, the median values for ESR and CRP were; 24.5 mm/hr (1-100) and 2 mg/dl (0-31), respectively. Half of the patients (n=25) were randomized to colchicine and the other half (n=25) to placebo. At the unblind period the results were assessed: patients treated with colchicine had lower ESR when compared to placebo in the first phase only (p=0.004). CRP, WBC and SAA levels were not statistically different neither in the first phase nor after the cross-over period. However, the number of attacks were significantly less in the colchicine group (median 0 attack) when compared to the placebo group (median 1 attack) (p=0.011).
In the study group, 13 patients were homozygous, 11 patients were compound heterozygous and seven patients were heterozygous for MEFV mutation. The rest of the patients (38%) did not carry any MEFV mutations.
Conclusion: The number of attacks were less in the colchicine group however, the lack of difference in the laboratory parameters suggest a marked placebo effect.
Disclosure of interest: None declared.

A59

PW01-006 – The effect of colchicine on physical growth in FMF

N Cakar[1*], TC Yoldas[2], B Acar[1], N Uncu[1], S Cayci[1]
[1]nephrology and rheumatology, Turkey; [2]Ministry of Health Ankara Childrens Hospital, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A59

Introduction: Familial Mediterranean fever is the most periodic fever sendrome and characterized by reccurent fever, abdominal pain, chest pain, arthritis/ arthralgia and erizipel like rash and self limited attacks with fever and serozal inflomation also elevated acute phase reactants. As with other chronic inflammatory diseases in patients with AAA growth may be negative effects.
Objectives: The aim of the study was to evaluate the growth parameters after treatment with colchicine in patients with newly diagnosed by examining prepubertal, along with other identifying characteristics of the disease, taking into account the status of inflammation subclinic to investigate the effects on the growth of disease severity and activity.
Methods: In the study, patients' disease activity, attack frequency, colchicine dose, laboratory studies of control, height and weight which measured with a standard stadiometre, were recorded by a standard person. Growth rates detected by determining the height and weight SDS 's (Z-score) with an interval of six months. All measurements and evaluations were made on 51 prepubertal patient.
Results: Patients, 22 (43.1%) were male and 29 (56.9%) were female with age of disease onset 5 (1-10) years, age of diagnosis and onset of therapy were 6.4 (1.3 to 11) years. According to Tel Hashomer criteria, severity score of the disease was 7 (4-10). When compered of height and weight of the patients at the beginning and at the one year of colchicine treatment a significant increase was found (p<0,05). Height SDS 's increased from -0.6 to -0.2; weight SDS' s increased from -0.6 to -0.4. The participants' BMI SDS didn't showed a significant increase, both the weight and height SDS proceedings of the increase was associated with, so it is possible there was no significant increase. The patients', who had no episode, height and weight SDS increased more than who had episode. There was no effect of disease severity on growth (p> 0.05). Also height gain was not affected from elevated acute phase reactans at the attack free period, weight gain did. Body weight and body surface of the patients receiving doses of colchicine were averaged 0.03 ± 0.02 mg/kg/day was 0.98 ± 0.045 mg/m^2. Colchicine dose was the same in patients with or without any attack.
Conclusion: In this study, the positive effects of colchicine on the growth in prepubertal patients with newly diagnosed AAA was found. The prevention clinical episodes was enough for height growth but the prevention clinical episodes and subclinical inflammation was necessary for increase of weight.
Disclosure of interest: None declared.

A60

PW01-007 – Colchicine brand switching in FMF patients

J Jagger[1*], J McGonagle[2]
[1]Medicine, University of Virginia, Charlottesville, Virginia, US; [2]Pediatrics, Crotched Mountain Rehabilitation Center, Greenfield, New Hampshire, United States
Pediatric Rheumatology 2013, **11(Suppl 1):**A60

Introduction: In July of 2009 the U.S. Food and Drug Administration enacted new regulation of colchicine under the "Unapproved Drugs Program." Like other old drugs that were on the market before the existence of the FDA, colchicine had never been subjected to FDA-required safety and efficacy trials. One company elected to put colchicine through the FDA's approval protocol and when approval was granted in 2009 the FDA announced a ban on five unapproved brands of colchicine on the market and gave proprietary rights to one approved brand. The sudden brand changes that followed this regulation led to a therapeutic crisis for FMF patients in the U.S. and coincidentally revealed heretofore unrecognized patterns in patient response to different brands of colchicine.
Objectives: To document and characterize the brand specific response of individual FMF patients participating in FMF support groups on the internet and determine the potential of multiple brand exposure for improving the effectiveness of colchicine by reducing intolerance and resistance rates.
Methods: An online survey of FMF patients participating in patient support groups on Facebook and Yahoo was conducted. Patients residing in the U.S. were selected and were asked to report each brand of colchicine they had ever taken and to indicate for each brand whether their response was satisfactory; unsatisfactory, but kept taking it; or unsatisfactory and discontinued it.
Results: 40 FMF patients reported taking 11 brands of colchicine; a total of 101 individual/brand trials. Of 31 patients taking 2 or more brands, 3 brands was the average number tried. FMF patients revealed a highly idiosyncratic response to different brands of colchicine. Adverse responses and ineffectiveness were common for all brands ranging from 81% (FDA approved brand) to 30% (FDA banned brand). A critical finding was that all 9 patients discontinuing a brand for ineffectiveness ("colchicine resistance") had a satisfactory response to another brand. Despite the frequency of adverse responses (intolerance or ineffectiveness) to individual brands, all patients with exposure to 2 or more brands reported a satisfactory response to at least one brand.

Conclusion: Intolerance and ineffectiveness (i.e. "colchicine resistance") are not characteristics of FMF patients but are idiosyncratic brand responses. Most FMF patients could achieve a satisfactory therapeutic response to colchicine if a minimum of three independent brands were available to them. Wider availability of colchicine brand options are needed for all FMF patients. Before resorting to alternative higher-risk drugs, FMF patients should be shown to be intolerant to 3 independent brands of colchicine. Although this is a clinically simple therapeutic strategy, the greater challenge is the maze of regulations governing the importation of pharmaceuticals across national borders.
Disclosure of interest: None declared.

A61

PW01-008 – The inflammasome and secretory pathways in FMF

B Orak[1*], T Kallinich[1], M Lieber[1], H von Bernuth[2], H Wittkowski[3], D Foell[4]
[1]Paediatric Rheumatology, Charite University Medicine Berlin, Germany; [2]Paediatric Rheumatology and Immunology, Charite University Medicine Berlin, Berlin, Germany; [3]Paediatric Rheumatology, University Hospital of Muenster, Germany; [4]Paediatric Rheumatology, University Hospital of Muenster, Muenster, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A61

Introduction: Aberrant inflammasome priming and dysregulated secretory pathways contribute to parallel IL-18 and S100A12 hypersecretion from neutrophils in instable FMF.
Objectives: The study was performed to assess the ex vivo inflammasome activity in granulocytes from patients with unstable FMF. Furthermore, the secretion of S100A12 molecules after various kinds of cell stimulation was assessed.
Methods: 6 turkish patients with the clinical diagnosis of FMF exhibiting homocygous or combined heterozygous mutations within the MEFV gene were included. Patients still exhibited clinical symptoms and elevated inflammation markers despite sufficient colchicine therapy ("instable disease"). Healthy probands served as controls. Their health status was assessed by a standardized questionnaire. All patients and controls gave written consent.
25 – 30 ml blood was drawn and PBMC and granulocytes separated by a two density gradient centrifugation. 5 x 106 cells were stimulated with (i) mock, (ii) PMA (10nM), (iii) LPS (10ng/ml) and (iv) LPS (10ng/ml) + ATP (1mM) (later substance for the last 30 minutes). In a similar approach cells were treated with additional colchicine (5µg/ml) for the whole incubation time. Supernatant was gained and frozen at -20oC after 5 hours. ELISA for S100A12, IL-18 and caspase-1 were performed according to standard protocols.
At the time of blood drawing high sensitivity CRP was measured.
Results: Compared to controls even unstimulated granuloytes from FMF patients with instable disease secreted significantly more S100A12 (mean controls 43ng/ml vs. mean patients 327ng/ml, p <.01), IL-18 (0pg/ml vs. 274pg/ml, p <.01) and caspase-1 (10pg/ml vs. 81pg/ml, p <.01). Stimulation also induced enhanced secretion of S100A12 (PMA: 61ng/ml vs. 336ng/ml, p <.01; LPS: 74ng/ml vs. 247ng/ml, p <.01; LPS/ATP: 94ng/ml vs. 252ng/ml, p <.01), IL-18 (LPS: 3,6pg/ml vs 176pg/ml, p <.05; LPS/ATP: 7pg/ml vs. 198pg/ml, p <.05) and caspase-1 (LPS: 23pg/ml vs. 72pg/ml, p <.01; LPS/ATP: 32pg/ml vs. 69pg/ml, p <.05). Furthermore, supplementary colchicine significantly suppressed the hypersecretion of S100A12, IL-18 and caspase-1.
Conclusion: The spontaneous release of IL-18 and caspase-1 demonstrated the constant inflammasome activity in patients with instable FMF. It was not be further induced by LPS/ATP stimulation. S100A12, although not processed by the inflammasome, was also secreted in high amount from FMF neutrophils, irrespective of further stimulation. Taken together, these data indicate that FMF neutrophils show a spontaneous hypersecretion of S100A12 and IL-18 which is only partially related to aberrant inflammasome activation (shown by parallel Caspase-1 release) but may also dependent on dysregulated alternative secretion (inhibited by colchicine in vitro).
Disclosure of interest: None declared.

A62

PW01-009 – Markers of inflammation in adult FMF patients

A Giese[1*], H Wittkowski[2], A Örnek[1], M Kurucay[1], E Lainka[3,4], BF Henning[1]
[1]Central Patient Admission Unit / Emergency Department, Marienhospital Herne, Ruhr-University Bochum, Herne, Germany; [2]Department of Paediatrics, University of Münster, Münster, Germany; [3]Department of Paediatrics, University Medical Centre Essen, Germany; [4]AID-NET Autoinflammatory disorders (AID) in children: Genetics, disease mechanisms, diagnostic markers and therapeutic targets, Supported by the German Ministry of Education and Research (BMBF 01GM08104), Essen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A62

Introduction: The therapeutic goal in Familial Mediterranean Fever (FMF) is to prevent attacks of clinically overt disease as well as to stop or reverse the development of amyloidosis and subsequent organ damage. The dosage of colchicine treatment is therefore generally adjusted according to clinical information and biochemical markers of subclinical inflammation.
Objectives: To evaluate whether biochemical markers of inflammation help to differentiate between healthy subjects and FMF patients with or without clinically apparent attacks among adult Turkish migrants living in Germany.
Methods: 40 consecutive patients suffering from FMF according to the Livneh criteria and 40 healthy controls (C) were included into the study in Herne, Germany. All participants were Turkish migrants aged ≥18 years. Patients were excluded if they reported symptoms of FMF ≤7 days prior to inclusion. Frequency of FMF attacks during the 3 months preceding the study inclusion was assessed. Patients were grouped into patients without (F-) or with (F+) attacks. The following markers were determined: erythrocyte sedimentation rate (ESR, ref: <20mm/h), C-reactive protein (CRP, ref: <0.5mg/dl), serum amyloid A (SAA, ref: <0.5mg/dl), fibrinogen (FI, ref: 2.38-4.98 g/l) and S100A12 (ref: <120 ng/ml).
Results: C (n=40) / F- (n=14) / F+ (n=26) showed the following characteristics (continuous variables are depicted as mean ±standard deviation); age: 34.6±10.7 / 35.3±8.2 / 35.2±11.2 years, female gender: 52.5 / 57.1 / 61.5 %. Age at FMF onset and daily colchicine dose in F- / F+ was 8.7±5.2 / 11.9 ±7 years and 1.4±0.7 / 1.3±0,9 mg/day. Biochemical markers were as follows for C / F- / F+; ESR: 16.3±12.8 / 26.1±25.1 / 28.7±16.2 mm/h, CRP: 0.35±0.76 / 0.69±0.92 / 0.83±0.97 mg/dl, SAA: 0.92±3.6 / 2.1±3.0 / 2.8±4.8 mg/dl, FI: 2.8 ±0.5 / 2.9±0.6 / 3.22±0.6 g/l, S100A12 was available only for F- / F+ and amounted to 746±1072 / 3642±9116 ng/ml. C and F+ significantly differed concerning ESR, CRP, SAA and FI but not concerning age and gender. No significant difference could be detected between F- and F+ concerning any of the parameters studied.
Conclusion: Among Turkish migrants living in Germany FMF patients with ≥1 attack in 3 months (F+) show significantly higher ESR, CRP, SAA and FI compared to healthy controls (C). ESR, CRP, SAA and FI did not permit to differentiate between C and FMF patients without attacks (F-) nor between F- and F+. Between F- and F+ no significant difference in S100A12 levels could be detected.
Disclosure of interest: None declared.

A63

PW01-010 – The effect of pregnancy on disease course in FMF

T Eviatar, N Zaks, OL Kukuy, A Livneh[*], M Lidar
Sheba Medical Center, Ramat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A63

Introduction: FMF is the most common of the hereditary periodic fever syndromes. It usually begins in the first two decades of life, and as such, has a significant impact on women in their reproductive years. To date, only one retrospective study, investigating the course of FMF in pregnancy, has been published.
Objectives: To examine the course of disease in FMF patients, while pregnant, and to evaluate the association of pregnancy with attack frequency and severity.
Methods: All pregnant FMF patients treated at the FMF clinic of the Sheba Medical Center from May 2010 onwards, consenting to participate in this observational study, were studied prospectively for the occurrence of attacks. Attacks were recorded by patients in diaries provided on enrollment and relied by phone to a study coordinator on a monthly telephone call or on a pre-specified physician visit, whichever occurred first. In addition to noting attack occurrence, patients were instructed to record attack severity (on a 1-10 scale), attack location, duration, medications taken during the attack, colchicine dose prior to and during the attack, as well as ancillary signs and symptoms related to their FMF such as the occurrence of exertional leg pain.

Results: We present results of 28 pregnancies in 24 patients. Average attack rate remained unchanged throughout the pregnancy and the 9 month follow-up period post delivery (Average attack rates per 3 months, in the year before pregnancy vs. 1st, 2nd and 3rd trimester of pregnancy as well as the 9 months post delivery are 2.42 95%CI: 1.3-6.1, 2.4 95%CI:0.8-4, 2.2 95% CI:0.9-2.5, 2.2 95%CI:1.1-4.3 and 1.94 95%CI 0.2-3.6, respectively).That being, an amelioration in attack rate was experienced in 25% of the pregnancies including 10.7% of pregnancies in which attack rate decreased by more than 80% compared to the pre-pregnancy value. Conversely, in 28.5% of pregnancies patients experienced more frequent attacks. No significant changes in attack severity or location, nor in colchicine dose were noted. Early delivery was indicated in about 10% of cases including a single patient with pre-existing proteinuria, and increasing urinary protein levels during the 3rd trimester. Pregnancy outcome was favorable in all cases.
Conclusion: Pregnancy in FMF is safe for patients and their offspring. Odds for amelioration, worsening or endurance of prior course are similarly shared.
Disclosure of interest: None declared.

A64

PW01-011 – Exertional leg pain and spondyloarthropathy on FMF

Y Rosman, I Eshed, A Livneh*, M Lidar
Sheba Medical Center, Ramat Gan, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A64

Introduction: Exertional leg pain (ELP) is a characteristic musculoskeletal manifestation of FMF which forms one of the minor criteria for its diagnosis. Despite being highly prevalent, the pathogenesis of this unique phenomenon has yet to be elucidated. In a previous pilot study we have described MRI changes compatible with enthesopathy in the feet and ankles of FMF patients with ELP.
Objectives: The primary objective of the study was to define the frequency and characteristics of exertional leg pain in a large cohort of FMF patients. Secondary objectives were to evaluate for additional signs and symptoms of spondyloarthropathy in this patient population and to assess for the overall frequency of spondyloarthropathy in FMF.
Methods: All consecutive, consenting patients, aged 18-55, arriving at the FMF outpatient clinic and fulfilling the Tel Hashomer criteria for FMF, were included in the study. Patient allocation into study or control groups was based on the presence or absence of ELP, respectively. Following a detailed clinical, laboratory and genetic workup, randomly selected patients (in a ratio of 1:3 in the study and 1:8 among the control groups) underwent an ankle MRI and plain films of the sacroiliac joints.
Results: The prevalence of ELP among the 170 FMF patients included in the study was 58.5%, with equal gender distribution. Patients with ELP suffered from more frequent attacks that manifested at an earlier age, and which, in addition to the hallmark abdominal attacks, was characterized by an excess of pleural, joint and febrile bouts.
M694V homozygosity was more prevalent among the study group whereas HLAB27 carriage was a rarity in both groups. The presence of sacroiliitis on plain radiographs was evident in 40% of the study group patients compared to 14% of the controls, respectively, p=0.01. Furthermore, sacroiliitis grade 3-4 was noted in excess among the study group (16.6% vs. 2.5%, respectively). ELP and male gender were independently associated with sacroiliitis on multivariate logistic regression analysis (OR 11.8, 95% CI 1.65-85.44, p=0.014 and OR 5.49, 95% CI 1.14-26.28, p=0.03, respectively). Signs compatible with enthesopathy on MRI of the ankle were observed in 73.5% of the study and 33.3% of the control group, p=0.04. Enthesopathy, characterized by 2 or more MR characteristic pathological findings was recorded in 55% of the study group and none of the controls. Multivariate logistic regression showed that ELP (OR 13.5, 95% CI 1.97-92.44, p=0.008) and male gender (OR 5.5 95%CI 1.21-24.98, p=0.027) were independently associated with signs of enthesopathy on MRI.
Conclusion: ELP was found to be one of the most common manifestations of FMF, second in frequency to peritonitis and on par with joint attacks. On top of being a marker for a more severe disease phenotype, ELP was found to be associated with local signs of enthesopathy as well as a higher frequency of sacroiliitis. As such, ELP may be regarded as a manifestation of spondyloarthropathy in patients with FMF.
Disclosure of interest: None declared.

A65

PW01-012 – Canakinumab in patients with FMF

S Ugurlu, E Seyahi, G Hatemi, A Hacioglu, H Ozdogan*
Department of Internal Medicine, Division of Rheumatology, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A65

Introduction: In a recent pilot study, it was reported that Canakinumab reduced the frequency of attacks in 9 patients with Familial Mediterranean Fever (FMF) resistant to colchicine with no apparent side effects [1].
Objectives: Here, we present our experience with Canakinumab in FMF patients with insufficient response to colchicine.
Methods: The charts of the patients with FMF who were on Canakinumab were evaluated retrospectively and the patients who had received 3 or more injections were asked to come to the clinic to assess the response and safety.
Results: There were 19 patients with FMF (13 F/6 M) who were receiving canakinumab for various indications. Here we report 10 (6 F/4 M) who had at least 3 injections. Three patients had concomitant diseases such as psoriasis, ankylosing spondylitis and polyarteritis nodosa. The indications for canakinumab (150mg) were colchicine resistancy in 7 patients (>1 attack/month), amyloidosis in 2 and injection site reaction due to anakinra in one. The mean age of the patients was 31.8 ± 16.47 years, while the disease duration was 22.0 ± 9.98 years. The mean colchicine dose was 2.28 ± 0.36 mg/day. The median injection number with canakinumab was 4 (range 3-7). Although injections were planned to be monthly, patients received the drug with irregular intervals due to shortage of the drug. The duration of canakinumab use was 4 ± 1.2 months. Eight of the patients had no attacks after canakinumab, while in two patients attack frequency was reduced more than 50%. In two patients with amyloidosis, proteinuria was stable in one and increased from 1.7g/d to 4.7g/d in the other. Six of the patients who were complaining of severe myalgia, improved significantly after treatment. According to patient global assessment nine patients reported significant improvement while only one, reported no change.
Canakinumab was tolerated well in general. None of the patients had injection site reactions. Although, the patient with psoriasis reported a flare in psoriatic plaques, the treatment was not interrupted and psoriasis was controlled eventually by local applications.
Conclusion: Canakinumab is effective in decreasing the frequency of attacks in colchicine-resistant FMF patients. In spite of the small number of patients, and short duration of follow-up, the side effect profile seems favorable. There is need for larger trials to further evaluate its efficacy on amyloidosis.
Disclosure of interest: None declared.
Reference

1. Gul A, Ozdogan H, Erer B, Ugurlu S, Davis N, Sevgi S, Kasapcopur O: **Efficacy and Safety of Canakinumab in Adults with Colchicine Resistant Familial Mediterranean Fever.** *Arthritis Rheum* 2013, **64(10)**: S322.

A66

PW01-013 – Localization of alternative pyrin isoforms

E Tahir Turanli[1,2*], S Erdemir[2], G Celikyapi Erdem[2]
[1]Department of Molecular Biology and Genetics , Istanbul Technical University, Turkey; [2]Molecular Biology-Biotechnology and Genetics Research Center, Istanbul Technical University, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A66

Introduction: The importance of MEFV gene protein, Pyrin/Marenostrin (P/M) in the inflammatory pathway is well established. P/M is expressed in neutrophils, eosinophils, monocytes, dendritic cells and synovial fibroblasts. There are many MEFV transcripts which are generated by alternative splicing events including deleted exons 2,3,4,5,7 and 8 in several combinations or individually. Some of these transcripts (2a, 2d, 8ext, 2d/8ext and 2d/9ext) were shown to be expressed as protein isoforms in leukocytes. A previous study carried out by our group has shown that exon 2 deleted form (d2) in leukocyte samples of FMF patients is expressed more than 400 fold compared to healthy control samples (p=0.026).

Objectives: Based on the hypothesis that different localizations and functions for full length and MEFV alternatively spliced transcripts, this study aimed to determine the localization differences between full-length P/M and P/M-d2 protein isoforms in neutrophil-like cells *in vitro*.
Methods: Two GFP-tagged plasmids which are are pCMV6-AC-GFP + MEFV-fl (MEFV-fl-GFP) and pCMV6-AC-GFP + MEFV-d2 (MEFV-d2-GFP) were transfected to HL-60 (Human promyelocytic leukemia cells) cell lines and examined via confocal microscopy. Subsequently, six-day incubation with 1.75% DMSO was performed to differentiate HL-60 cells to neutrophil-like cells. These cells were also transfected with same plasmids and proteins were observed through confocal microscopy technique.
Results: Transfection studies showed that MEFV-fl-GFP was cytoplasmic and MEFV-d2-GFP was nuclear in HL-60 cell line. On the other hand, both MEFV-fl-GFP and MEFV-d2-GFP were localized in cytoplasm of neutrophil-like cells.
Conclusion: In previous studies, cellular localization of P/M-fl and P/M-d2 was investigated in several cell lines through using transfection methods or P/M antibody. Transfection studies showed that full-length P/M was generally cytoplasmic and 2Δ isoform was the only isoform which can enter nucleus but may also localize in cytoplasm. However localization studies using P/M antibodies, which cannot currently distinguish between different isoforms, showed that although native P/M consists of predominantly full-length type, protein was also observed in the nucleus of neutrophils. Our localization results of P/M-fl and P/M-d2 in HL-60 cells were compatible with literature, they were observed in the cytoplasm and nucleus, respectively. On the other hand, both P/M-d2 and P/M-fl isoforms were found to be localized only in cytoplasm not in nucleus in the neutrophil-like cells. These findings had led us to suggest that post-transcriptional modifications for P/M-d2 that may occur during cell differentiation or possibly through inflammation such that its natural localization in the nucleus may point to its role in the inflammation maybe like a transcription factor.
Disclosure of interest: None declared.

A67

PW01-014 – MEFV methylation analysis in FMF and JRA diseases

E Tahir Turanli[1,2], AK Kirectepe[2*], Ö Kasapçopur[3]
[1]Department of Molecular Biology and Genetics, Istanbul Technical University, Turkey; [2]Molecular Biology-Biotechnology and Genetics Research Center, Istanbul Technical University, Turkey; [3]Department of Pediatric Rheumatology, Cerrahpasa School of Medicine, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A67

Introduction: MEFV is the first identified autoinflammatory gene related to Familial Mediterranean Fever (FMF) disease. We previously the tested the hypothesize of epigenetic involvement in FMF, mainly based on the occurrence of FMF in patients without mutations and decreased MEFV transcripts in leukocyte samples independent from mutations. Our study showed that higher methylation level of MEFV second exon CpG island in FMF patients compared to healthy controls (p=0.049) and negative correlation between methylation and expression levels in leucocytes (cor=-0.29, p=0.041 in both groups, cor=-0.36, p=0.035 in FMF samples).
Objectives: As there are studies suggesting that MEFV might be related not only to FMF, additionally to other inflammatory disorders, we wanted to know our proposed epigenetic involvement hypothesis specificity to FMF. It has also been known that methylation of intronic and exonic sites has a role on regulation of expression by influencing transcription elongation. In this study we aimed to compare CpG island methylation level of MEFV gene in FMF and Juvenile Rheumatoid Arthritis (JRA) patients.
Methods: DNA was isolated from venous blood of age-gender matched FMF (N=20) and JRA (N=17) patients in attack-free period, who are diagnosed and followed up at Istanbul University, Cerrahpasa Medical Faculty, Department of Pediatric Rheumatology. The parents of children were informed and consent forms were fulfilled. Methylation levels were calculated according to the protocol of OneStep qMethyl Kit (Zymo), which is a real-time PCR procedure based on methylation specific restriction enzyme digestion. The methylation levels were compared between groups using *student-T* test analyses.
Results: First intron and part of the second exon methylation level of MEFV gene were analyzed in both FMF and JRA groups and there was no significant difference between two groups.
Conclusion: In this preliminary study we have observed similar MEFV intron 1 and part of exon 2 methylation levels between FMF and JRA patients. In combination with previous studies pointing MEFV involvement in other inflammatory disorders such as JRA and BS, our findings may further support the importance of MEFV in inflammatory pathway and possibly not only through genetic mechanisms but also by means of epigenetic regulations.
Disclosure of interest: None declared.

A68

PW01-015 – Canakinumab in adults with colchicin resistant FMF

A Gül[1*], H Özdoğan[2], Ö Kasapçopur[2], B Erer[1], S Uğurlu[2], N Davis[3], S Sevgi[4]
[1]Istanbul Faculty of Medicine, Turkey; [2]Cerrahpaşa Faculty of Medicine, Istanbul University, İstanbul, Turkey; [3]Novartis, East Hannover, NJ, United States; [4]Novartis, İstanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A68

Introduction: Familial Mediterranean fever (FMF) is associated with variations in the MEFV gene resulting in proteolytic activation of IL-1β through the inflammasome complex. There is no established treatment available for those resistant or intolerant to standard of care colchicine treatment. Canakinumab, a fully human selective anti-IL-1β monoclonal antibody with a half-life of ~4-weeks binds to human IL-1β and neutralizes its proinflammatory effects.
Objectives: To evaluate the efficacy and safety of canakinumab in adolescents and adults with FMF who are resistant or intolerant to colchicine.
Methods: FMF patients with ≥1 attack/month in the preceding 3-months despite the highest tolerated colchicine dose were eligible to enter a 30-day run-in period. Those with an attack in the run-in period advanced to a 3-month treatment period to receive canakinumab 150mg sc every 4-weeks starting at the next attack in the following month. Patients then followed-up for up to 2 months or until the next attack. Attacks were confirmed by presence of fever, serositis, and elevated CRP. Primary efficacy outcome was the proportion of patients with ≥50% reduction in time-adjusted attack frequency in the treatment vs pre-treatment periods. Secondary objectives included the percent of patients with no attacks in the treatment period, time to next attack after the last canakinumab dose, and changes in the quality of life by SF-36. Safety was assessed by AEs and laboratory values at each visit.
Results: Thirteen patients enrolled in the *run-in* and 9 (median age 22 yrs, range 12-34 yrs) entered *treatment* periods. Only 1 patient had an attack (peritonitis) during the treatment period and all had a ≥50% reduction in their time-adjusted pre-treatment attack rate. Median baseline elevated CRP (58mg/L) and serum AA (162mg/L) levels normalized (CRP, 2.5mg/L; SAA, 5.8mg/L) by Day 8 and remained low throughout the study. The Physical and Mental Component scores of the SF-36 improved from a median baseline of 32 and 38 to 81 and 78 at Day 8 respectively, and continued to improve throughout the treatment period. Five patients had an attack in the follow-up period, which occurred a median 71 days (31-78 days) from the last canakinumab dose. Compared to baseline, the physician and patient global assessment of FMF control improved with treatment with overall the response to treatment reported as Very Good by both physicians (9/9) and patients (7/9) at study end. Eight patients reported at least one adverse event (AE) with headache (n=4) and upper respiratory tract infection (n=2) being the only AEs reported in more than 1 patient. No one discontinued early from the trial.
Conclusion: In this pilot study, canakinumab was found to be effective at controlling the attack recurrence in FMF patients resistant or intolerant to colchicine. AEs were similar to previous canakinumab trials and were managable. Further studies are warranted to explore the role of canakinumab in the treatment of FMF.
Disclosure of interest: A. Gül Grant / Research Support from: Novartis, Xoma, Servier, Consultant for: Novartis, Xoma, H. Özdoğan Grant / Research Support from: Novartis, Consultant for: Novartis, Ö. Kasapçopur Grant / Research Support from: Novartis, Consultant for: Novartis, B. Erer: None Declared, S. Uğurlu: None Declared, N. Davis Employee of: Novartis, S. Sevgi Employee of: Novartis.

A69

PW01-016 – Are different disease subtypes present in FMF

S Akar[1], T Kasifoglu[2], D Solmaz[1], S Bilge[2], I Sari[1*], M Tunca[1]
[1]Rheumatology, DEUTF, Izmir, Turkey; [2]Rheumatology, Eskisehir Osmangazi University, Eskisehir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A69

Introduction: Familial Mediterranean fever (FMF) is an auto-inflammatory disorder characterized by self limited attacks of fever and serositis. The disease expression may be different in different ethnic groups and patients with certain MEFV mutations may be prone to have more severe disease and a greater probability of developing amyloidosis (1). Recently we showed that amyloidosis is the only predictor of mortality in Turkish FMF patients (2), however clinical subtypes with different clinical and genetic characteristics have been never identified previously.
Objectives: The aim of this study was to evaluate whether there are clinical subgroups, which may have different prognosis, among FMF patients.
Methods: The cumulative clinical features of a large group of FMF patients (1168 patients, 575 female [49.2%] and mean age was 35.3 ± 12.4 years) were studied. To analyse our data and identify groups of FMF patients with similar clinical characteristics, a two-step cluster analysis using log-likelihood distance measures was performed. For clustering the FMF patients, we evaluated the following variables: gender, current age, age at symptom onset, age at diagnosis, the presence of major clinical features (fever, peritonitis, pleuritis, arthritis, erysipelas like erythema [ELE], febrile myalgia, amyloidosis), variables related with therapy (the dosage of colchicine, compliance with therapy, and the presence of attacks despite colchicine), the family history for FMF and for renal failure and the presence of M694V allele.
Results: Three distinct groups of FMF patients were identified. Cluster 1 was characterized by high prevalence of arthritis, pleuritis, ELE, and febrile myalgia. The dosage of colchicine and the frequency of amyloidosis were lower in cluster 1. Patients in cluster 2 had earlier age at symptom onset and diagnosis. Other characteristics of cluster 2 were high frequency of arthritis, amyloidosis, M694V allele and family history for FMF. This group of patients was using highest dose of colchicine. The cluster 3 was characterized by the lowest frequency of M694V allele, ELE, arthritis, protracted febrile myalgia. The colchicine resistance was also lower in cluster 3. The mean age and age at diagnosis was the highest in cluster 3.
Conclusion: Patients with FMF could be clustered into distinct patterns of clinical and genetic manifestations and these patterns may have different prognostic significance.
Disclosure of interest: None declared.

A70

PW01-017 – Urine MMP-3 level as a biomarker for in FMF attack

B Sozeri[1*], N Dincel[2], E Yilmaz[2], S Mir[2]
[1]pediatric rheumatology, Ege University, Izmir, Turkey; [2]pediatric nephrology, Ege University, Izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A70

Introduction: Matrix metalloproteinase-3 (MMP-3) has been implicated in experimental and clinical models of human inflammatory conditions. Increased levels of MMPs have been shown in serum and other body fluids such as synovial fluid in inflammatory conditions including ankylosing spondylitis, rheumatoid arthritis and juvenile idiopathic arthritis (JIA). Familial Mediterranean fever (FMF) is an autosomal recessive, inherited, autoinflammatory disease characterized by recurrent, self-limited bouts of fever and localized inflammation, usually involving the peritoneum, pleura, joints or skin.
Objectives: To investigate whether level of urine matrix metalloproteinase-3 (MMP-3) can serve as a biomarker for monitoring and predicting attack in patients with FMF in daily clinical practice.
Methods: We studied 50 (28 females, 22 males) patients who diagnosed with FMF according to Tel Hashomer criteria and 32 healthy (21 females, 17 males) controls. We determine all FMF subjects both in attack period (FMF-AP) and attack free period (FMF-AFP) groups. Serum and urine samples were obtained within the first 6–24 h of the AP, and 10 days later after the attack (AFP). The serum samples were measured on the same day while urine samples were collected on ice and divided into aliquots and frozen immediately and stored at -80°C until ready for assay.
Results: The mean age at onset of symptoms was 57, 26±33.5 months. The most common symptom seen during the attacks was: fever (n=40, 80%) abdominal pain (n=36, 72%), arthritis (n=20, 40%) and others (myalgia, erysipelas like lesion, vasculitis, etc.) (n=6, 12%). In the genotype distribution of patients, homozygous M694V mutation was seen mostly (n=14, 28%). During AP, urine MMP-3 levels of patients was higher as well as during AFP and controls (2,32±0,51 ng/mL, 0.89 ± 2,29 ng/mL and 1,24 ± 0,17 ng/mL, respectively, p=0.00). In attack period, urinary MMP levels were detected higher in patients with arthritis than others (p <0.05). In addition urinary MMP-3 levels were significantly higher in male compared to female patients (2, 29 ± 0,45 versus 2,24±0,57, respectively, p=0,00). The patients with M694V allele (n=29) had statistically significant high levels of urine MMP-3 levels than other patients (2.37±0,56 versus 1,99±,31, p=0,04, respectively) . Also, acute phase reactants (WBC, SAA, fibrinogen CRP, ESR) were higher in patients with M694V allele but no there were no statistically significant (p=0.89, 0.75, 0.86, 0.85, 0.7, respectively).
Conclusion: In this study we have focused on the presence and patterns of appearance of MMPs in the urine of subjects with FMF, and in healthy age-matched subjects. We showed that inflammation-specific MMP patterns may provide clinicians with valuable information in patients with FMF.
Disclosure of interest: None declared.

A71

PW01-018 – Circulating endothelial biomarkers in FMF

I Sari[1*], BO Pamuk[2], S Selcuk[2], G Gokce[2], DL Kozaci[3]
[1]Rheumatology, Dokuz Eylul University School of Medicine, Konak, Turkey; [2]Internal Medicine, Bozyaka Training and Research Hospital, Izmir, Turkey; [3]Biochemistry, Adnan Menderes University School of Medicine, Aydin, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A71

Introduction: Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease that affects the populations with certain ethnic backgrounds. It is characterized by self-limiting febrile attacks of polyserositis. In recent years, some studies reported that FMF patients had increased vascular wall alterations and damage which may be another clinical phenotype of the disease.
Objectives: In the present study, we extensively evaluated biomarkers related with endothelial damage in regularly treated and attack-free FMF patients.
Methods: Forty FMF patients and eighteen healthy controls with no known cardiovascular risk factors were included. All patients were receiving regular colchicine treatment and examinations were performed during attack-free periods. Serum samples were used for the determination of high sensitive C-reactive protein (hs-CRP), tissue factor (TF), tissue plasminogen activator (t-PA) and osteoprotegerin (OPG). Plasma samples were used for the determination of asymmetric dimethylarginine (ADMA) and thrombomodulin (TM).
Results: There were 40 FMF patients (21 M and 19 F, 31 [15-58] years) and18 healthy subjects (11 M and 7 F, 35.5 [19-46] years). The median disease duration was 15 (0.6-45) years. Age, sex distribution, waist circumference, body mass index, smoking status and serum lipids were similar between the patients and controls (P > 0.05). The concentrations of high sensitive C-reactive protein (hs-CRP) was significantly higher in FMF patients compared to controls (hs-CRP: 0.78 [0.03-20.2] vs. 0.15 [0.02-4.71], μg/ml, P = 0.03). Asymmetric dimethylarginine (ADMA), osteoprotegerin (OPG) and thrombomodulin (TM) concentrations were significantly lower in the patients' group compared to those of controls (ADMA: 2.56 [0.84-4.07] vs. 3.26 [0.88-3.63], umol/l, P = 0.04; OPG: 361.5 [50.5-1232] vs. 548.9 [193-1181], pg/ml, P = 0.01; TM: 2.69 [0.92-7.26] vs. 3.59 [2.8-8.3], ng/ml, P = 0.001 respectively). However, von Willebrand factor (vWF), tissue factor (TF) and tissue plasminogen activator (t-PA) levels were similar between the groups (P > 0.05).
Conclusion: In this study we showed that markers related with endothelial injury including ADMA, OPG and TM were significantly down-regulated in FMF patients who were on regular colchicine treatment during attack-free disease state.
Disclosure of interest: None declared.

A72

PW01-019 – MEFV gene mutations in 53 periodic fever patients

N Toplak[1*], M Debeljak[2], T Avcin[3]
[1]University Children's Hospital Ljubljana, Department of Allergology, Rheumatology and Clinical Immunology, University Medical Centre Ljubljana, Slovenia; [2]Genetic Laboratory, University Children's Hospital Ljubljana, University Medical Centre Ljubljana, Slovenia; [3]Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital Ljubljana, University Medical Centre Ljubljana, Ljubljana, Slovenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A72

Introduction: FMF is a very rare disease in Slovenia and so far only few cases were diagnosed. It is known that environment influences the clinical picture of FMF.
Objectives: We were interested if patients with PFAPA or PFAPA like clinical picture and patients with other periodic fevers followed in single tertiary centre at University Children's hospital in Ljubljana, Slovenia, have mutations in *MEFV* gene.
Methods: We collected clinical and laboratory data from periodic fever patients followed at our center from the beginning of 2006 to the end of 2012. Results of genetic testing for *MEFV* gene mutations were also collected. Genetic testing was performed in Genetic laboratory of University Children's Hospital Ljubljana. All 10 exons and exon/intron regions of *MEFV* gene were directly sequenced with ABI Prism 310 Genetic analyzer.
Results: From 2006 86 patients with periodic fevers were followed at our center; 4 adults and 82 children. The majority of them (80%) were diagnosed with PFAPA syndrome according to Marshall criteria. Genetic testing for *MEFV* mutations was performed in 53 patients (60%). In 20 (38%) patients no mutations were found (N/N). Twenty one patient was found to have R202Q/N genotype (two had clinical presentation of FMF) and another 6 were found to be homozygous for R202Q mutation (all with PFAPA phenotype). E148Q/R202Q was found in one patient with PFAPA phenotype. P369S/R408Q and R202Q were found in three patients. Among them one adult had undefined periodic fever and two children were having PFAPA phenotype with abdominal pains during attacks. All together 31 patients (58%) were found to have at least one R202Q mutation present in exon 2 of *MEFV* gene. One M694V/N and one K695R/N genotype was found in two patients with FMF phenotype.
One patient with R202Q/N genotype and negative genetic testing of exon 3 in *NLRP* gene was having clinical presentation of CAPS like syndrome and in one adult with TRAPS clinical picture R92Q mutation in *TNFRSF1A* gene was found and no mutations in *MEFV* gene.
To test if R202Q mutation is more common in periodic fever patients than in apparently healthy population we also tested 50 apparently healthy persons for R202Q. Twenty nine (64%) were positive for at least one R202Q mutation.
Conclusion: In our cohort of periodic fever patients, 33 patients (62%) were found to have mutations in MEFV gene. Majority of them were positive for R202Q mutation, mostly with PFAPA phenotype. The percentage of positivity for R202Q mutation was not higher than in apparently healthy population. We haven't found a single patient with typical clinical picture of FMF and 2 mutations. Two patients with typical clinical picture were heterozygous for *MEFV* gene mutations and two for R202Q mutation which is, according to our results, a polymorphism.
Disclosure of interest: None declared.

A73

PW01-020 – MEFV mutations carrier rate in Central Europe

M Debeljak[1*], N Toplak[2], N Abazi[3], M Kolnik[4], B Szabados[5], V Mulaosmanovic[6], J Radović[7], J Vojnović[7], T Constantin[5], D Kuzmanovska[3], T Avčin[2]
[1]Center for Medical Genetics, University Children's Hospital, University Medical Center, Ljubljana, Slovenia; [2]Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Center, Ljubljana, Slovenia; [3]University Children Hospital, Medical Faculty, Ss. Cyril and Methodius University, Skopje, Macedonia, the Former Yugoslav Republic of; [4]Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Center, Ljubljana, Slovenia; [5]Unit of Paediatric Rheumatology, 2nd Department of Pediatrics, Semmelweis University Budapest, Budapest, Hungary; [6]Children's Hospital University, Clinical Center , Sarajevo, Bosnia and Herzegovina; [7]Department of Pediatric Rheumatology, Faculty of Medicine, University Niš, Niš, Serbia
Pediatric Rheumatology 2013, **11(Suppl 1):**A73

Introduction: Familial Mediterranean fever (FMF) is an autosomal-recessive disorder characterized by recurrent attacks of fever and serositis common in eastern Mediterranean population. Over 160 mutations have been identified in MEFV gene responsible for FMF. The most common mutations in MEFV gene are E148Q, M694I, M694V, V726A and M680I. The distribution pattern of MEFV mutation along the Mediterranean Sea is not uniform; eastern populations have the highest number of carriers (20-39%), whereas western Mediterranean populations are practically unaffected.
Objectives: The aim of this study is to determine the carrier rate in healthy Macedonian, Serbian, Slovene, Bosnian and Hungarian population.
Methods: We screened 100 healthy subjects from all 5 populations. Exon 10 was PCR amplified and screening was performed with dHPLC. All amplicons with detected nucleotide changes were subsequently sequenced with ABI prism 310 genetic analyzer. Amplicons of exon 2 were directly sequenced.
Results: Heterozygous mutations were found in 4% of apparently healthy Hungarians, 7% of Slovenians, 8% of Bosnians, 11% of Serbians and in 16% of apparently healthy Macedonians. Mutations found in Hungarian population were as follows: V726A (1), K695R (3). Mutations found in Slovenian population were: V726A (1), K695R (5) and E148Q (1). Mutations found in Bosnian population were: V726A (1), K695R (6) and F756C (1). Mutations found in Serbian population were: E148Q (6), K695R (5). Mutations found in Macedonian population were as follows: E148Q (8), K695R (7) and M694V (1).
Conclusion: We found higher than expected carrier rate in all populations, from 4% to 16%. It is interesting to note that more than half (60%) of detected carriers in all analyzed populations has K695R mutation.
Disclosure of interest: None declared.

A74

PW01-021 – The phenotype of FMF due to deletion M694

HJ Lachmann[1*], DM Rowczenio[2], JA Gilbertson[2], JD Gillmore[2], AD Wechalekar[2], T Lane[2], PN Hawkins[2]
[1]University College London Medical School, London, UK; [2]National Amyloidosis Centre, University College London Medical School, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A74

Introduction: Autosomal dominant FMF due to deletion of residue 694 was first described in 3 British families in 2000. It has subsequently been reported in other patients of Northern European ancestry but the phenotype has not been fully characterised.
Objectives: To describe the presenting symptoms, complications and treatment responses of familial Mediterranean fever due to deletion M694.
Methods: We sought patients with a genetic finding of deletion M694 from our database and reviewed their case records.
Results: A total of 19 patients (11 M:8 F) had been found to carry the Del M694 variant. Clinical details were available on 16 patients who had been assessed at our centre. 13 were of white British ancestry, the other 3 were of Irish ancestry. 2 patients gave no relevant family history, 1 was adopted and unaware of any family details, 10 patients (from 5 kindreds) gave a history of similar symptoms in at least 1 relative and the final patient reported that his mother died of renal failure of unknown cause raising the possibility of AA amyloidosis although without suggestive symptoms. 13 patients had symptoms: median age at onset 18 years (range 6-48), median age at diagnosis 48.1 years, median attack duration 2.5 days with a median of 1 attack per month, all described fever with peritonitic abdominal pain, pleuritic symptoms occurred in 4 cases, and erysipelas like erythema rash in 2. 3 patients had an appendectomy and 2 cholecystectomy prior to diagnosis. 5 of 7 symptomatic women reported attacks with menstruation and 3 had partial remissions with oral contraception. 3 patients had presented with AA amyloidosis of unrecognised aetiology of whom only 1 was truly asymptomatic. 2 children detected as part of family screening also denied any symptoms. 11 patients are on colchicine with good clinical and inflammatory responses, median age at starting treatment was 50 years,

2 felt that oral contraceptives provided adequate symptom relief, 1 declined treatment.
Conclusion: Familial Mediterranean fever associated with deletion M694 is an autosomal dominant condition in Northern European Caucasians with variable penetrance. Disease onset appears slightly later but symptoms and colchicine responsiveness are very similar to classical autosomal recessive FMF. The high rate of AA amyloidosis (19%) may reflect the very delayed diagnosis and late initiation of colchicine treatment.
Disclosure of interest: None declared.

A75

PW01-022 – Dissociation between CRP and SAA in FMF

K Stankovic Stojanovic[1*], V Hentgen[2], S Georgin-Lavialle[1], S Fellahi[3], I Jeru[4], S Amselem[4], J-P Bastard[3], G Grateau[1]
[1]Internal Medicine, APHP, Hôpital Tenon, Paris, France; [2]Pediatrics, Hopital de Versailles, Le Chesnay, France; [3]Biochemistry Laboratory, APHP, Hôpital Tenon, Paris, France; [4]Genetic Laboratory, APHP, Hopital Trousseau, Paris, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A75

Introduction: An Israeli study previously showed that dissociation between normal C-reactive protein (CRP) and elevated serum amyloid A (SAA) could be observed in Familial Mediterranean fever (FMF). Considering that elevated SAA is predictive for AA amyloidosis, this study suggested that SAA could be a better tool in the diagnosis and therapeutic management of FMF.
Objectives: To analyze the dissociation between CRP and SAA in a large cohort of FMF adults and children in France.
Methods: CRP and SAA were systematically measured during the follow-up of consecutive attack-free FMF outpatients seen in a pediatric and an adult French reference center. Dissociations between CRP and SAA were defined by normal CRP (<5mg/L) and elevated SAA (group A), or elevated CRP and normal SAA (<10mg/L) (group B). Demographic data, genotype, clinical characteristics of FMF, and treatment were analyzed.
Results: 274 samples from 219 patients were analysed. The cohort had a median age of 24 years old [interquartile 15-35], 54% were female. Ethnic origins were: 60% non-ashkenasi Jews, 1% ashkenasi Jews, 4% mixed, 9.5% Arabs, 5% Armenians, 5% Turks, 3% Lebanese or Syrians, 1% Italians, 1% Portuguese or Spanish, 1% Caucasian. *MEFV* genotype was known in 181 patients (83%): 63.5% had 2 non-ambiguous mutations, 24% were simple heterozygous, 7% were compound heterozygous with one non-ambiguous mutation and one polymorphism, 5.5% had no mutation. Six patients had amyloidosis. 181 patients (83%) were treated with colchicine, 3 patients with interleukin-1 inhibitor. Elevated SAA (median=16.5mg/L [13;31] while normal CRP was found in 21 samples (13% samples of with normal CRP). Elevated CRP (median=9mg/L [7;11]) while normal SAA was found in 38 samples (22% samples of normal SAA). Age was significantly higher in group B comparing to group A or the group with no dissociation (33 years old versus 21 and 23 respectively, p=0.004). Colchicine dosage was significantly higher in group B comparing to the group with no dissociation (1.05mg/day versus 1.34, p=0.04). No statistical difference was found concerning genotype or Ethnic origin. Dissociation with high SAA and normal CRP was found in some patients with amyloidosis but the difference was not statistically different (p=0.08). Finally, for values of CRP above 30mg/L (30-63mg/L), corresponding SAA values were 1.5 to 6 times higher (53-683).
Conclusion: Dissociation between SAA and CRP was not frequent in our study. Genotype and ethnic origin were not predictive for this dissociation.
Disclosure of interest: None declared.

A76

PW01-023 – Ex vivo PBMC cytokine profile in FMF patients

JN Ibrahim[1,2*], R Jounblat[2], A Delwail[1], J Abou-Ghoch[3], N Salem[3], E Chouery[3], A Megarbane[3], J-C Lecron[1], M Medlej-Hashim[2]
[1]Laboratoire inflammation, Tissus épithéliaux et cytokines; EA4331, University of Poitiers, Poitiers, France; [2]ER030-EDST; Faculty of Sciences II, Lebanese University, Beirut, Lebanon; [3]Medical Genetics Unit, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon
Pediatric Rheumatology 2013, **11(Suppl 1):**A76

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder affecting mainly Jews, Armenians, Turks and Arabs. Recently, several pro- and anti-inflammatory cytokines have been studied in sera of Armenian and Turkish FMF patients during and in between crises. However, the information were limited and contradictory.
Objectives: This work aimed to evaluate the *ex vivo* and serum cytokine profile of Lebanese FMF patients during acute attacks and attack-free periods and to compare it with that of healthy controls in order to identify a specific cytokine ¨signature¨ and to understand the role of the inflammasome in this autoinflammatory disease.
Methods: The study included 34 FMF patients, of whom 9 were studied during both attack and remission and 25 healthy controls. Cytokine levels were evaluated in serum and supernatants of peripheral PBMC cultures with and without 24h stimulation of monocytes by LPS and T lymphocytes by anti-CD3/CD28 beads, by Luminex Multiplex ELISA.
Results: Ex vivo cytokine profile: The levels of pro-inflammatory cytokines IL-6 and TNF-α were higher in unstimulated and LPS stimulated PBMC supernatants of FMF patients in crises compared to the control group. Concentrations were comparable between FMF patients during and between crises.
There was no difference in spontaneous IL1-β and IL-1 α release by PBMCs of FMF patients and controls. However, in response to LPS stimulation, levels of these cytokines were found higher in PBMC supernatants of FMF patients during crises compared to those in remission and to the controls. In contrast, no difference was found in IL1-RA levels between FMF patients and controls in all conditions.
Regarding Th1 and Th2 cytokines, IFN-γ and IL-4 levels were lower in unstimulated and anti-CD3/CD28 stimulated PBMCs supernatants of FMF patients during and between crises compared to the controls. Moreover, lower levels of those cytokines were detected in culture supernatants of FMF patients during crises compared to those in remission after T cell stimulation.
For Th17 cytokines, IL-17 was higher in anti-CD3/CD28 stimulated PBMC supernatants of FMF patients during crises compared to the control group. After T cell stimulation, PBMCs from FMF patients in remission release more IL-22 than PBMCs from control subjects.
Finally, no difference in IL-10 levels was detected between FMF patients and controls.
Serum cytokine profile: Except IL-6, all cytokines tested, were almost not detected in the serum of patients and controls.
Conclusion: The cytokine changes observed in FMF patients and characterized by a continuous pro-inflammatory Th17 and IL-1 family cytokine activation and a reduced Th1 and Th2 response, suggest an ongoing subclinical inflammation and represent a specific cytokine ¨signature¨ to FMF patients. In addition, these results do not show a particular involvement of the inflammasome in FMF physiopathology. Finally, we suggest that *ex vivo* study represents a novel and interesting approach to evaluate the cytokine involvement in FMF patients.
Disclosure of interest: None declared.

A77

PW01-024 – Phenotypic analysis of a MEFV negative FMF cohort

A Soriano[1], D Rigante[2], L Cerrito[1], C Fonnesu[1], L Sicignano[1], A Gallegos[3], R Manna[1*]
[1]Periodic Fevers Research Centre, Catholic University of Sacred Heart, Rome, Italy; [2]Department of Pediatrics, Catholic University of Sacred Heart, Rome, Italy; [3]Nuestra Senora del Prado Hospital, Talavera de la Reina, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A77

Introduction: Familial Mediterranean fever (FMF) is an inherited autosomal recessive disorder, ethnically restricted and commonly found among individuals of Mediterranean descent, caused by MEditerranean FeVer gene (*MEFV*) mutations on the chromosome 16. It is the most frequent periodic febrile syndrome among autoinflammatory syndromes. Eighty % of patients with FMF have *MEFV* mutations, while around 20% do not have mutations.
Objectives: We analysed epidemiological and clinical characteristics, as well as treatment schedules of a large cohort of FMF patients without any *MEFV* mutations, who responded to colchicine, in order to identify further clinical features of this specific subgroup.
Methods: Epidemiological and clinical details of 344 patients attending the Periodic Fevers Research Centre in a period of 15 years were analysed. We selected patients without *MEFV* mutations, in whom diagnosis was established by the Tel-Hashomer criteria. We finally compared the clinical findings of *MEFV*-negative population with the *MEFV*-positive one.

Results: Genetic testing by *MEFV* analysis was performed in all patients (n = 344); 41 patients (14%, 20 males and 21 females) negative for *MEFV* mutations were selected and studied. Similarly with *MEFV* positive patients, in our case-series, most *MEFV*-negative ones came from Southern and Central Italy. The mean age of FMF onset was 21.8 years, differently from what observed in *MEFV*-positive population, in which the mean age was 15. The frequency of attacks went from less than 1 attack/month (in 26%) to 1-2 attacks/month (in 54%) and more than 2 attacks/months (in 19.5%). The mean duration of each attack was 83.9 ± 8.91 hours. The typical clinical signs of FMF attacks were: fever (T max 39.4°C±0.12, present in 100% of patients), articular pain (76%), abdominal pain (63.4%), oral aphthosis (44%), and chest pain (37%). Thirty-one out of 41 patients had joint involvement in terms of arthritis (21.5%), arthralgias (25%), arthromyalgias (32%), and myalgias (21.5%). Attacks were controlled with a mean dose of colchicine of 1.5 mg/day in all patients (vs a mean dose of 1.3 mg/die in the *MEFV*-positive population). No statistically significant difference was detected in terms of frequency and duration of attacks, as well as in symptoms distribution and colchicine dosage between *MEFV*-negative and positive populations.
Conclusion: Analysis of our MEFV-negative series of Italian patients revealed a higher prevalence of late-onset FMF, whereas the percentage distribution of symptoms was similar to *MEFV*-positive patients. These results support the hypothesis of involvement of other low-penetrance genetic systems in the FMF clinical expression.
Disclosure of interest: None declared.
References

1. Soriano A, Manna R: **Familial Mediterranean fever: new phenotypes.** *Autoimmun Rev* 2012, **12**:31-7.
2. Rigante D: **The fresco of autoinflammatory diseases from the pediatric perspective.** *Autoimmun Rev* 2012, **11**:348-56.
3. Manna R, Cerquaglia C, Curigliano V, Fonnesu C, Giovinale M, Verrecchia E, Montalto M, De Socio G, Soriano A, La Regina M, Gasbarrini G: **Clinical features of familial Mediterranean fever: an Italian overview.** *Eur Rev Med Pharmacol Sci* 2009, **13(Suppl 1)**:51-3.

A78

PW01-025 – Definition of colchicine resistance in FMF

E Demirkaya, C Acikel, A Tufan, A Kucuk, A Berdeli, A Gul, AM Onat, A Delibas, A Duzova, A Dinc, O Yavascan, O Kasapcopur, B Makay, B Goker, B Sozeri, B Kisacik, E Comak, E Unsal, E Erken, E Gunal, E Baskin, F Yalcinkaya, F Yildiz, F Gok, G Basbozkurt*, G Ozcelik, G Demircin, H Poyrazoglu, H Erdem, H Direskeneli, H Ozer, H Ozdogan, I Simsek, I Dursun, I Gokce, M Tunca, M Gurgoze, N Cakar, N Akinci, N Ayaz, O Donmez, O Ozkaya, R Topaloglu, S Kavukcu, S Yuksel, S Akar, S Bakkaloglu, S Emre, S Senel, S Erten, S Yavuz, S Kalman, T Kasifoglu, U Kalyoncu, Y Tabel, Z Ekinci, S Ozen,
Turkish FMF Study Group,
FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology (FAVOR)
Turkish FMF study group, FAVOR, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A78

Introduction: Familial Mediterranean fever (FMF) is an autoinflammatory disorder, characterized by periodic fever and serosal inflammation. Colchicine is effective in controlling the attacks and preventing the development of amyloidosis. About 5-10% of the patients do not respond to colchicine.
Objectives: In this study it was aimed to determine the set of criteria for the diagnosis of resistance to colchicine.
Methods: This study was planned with Delphi technique sent to 70 experts on FMF and 59 of them approved to attend. In the first Delphi round, clinical and laboratory findings indicating colchicine resistance and the protocol which would define resistance to treatment and exclusion criteria were defined. Based on the results of the first Delphi, a second Delphi form which included 5 evaluation questions was developed. In this latter form the questions to be used in order to define complete response, partial response and non-response were tried to be determined.
Results: In the first Delphi round, persistence of the frequency, severity and the duration of episodes in spite of the treatment with adequate dosage ranked the highest. Laboratory findings that are thought as the best indicator of resistance to treatment were high levels of acute phase reactants (In order of frequency; CRP, ESR, SAA and fibrinogen).
In the second Delphi round, among 57 experts, 35 experts reported the frequency of attacks while 10 experts reported the duration and 9 reported the severity of the disease as an indicator for the assessment of response to colchicine treatment.
For a complete response, normal CRP levels were required by 54 experts while 43 experts reported that 50% decrease in CRP levels could be accepted as partial response to treatment. Appropriate duration for the assessment of response to colchicine treatment was determined as 3 to 6 months by 34 experts. It was also stated that the highest dose for age and weight should be given in order to state colchicine resistance. Thirty three experts stated that the patient should be attack free for a complete response to colchicine treatment. For defining the partial response to treatment, a 50% decrease in attack frequency was the most favored choice.
Conclusion: Assessing the colchicine resistance via concrete and agreed scale will provide a reliable data. The study is to be finalized soon with an expert panel.
Disclosure of interest: None declared.

A79

PW01-026 – Validation of pediatric diagnostic criteria in FMF

E Demirkaya[1], S Ozen[2], C Saglam[1*], T Turker[3], A Duzova[4], P Woo[5], I Konè-Paut[6], M Doglio[7], G Amaryan[8], J Frenkel[9], Y Uziel[10], A Insalaco[11], L Cantarini[12], M Hofer[13], S Boiu[14], C Modesto[15], A Bryant[5], D Rigante[16], E Papadopoulou-Alataki[17], S Guillaume-Czitrom[18], N Ruperto[7], M Gattorno[7], PRINTO and Eurofever Project
[1]FMF Arthritis Vasculitis and Orphan Disease Research Center (FAVOR), Gulhane Military Medical Faculty, Ankara, Turkey; [2]Pediatric Rheumatology, Hacettepe University, School of Medicine, Ankara, Turkey; [3]Epidemiology, Gulhane Military Medical Faculty, Ankara, Turkey; [4]Rheumatology, Istanbul University, Istanbul, Turkey; [5]Pediatric Rheumatology, UCL, London, UK; [6]Pediatric Rheumatology, University of Paris SUD, Paris, France; [7]Pediatric Rheumatology, Ospedale Gaslini, Genoa, Italy; [8]National Pediatric Familial Mediterranean Fever Centre, Institute of Child and Adolescent Health, Yerevan, Armenia; [9]Pediatrics, University Medical Center Utrecht, Utrecht, the Netherlands; [10]Pediatrics, Meir Medical Centre, Kfar Saba, Israel; [11]Reumatologia, Ospedale Pediatrico Bambin Gesù, Rome, Italy; [12]Rheumatology, Policlinico le Scotte, University of Siena, Siena, Italy; [13]Pediatric Rheumatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [14]Pediatric Rheumatology, Université Paris-Descartes, Paris, France; [15]Reumatologia, Hospital Valle de Hebron, Barcelona, Spain; [16]Pediatrics, Università Cattolica Sacro Cuore, Rome, Italy; [17]Fourth Department of Pediatrics, Aristotle University of Thessaloniki Papageorgiou Hospital, Thessaloniki, Greece; [18]Pediatric Rheumatology, Bicêtre-Hôpitaux Universitaires Paris-Sud, Bicetre, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A79

Introduction: The diagnosis of FMF is made clinically and may be confirmed by identifying mutations in the *MEFV* gene. The most commonly used diagnostic criteria for FMF are those of Tel Hashomer, which have been established in the Jewish adult population. Recently, a Turkish group (Yalcinkaya-Ozen's diagnostic criteria) proposed new criteria for diagnosis of FMF in children.
Objectives: We analyzed the validity and reliability of Yalcinkaya-Ozen's diagnostic criteria for childhood FMF in a large international registry.
Methods: The study group consisted of 339 FMF patients diagnosed according to Tel Hashomer criteria. A control group of 377 patients were diagnosed other periodic fever syndromes including MKD, TRAPS, CAPS and PFAPA syndromes. Both groups were evaluated according to the Tel Hashomer criteria and the new set of diagnostic criteria proposed to use in childhood FMF. The diagnostic performance of both criteria was assessed by multiple logistic regression analysis.
Results: The sensitivity and specificity of Tel Hashomer criteria in our study were 35.1% and 97.7 %, respectively. The presence of two or more of these new five criteria diagnosed FMF with a high and sufficient sensitivity of 87.4 % and the NPV of 74.8%. When we used at least three Yalcinkaya-Ozen's criteria, the discrimination of the diseases other than FMF reached the highest specificity of 88.2% and the PPV of 82.9% however the sensitivity was compromised. In case of all the new set of criteria were met, the sensitivity and specificity were 99.6% and 5.6%, respectively with a PPV of 94.1% and an NPV of 49.2% . Our study showed that ethnicity had no impact on the validation.

Conclusion: Tel Hashomer diagnostic criteria was found to have high specificity, whereas Yalçınkaya-Ozen's criteria has a higher sensitivity for the diagnosis of FMF with the combination of at least any two out of these five criteria are met. The small number of patients with amyloidosis or erysipelas like erythema and the response to colchicine therapy constituted the drawbacks in assessing the patients with Tel Hashomer criteria.
Disclosure of interest: None declared.

A80

PW01-027 – Predictors and survival of FMF related amyloidosis

E Demirkaya[1*], I Yilmaz[2], C Acikel[1], M Saglam[3], H Unal[2], M Gok[2], A Polat[1], H Cetinkaya[2], T Eyileten[2], S Sari[3], AO Yildirim[1], Y Oguz[2], A Vural[2], JJ Carrero[4], FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology (FAVOR)
[1]FMF Arthritis Vasculitis and Orphan Disease Research Center, Gulhane Military Medical Academy, Ankara, Turkey ; [2]Nephrology, Gulhane Military Medical Academy, Ankara, Turkey ; [3]Radiology, Gulhane Military Medical Academy, Ankara, Turkey; [4]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Solna, Sweden
Pediatric Rheumatology 2013, **11(Suppl 1):**A80

Introduction: Amiloidosis is most fatal complication of FMF. Former studies recognize that endothelial functions were severely impaired in patients who have amyloidosis than the patients who have other glomerulopathies. Asymmetric dimethyl arginine (ADMA), the endogenous inhibitor of nitric oxide synthase is possibly a causative or predictive factor in endothelial dysfunction in humans.
Objectives: Compare the amyloidosis group to only proteinuria group for biochemical, demographic and some other features such as flow-mediated dilation (FMD), to understand which markers may affect or help prediction of amyloidosis. We also evaluated the effects of elevated ADMA levels and impaired FMD responses on the survival time of CVD free period in two distinct groups with severe proteinuria, secondary amyloidosis (SA) versus primary glomerulopathy. We proposed that increased ADMA synthesis in amyloidosis induced endothelial damage may contribute part of the mechanism by which proteinuria increases cardiovascular morbidity and mortality.
Methods: Study was part of a cohort study. The amiloidosis and proteiuria groups were followed up for predictive factors. All enrolled subjects were evaluated by standard physical examination, chest X-ray, baseline electrocardiogram, two-dimensional echocardiography, and routine biochemical laboratory tests, including liver and kidney function tests and 24-hour urinary protein measurements. FMD and venous blood samples were taken following a 2 week wash-out period, during which time no vasoactive drugs (including colchicines) were given. Measurements of serum ADMA and SDMA were done using HPLC.
Results: The data of 102 patients with proteinuria due to primary glomerulopathy and 98 patients with amyloidosis due to FMF were assessed. Median age of diagnosis in patients with amyloidosis was 16 (min.-max.: 6-25) and 71.4 % of patients were 18 or younger at the date of diagnoses of amyloidosis. Patients with amyloidosis provided higher levels of SDMA and ADMA ($p < 0.01$) and lower FMD percentage when compared to patients with glomerulonephritis ($p < 0.01$). Inflammatory markers such as high sensitivity C-reactive protein (hsCRP) and pentraxim-3 were statistically different between groups and higher among patients with amyloidosis.
Conclusion: Inflammatory markers such as hsCRP and pentraxim-3 were statistically different between groups and higher among patients with amyloidosis. The mortality and the cardiovascular event rate was much higher in patients with secondary amyloidosis.
Disclosure of interest: None declared.

A81

PW01-028 – Developing a new severity score for FMF

E Demirkaya[1], C Acikel[2*], A Gul[3], M Gattorno[4], E Ben-Chetrit[5], H Ozdogan[3], P Hashkes[6], A Polat[1], O Karadag[7], A Livneh[8], S Ozen[9],
FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology (FAVOR)
[1]Pediatric Rheumatology, Gulhane Military Medical Academy, Ankara, Turkey; [2]FMF Arthritis Vasculitis and Orphan Disease Research Center, Gulhane Military Medical Faculty, Ankara, Turkey; [3]Rheumatology, Istanbul University, Istanbul, Turkey; [4]Pediatric Rheumatology, Ospedale Gaslini, Genoa, Italy; [5]Rheumatology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [6]Pediatric Rheumatology, Shaare Zedek Medical Center, Jerusalem, Israel; [7]Rheumatology, Hacettepe University, School of Medicine, Ankara, Turkey; [8]Medicine, Sheba Medical Centre, Ramat-Gan, Israel; [9]Pediatric Rheumatology, Hacettepe University, School of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A81

Introduction: Severity is a basic feature that defines the prognosis of a disease. FMF presents with a variety of clinic and laboratory manifestations affecting severity, and evaluation of prognosis is an elusive task. Different severity scores have been previously proposed for FMF, and commonly used ones include Mor, Pras and Tel Hashomer severity scores however recent studies showed that there is no consistency among them.
Objectives: The aim of this study was to develop and validate a new set of criteria for the assessment of disease severity for FMF patients by a multicenter study.
Methods: Delphi survey technique was used for the initial phase of the study. A panel of experts including twenty-four FMF specialists from 16 countries participated in the survey. The first Delphi round aimed to identify all clinical and laboratory features considered to be associated with the severity of FMF. In the second round, 33 structured questions were developed to collect expert opinions about FMF severity. At the third and the last rounds, the expert panel was asked to evaluate the answers given to the questions in the previous round. After all rounds, a subgroup of the expert panel (ten experts and one facilitator) gathered in a consensus meeting (NGT) on November 13, 2012 in Washington DC, USA. In this meeting, the results of all previous rounds and items for the candidate criteria and their standard definitions were discussed.
Results: In Delphi rounds, three of the mostly reported clinical items were 'response to colchicine treatment', 'frequency of attacks' and 'presence of arthritis' and laboratory parameters were high levels of Serum amyloid A (SAA), C-reactive protein (CRP), and the MEFV gene mutations. At the consensus meeting, items revealed through the second and the third rounds were reevaluated, and the following nine items were selected for the final set of severity criteria: presence of a chronic sequel (including amyloidosis, growth retardation, anemia, splenomegaly), organ dysfunction (nephrotic-range proteinuria), organ failure (cardiac, renal, thyroid etc.), frequency of attacks (average number of attacks between 1-2 per months), increased acute phase reactants (CRP, SAA, ESR, fibrinogen) during the attack-free period (at least 2 weeks after the last attack in 2 occasions one-month apart), involvement of more than 2 sites during an individual acute attack (pericarditis, pleuritis, peritonitis, synovitis), more than 2 different types of attack during the course of the disease (isolated fever, pericarditis, pleuritis, peritonitis, synovitis, erysipelas like erythema, scrotal involvement, vaginitis, myalgia etc.), duration of attacks (more than 72 hours in at least 3 attacks during one year), effort-induced leg pain (pain following standing or excercising, excluding other causes).
Conclusion: The panel of experts agreed on the nine items to be used in the severity criteria for FMF, and results of the validation study are being waited for final definition and weights of the items.
Disclosure of interest: None declared.

A82

PW01-029 – Relationship between apoptotic alterations and inflammation in familial Mediterranean fever

G Mkrtchyan*, A Boyajyan
Institute of Molecular Biology of NAS RA, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A82

Introduction: A number of studies indicated that alterations of apoptosis and its regulation together with upregulated inflammatory response are involved in pathogenesis of familial Mediterranean fever (FMF). However, molecular and cellular mechanisms responsible for abnormalities in apoptosis and their relationship with autoinflammatory responses in FMF are not clear.
Objectives: In the present study we determined the levels of annexin-A5, ficolin-H, and ficolin-L proteins, in the blood of patients with FMF and

healthy controls. Assessment of correlation between the levels of these proteins was also performed. Annexin-A5 is a marker of apoptosis; ficolin-H and ficolin-L are components of the complement cascade. Binding of ficolins to the surface of apoptotic cells may activate the complement lectin pathway, as well as phagocytosis of apoptotic cells. Thus, ficolins may act as both inflammatory mediators and opsonins.
Methods: Forty four FMF-affected subjects and 50 healthy controls were involved in the study. The enzyme linked immunosorbent assay was used to measure blood serum levels of annexin-A5, ficolin-H, and ficolin-L proteins in patients and controls. Statistical approaches included Mann-Whitney U test and Spearman correlation analysis.
Results: Significantly increased levels of annexin-A5, ficolin-H, and ficolin-L proteins were detected in FMF-affected subjects as compared to healthy controls ($p<0.05$). The data obtained provided further evidence on the increased rate of apoptosis in FMF and demonstrated the involvement of the complement lectin pathway in FMF-associated inflammatory reactions. In addition, in case of FMF a positive correlation between the levels of annexin-A5 and ficolin-H, as well as between the levels of annexin-5 and ficolin-L. No correlation between the measured parameters was detected in healthy subjects group. These results indicated that the detected correlation in conditioned by FMF-associated pathologic processes, namely increased rate of apoptosis and upregulated inflammation.
Conclusion: The obtained results suggest that the pathogenesis of FMF is characterized by increase rate of apoptosis associated with hyperactivation of the complement lectin pathway. Based on the obtained data we also concluded that apoptotic alterations and upregulated inflammation in FMF are interrelated.The obtained results suggest that the pathogenesis of FMF is characterized by increase rate of apoptosis associated with hyperactivation of the complement lectin pathway. Based on the obtained data we also concluded that apoptotic alterations and upregulated inflammation in FMF are interrelated.The obtained results suggest that the pathogenesis of FMF is characterized by increase rate of apoptosis associated with hyperactivation of the complement lectin pathway. Based on the obtained data we also concluded thatapoptotic alterations and upregulated inflammation in FMF are interrelated.
Disclosure of interest: None declared.

A83

PW01-030 – Pulmonary manifestations of FMF

AV Sargsyan[1], AR Davtyan[2], YS Sargsyan[3*]
[1]Internal Medicine, Yerevan State Medical University, Yerevan, Armenia; [2]ArtLab Diagnostic Center, Artashat, Armenia; [3]General Medicine Student, Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A83

Introduction: Familial Mediterranean Fever (FMF) is an autoinflammatory disease. It is associated with vasculitis, pulmonary hemorrhage, infiltrates, and pulmonary hypertension due to amyloidosis. These complications however have been reported only rarely.
Objectives: The aim of our study was to investigate pulmonary consequences of FMF.
Methods: The study cohort involved 155 FMF patients (male/female 87/68). Mean age was 33,6±11,8 years in the patients group without renal amyloidosis (45 men, 35 women, n=80) and 37,8±7,4 years in the patients group with amyloidosis (42 men, 33 women, n=75). All the patients had symptoms related to the respiratory system, such as pleuritic chest pain with or without cough, dyspnea, chest tightness and frequent pneumonias. 28 patients had a history of tobacco use. Most of the patients (122) had M694V mutation, and the rest had other mutations. All the patients were on colchicine treatment at the time of the study with the exception of 2 hemodialysis patients. Laboratory tests, including CRP, SAA and capillary blood gases, ECG and chest X-ray were carried out on all the patients. 50 patients underwent Doppler echocardiography and 25 HRCT scan of the chest.
Results: Mean C-reactive protein (CRP) and serum amyloid-A (SAA) were 17.74±13.74 mg/L vs 11,88±13.79 mg/L and 33±66.6 mg/L vs 5.25 ±4,45mg/L, respectively, and significantly higher in the patients group with renal amyloidosis than the mean values of the patients group without amyloidosis (P<0,000). Blood gases values (mean±SD) were within normal ranges in patients without amyloidosis, and were slightly decreased in amyloidosis patients group (PO_2 83.6±8.95 mm Hg, PCO_2 39.4±3.6 mmHg, O_2Sat 94.6±3.38% vs. PO_2 74±11.36 mm Hg, PCO_2 35.3±4.5 mm Hg, O_2Sat 90.1±10.26%, P<0.000). Infiltrates, ground-glass opacities, reticulonodular pattern, pleural effusion and pleural thickening, lymphadenopathy, dilatation and hypertrophy of right ventricle and increased pulmonary artery systolic pressure were more frequent findings in the patients group with amyloidosis than in the group without it, though in the group without amyloidosis they occurred as well.
Conclusion: Our results suggest that patients with FMF and amyloidosis tend to have hypoxemia. The latter could contribute to pulmonary complications in FMF patients. On the other hand, it is possible that FMF patients without renal amyloidosis experience pulmonary manifestations and develop pulmonary complications. Respiratory symptoms in FMF patients without renal amyloidosis probably result from ongoing inflammation and early vascular alteration. Hypoxemia is a sign of advanced disease.
Disclosure of interest: A. Sargsyan Consultant for: clinical and lab tests, A. Davtyan Consultant for: lab tests, Y. Sargsyan Consultant for: biochemical tests and statistics.

A84

PW01-031 – Treatment of FMF in middle and old age

A Ayvazyan
Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A84

Introduction: Current recommendations for the treatment of familial Mediterranean fever (FMF) are based largely on the observation of FMF patients receiving colchicine therapy in childhood and young age. The adequate colchicine therapy led to more and more patients survive to that age. In addition, there are national peculiarities of FMF. For example, in Armenia, even before the massive use of colchicine therapy, many patients survived to middle and old age.
Objectives: We have investigated the corse of FMF in middle and old age, the incidence of miocardial infarction and the outcomes in case of myocardial infarction.
Methods: Follow-up during 10-30 years.
Results: Our research has shown that the risk of amyloidosis decreases with age, and the ability of colchicine to prevent attacks of FMF increases with age. Our research has also shown that in the absence of regular colchicine therapy increases the risk of myocardial infarction. In addition, myocardial infarction in patients with FMF is more severe, with a higher risk of death. With age the incidence of many diseases is increased, but their co-therapy with colchicine has not been studied.
Conclusion: To date, may be recommended in middle and old age to take colchicine at a dosage that fully prevents the attacks of FMF in young age. If treatment is initiated in middle or old age, the dosage of colchicine should be higher than necessary to control the attacks of illness and indicators of inflammation. Untreated in young age FMF should be considered as a risk factor for CHD. Patients with FMF in the case of acute myocardial infarction should be observed over a long time and prevention of complications should be more intense. Urgently need to be initiated the multi-center and national studies on the combined treatment of FMF and the most common diseases in middle and old age.
Disclosure of interest: None declared.

A85

PW01-032 – FMF-like state: genetic factors unrelated to MEFV

D Babikyan[1*], I Jeru[2], B Copin[2], H Hayrapetyan[1], S Amselem[2], T Sarkisian[1]
[1]Center of Medical Genetics and Primary Health Care, Yerevan, Armenia; [2]Hôpital Armand Trousseau, Paris, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A85

Introduction: FMF is considered an autosomal recessive autoinflammatory syndrome caused by single gene (MEFV) mutations. Recently, it has been known that also heterozygous mutation carriers can suffer from a mild or incomplete form of FMF, named FMF-like disease. Among Armenians, who have relatively high carrier rate of MEFV mutations, single mutation has been detected in about 1/5 of symptomatic cases. Thus, one cannot exclude the influence of other modifier genes and/or environmental factors which

can contribute to the variable penetrance and to the phenotypic variability of FMF-like disease.
Objectives: The aim of our ongoing project is to better describe the genetic basis of the FMF-like condition unrelated to MEFV by identifying genetic variations in patients with single MEFV mutation.
Methods: From records of more than 8,000 FMF patients we analyzed 105 affected heterozygous sporadic patients with definite diagnosis of FMF and extended Armenian family with variable clinical presentations. All cases were screened for full MEFV gene sequence variations and MEFV-linked five microsatellites. Whole-genome genotyping assay was applied for selected sporadic cases and 30 familial symptomatic and asymptomatic cases. Data are analyzed with Illumina GenomeStudio for LOH regions and with MERLIN for linkage analysis.
Results: Genotype-phenotype analysis performed among one and two mutation carriers has showed high probabilities for the presence of major FMF symptoms in heterozygous individuals. Further analysis of selected 12 sporadic cases has revealed a LOH at a region encompassing genes involved in the NF-κB activation in two unrelated FMF-like sporadic patients.
Later, we authenticated autosomal dominant and autosomal recessive patterns of inheritance by finding a single mutated allele or homozygous/compound heterozygous genotypes confirmed by microsatellite analysis in several affected members of the selected family. Four of five sibs of healthy and not-related parents were carriers of a single M694V mutation, where two sibs were diagnosed with FMF and other two were asymptomatic, indicating 50% disease penetrance. Notably, the autosomal dominant inheritance was observed also in the offspring of two affected sibs with 25% (1/4) and 100% (3/3) penetrance. However, in both generations with autosomal dominant FMF we did not find a common MEFV haplotype.
Conclusion: Parallel to the rising evidence of the association of a single MEFV mutation with only mild FMF symptoms, possibie explanation for definite phenotype of FMF in simple heterozygous patients does not exclude the association of other mutations or polymorphisms in relevant genes acting in synergy and affecting the course of FMF-like disease. Despite of the complexity of ongoing linkage analysis complicated with two consanguineous marriages and members with atypical or uncertain phenotype, co-existence of two patterns of the inheritance in the same family speculates on heterogeneous genetic basis of FMF in single mutation carriers. Preliminary finding of one candidate LOH region in sporadic cases and absence of common MEFV haplotype encourage further search of genetic variations in the genes of the inflammatory pathway acting in combination with MEFV and changing the severity of the kaleidoscopic clinical phenotype in simple carriers.
Disclosure of interest: None declared.

A86

PW01-033 – Phenotype – genotype in Armenian children with FMF

G Amaryan[1*], T Sarkissian[2], T Sarkissian[2], H Hayrapetyan[2], H Hayrapetyan[2], A Tadevosyan[3], A Tadevosyan[3]
[1]National Pediatric Centre for Familial Mediterranean Fever of "Arabkir" Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan State Medical University, Armenia; [2]Centre of Medical Genetics and Primary Health Care, Yerevan, Armenia; [3]Yerevan State Medical University, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A86

Introduction: Familial Mediterranean Fever (FMF) is an ethnic disease for Armenian population and represents a significant health care problem. Frequency of carriers of *MEFV* mutations is 1:3, and the prevalence of FMF is rather high (14-100:10000). During the period between 2003 and 2012 there was a 4.5-fold increase of the total number of children with FMF. Many of these cases have severe clinical picture and atypical course, which may complicate timely diagnosis.
Objectives: To establish phenotype-genotype correlations in Armenian children with FMF.
Methods: We analyzed a group of 715 children with FMF (438 boys and 277 girls, mean age: 8.64±0.17), including sporadic (51.8%) and familial cases (48.2%). The diagnosis was based on the Tel-Hashomer criteria, the "Guidelines for the genetic diagnosis of hereditary recurrent fevers"(2011) and molecular-genetic detection of 12 *MEFV* mutations common for Armenians. The statistical analysis was performed using Epi-Info 2000 software. For comparison of two nominal variables in table "two by two" Yaet's corrected for continuity chi-square test was used, significance level $p<0.05$.
Results: FMF manifestation during the first decade of life were determined in 95.2% of patients (mean age of onset 3.5±0.1, mean age of diagnosis 5.25±0.15). FMF onset before 5 years was in 78.6%. In 96.5% of patients severe phenotype with polyserositis was associated with *MEFV* mutations in exon 10 (M694V- 58.1%; V726A- 20.4%; M680I- 15.7%). In contrast, mild or atypical FMF phenotypes without polyserositis were found in heterozygous carriers of one *MEFV* mutation in exons 2 and 3 (E148Q and P369S, respectively). We suggest that our patients with FMF developed pleurisy more frequently (81.7%), also myalgia (37.5%), pericarditis (13.8%), and rare skin lesions (13.4%) mostly as erysipelas-like erythema, ELE, (10.8%) in comparison with other populations (Jews, Turks, Arabs).
Among concurrent pathologies, we found Henoch-Shonlein purpura (HSP,1.5%), protracted febrile myalgia (PFM, 2.7%) as well as the higher than expected frequencies for juvenile idiopathic arthritis (JIA, 4.7%), non-amyloid renal involvements (NARI, 1.1%) and ulcerative colitis/Crohn's disease (UC/CD, 1%). The development of serositis, splenomegaly, ELE and vasculitis was associated with *M694* homozygous and compound-heterozygous genotypes. Early manifestation and severe FMF attacks were detected in patients carrying *M694V*genotypes, whereas the M680I and V726A mutations were associated with relatively mild clinical features. Adhesive intestinal obstruction (AIO), was detected in 3.2% of patients, in some cases as the first and only FMF manifestation, especially in the *M694V*carriers.
Conclusion: Taking into account the high prevalence of FMF in Armenia, *MEFV* mutation screening is recommended not only for patients with atypical symptoms resembling FMF, but also for patients with FMF-associated vasculitis (HSP, PFM) as well as co-existed immune diseases (JIA, NARI, UC/CD) and AIO. In addition to improving the early diagnosis of FMF and preventing the development of amyloidosis, this can be especially important in the patients resistant to conventional treatments for the aforementioned FMF-associated and other concurrent diseases.
Disclosure of interest: None declared.

A87

PW01-034 Clinical-genetic investigation of FMF in Armenia

H Hayrapetyan[1], G Amaryan[2], A Yeghiazaryan[1], T Sarkisian[1*]
[1]Center of Medical Genetics and Primary Health Care, Yerevan, Armenia; [2]Arabkir Joint Medical Centre - Institute of Child and Adolescent Health, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A87

Introduction: Familial Mediterranean Fever (FMF) is the most common hereditary disorder among Armenians. The establishment of clinical and genetic testing of FMF in the Center of Medical Genetics and Primary Health Care (CMG) was mostly forced by the high social and public health problems concerning a huge cohort of patients.
Objectives: Prevalence of FMF is 14-100/per 10000 in different regions of Armenia. Frequency of carriers of MEFV mutations is 1:3 in Armenians. And, the increase of the incidence of FMF and related disorders is caused by genetic drift and geographical isolation.
Methods: Molecular genetic detection of 12 MEFV mutations accounting for 98,71% of patients compared to healthy individuals revealed the most frequent genotypes and genotype-phenotype correlations.
Results: Heterozygote carriers associated with abortive and mild FMF features is 18,72%, and 1.29% of patients with clinical features of FMF are without mutations. In some FMF patients "mild" MEFV mutations are associated with inflammatory attacks (P369S: 0.49%; E148Q: 5.09%; A744S: 0.74%). Genotypes E148Q/A744S and E148Q/P369S are found rarely.
We have revealed the complex FMF cases with following concurrent morbidity: epilepsy (M694V/M694V; V726A/M680I); Sjogren syndrome (M694V/M694V); bronchial asthma (M694V/V726A, V726A/M680I, M680I); b-thalassemia (M694V/M694V); hyperthyroidism (M694V/M680I); Tourette syndrome (M694V/M694V); Ulcerative colitis (M694V/M694V); renal amyloidosis and multiple sclerosis (M680I/M680I); ankylosing spondilitis-like syndrome in about 20% of FMF patients (predominantly M694V/M694V), etc.
We have shown that particular mutations have significant correlation with renal amyloidosis (RA). In frames of International Meta-FMF project we compared our data with the FMF morbidity among the other populations. We confirmed that M694V mutation is a high risk factor of RA in patients in Armenia, Israel, Lebanon, but not associated with RA in Turkey. M694V homozygous genotype of MEFV in FMF patients with RA is significantly higher than in patients without RA. The risk of male patients to develop

RA is four times higher than that of female patients. SAA (Serum Amyloid A) a/a homozygous genotype is also associated with a seven-fold increased risk of developing RA, compared to other SAA1 genotypes. The presence of only one SAA1 a/a allele does not suggest an increased susceptibility to RA. In our cohort of FMF patients the adequate colchicine-therapy may delay RA progression. In a few cases, the effect of colchicine remains controversial. M694V homozygotes present a more severe phenotype and show a limited response to colchicine at the nephrotic stage of RA. In contrast, FMF patients with other genotypes still have a good chance to escape the nephrotic syndrome and to maintain renal function.
Conclusion: As a result of our 16-year experience, the CMG holds the largest DNA Biobank of FMF (more than 18000 samples). The number of patients visiting CMG is dramatically increasing due to complex clinical and genetic examinations, assessment of efficiency of colchicine treatment, prognosis of development of complications, including renal amyloidosis, counseling of families, professional and public awareness. Genetic counseling of FMF patients and their families is performed for the disease risk estimation for future generations.
Disclosure of interest: None declared.

A88

PW01-035 – Mutations in PB30.2D and complexing with caspase-1

K Nazaryan[1,2], G Arakelov[1*]
[1]Russian-Armenian (Slavonic) University, Yerevan, Armenia; [2]Institute of Molecular Biology, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A88

Introduction: Mutations M680I, M694V and V726A of Pyrin - the product of MEFV gene - are localized at the domain B30.2 (PB30.2D) and responsible for manifestation of the most widespread and severe forms of FMF. From the other hand, it is well known that malfunction of the pyrin-caspase-1complex is the main reason for inflammation during FMF. Therefore, we suggest that comparative investigation of normal and mutated pyrin and caspase-1 interaction will help to reveal possible link between those mutations and structural changes that influence formation of pyrin-caspase-1 complex.
Objectives: From abovementioned the goal of current study was to detect possible changes in the tertiary structure of B30.2 and to show structural consequences that influence formation of pyrin-caspase-1 complex.
Methods: 3D structures of M680I, M694V and V726A was build up with the help of Rosetta software, caspase-1and B30.2 files obtained from PDB. Alignment of native B30.2 with three mutant forms has been done by VMD programme. In silico molecular modeling interaction experiments between native and mutated B30.2 and caspase-1 has been performed by CHARMM software in in Effective Energy Function 1 environment using 24-node computer cluster. Each molecular modeling time was 110ns with iteration 2fs.
Results: Influence of mutations on B30.2 tertiary structure has revealed the following structural changes: M680I – induces conversion of β-sheet into loop in position THR663-TRP665, loop - β-sheet in THR707- LEU710 and loop - α-helix in LYS765 – ALA768 site. Alignment of the native structure with mutation has RMSD=1,137 Å. M694V – conversion of the loop into β-sheet in the sites LYS695-GLU696 and THR707 – LEU709, RMSD=1,699 Å. V726A – conversion β-sheet into loop in the sites TRP655 – VAL657, loop - β-sheet in THR707- LEU710 and ARG725 – GLY727, loop - α-helix in ASP762 – LYS765, RMSD=1,808 Å. Molecular modeling of B30.2-caspase-1 dynamic interaction has shown significant differences between interaction energy of normal and mutated domains. Decrease in the minimal and average complex formation energy in the case of V726A (71 and 69% in comparison to the normal domain), no changes for M680I (99 and 97.4%) and increase for M694V (136.5 and 126,5%). At the same time maximal energy values have not shown any considerable differences.
Conclusion: Summarizing results of impact of the mutations on the B30.2-caspase-1 complex formation we came to the conclusion that dramatic changes in the tertiary structures which reflected in the shifts of binding sites and differences of interaction energy have notable influence on the complex formation, which in its turn should affect process of IL1β activation a trigger stage of inflammation process.
Disclosure of interest: None declared.

A89

PW01-036 – Renal replacement therapy in patients with FMF

M Papazyan[1*], M Voskanyan[1], H Nazaryan[1], S Babloyan[2], A Sarkissian[1]
[1]Nephrology, "Arabkir" Medical Centre, Yerevan, Armenia; [2]Paediatric Surgery & Urology, "Arabkir" Medical Centre, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A89

Introduction: Renal amyloidosis (RA) of Familial Mediterranean Fever (FMF) – although largely preventable –still is a major health problem in Armenia and an important cause of death.
Objectives: The **aim** of the study is to investigate the long-term outcome of patients with renal amyloidosis on RRT.
Methods: From January 2002 till September 2012 279 patients were admitted to our centre for Renal Replacement Therapy (RRT), of whom 40 (14.3%) had RA of FMF. Their mean age was 31.4±12.7 (range 12.6–52.9), 60% were males. Mean duration of hemodialysis (HD) was 1.6±1.7 years (range 0.1-6.0).
Results: Hemodialysis: Of the 28 patients not undergoing renal transplantation (Tx) in Armenia, 9 died (systemic amyloidosis - 5, heart attack - 2, stroke -2); 15 moved to another country and 4 remained on dialysis. Over half of the 25 pts with minimum observation period of 6 mo were resistant to EPO. One third died, mainly due to cardiovascular complications and systemic amyloidosis.
Living related donor Tx was done after 8 mo (median) of HD in 12 patients aged 38 ± 11.6 years, i.e. 12.5% of all Tx (96) done at the same period. In addition to standard immunosuppression all received low dose colchicine (0.6-1.2 mg/day). Main complications were rejection (8), delayed graft function for tubular necrosis (2), lymphocele (2), CMV disease (2) and tuberculosis (1). Additional problems included diarrhea (colchicine, MMF, generalized amyloidosis; 9) and severe neuropathy due to interaction of cyclosporine with colchicine (1). Interaction of colchicine with CNI/MMF (neuropathy,diarrhea) required reduction of immunosuppression in some patients, resulting in higher rejection rate. One patient died of generalized amyloidosis and 1 kidney was lost after reduction of immunosuppression due to tuberculosis. Ten patients have good renal function.
Conclusion: The number of patients with amyloidosis of FMF requiring RRT in Armenia is alarming. Prevention of RA by early diagnosis and early intervention (colchicine) must be intensified. Outcome of HD was poor in contrast to renal Tx. Results after renal Tx were much better than for HD and did not differ greatly from non-FMF pts, apart from drug interaction.
Disclosure of interest: None declared.

A90

PW01-037 – Amyloidosis probability depending on MEFV type

A Simonyan[1*], A Ayvazyan[1], V Vardanyan[1], L Kozlovskaya[2], V Rameev[2]
[1]yerevan State Medical University, Yerevan, Armenia; [2]Moscow Medical Academy after I.M. Sechenov, Moscow, Russian Federation
Pediatric Rheumatology 2013, **11(Suppl 1):**A90

Introduction: Familial Mediterranean fever (FMF) is a hereditary inflammatory disorder. FMF is an autoinflammatory disease caused by mutations in MEFV, a gene which encodes a 781–amino acid protein denoted pyrin. AA-amyloidosis is the main complication of FMF.
Objectives: To access the relationship between MEFV genotype and occurrence of amyloidosis in patients with Familial Mediterranean Fever (FMF).
Methods: 69 FMF patients (37 with amyloidosis, 32 – without amyloidosis) were investigated. All 69 patient underwent molecular-genetic investigation (PCR method), 9 different mutant combinations of MEFV gene were detected: 3 homozygous - M694V/M694V(AA) in 25 patients, M680I/M680I (CC) - in 4, V726A/V726A(BB) in 1, 5 compound heterozygous - M694V/V726A(AB) in 17, M694V/M680I(AC) in 9, V726A/M680I(BC) in 5, V726A/R761H(BD) in 1, M680I/R761H(CD) in 1, and 1 heterozygous - M694V/U(AU) in 6 patients.
Results: 3 of 9 detected MEFV genotypes (V726A/V726A, V726A/R761H и M680I/R761H) were not revealed in FMF patients with amyloidosis, but in the present investigated group these genotypes were sporadic (3 patients), so that it's not possible to decline the probability of amyloidosis development in these genotypes unambiguously. The rest 6 genotypes (M694V/M694V, M694V/U, M694V/M680I, M694V/V726A, V726A/M680I,

M680I/M680I) were found either in patients with amyloidosis or without. Particularly M694V- genotypes were distributed approximately equally among 2 subgroups of patients. M694V/M694V-genotype appeared to be more frequent in patients with amyloidosis – in 17 of 37 patients (45,9%) in comparison with patients without amyloidosis – in 8 of 32 patients (25%). Nevertheless, detected difference proved to be non-significant (χ^2=12,51171, p=0,12981).

Conclusion: Our investigation hadn't revealed significant relationship between carriage of definite FMF genotype and development of amyloidosis. The absence of genetic relationship between FMF and amyloidosis may be explained by the fact that MEFV and SAA genes are located on different chromosomes – 16 and 11 respectively, and the carriage of the mentioned genes has unlinked character.

Disclosure of interest: None declared.

A91

PW01-038 – Genomewide association study of Still's disease

MJ Ombrello[1*], EF Remmers[2], E Zeggini[3], W Thomson[4], DL Kastner[2], P Woo[5], International Childhood Arthritis Genetics Consortium (INCHARGE)
[1]Translational Genetics and Genomics Unit, NIAMS, Bethesda, MD, USA; [2]Inflammatory Disease Section, NHGRI, Bethesda, MD, USA; [3]Applied Statistical Genetics Group, Wellcome Trust Sanger Institute, Hinxton, UK; [4]Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, UK; [5]Center of Paediatric and Adolescent Rheumatology, University College London, London, UK

Pediatric Rheumatology 2013, **11(Suppl 1):**A91

Introduction: Still's disease or systemic juvenile idiopathic arthritis (sJIA) is a severe inflammatory disease of childhood characterized by periods of daily spiking fever, evanescent skin rash, severe arthritis, serositis, lymphoid hyperplasia, and, in up to half of cases, macrophage activation syndrome. Although thought to have a genetic component, the causes of sJIA are unknown.

Objectives: To identify genetic factors that contribute to sJIA susceptibility.

Methods: We generated single nucleotide polymorphism (SNP) genotypes from the genomic DNA of 988 children with sJIA and 514 healthy control subjects. These data were combined with SNP genotypes *in silico* from 7370 additional healthy control subjects. After dividing the dataset into 9 strata by country of origin, we excluded samples and markers that did not meet our strict quality requirements. We performed haplotype phasing with ShapeIT, SNP imputation with IMPUTE2, and association testing with SNPTEST independently in each stratum. The results of association testing were subjected to fixed- and random-effects meta-analyses with GWAMA. A second round of more intensive "deep imputation" was performed in each region with p_{meta}<1E-7. Using the directly genotyped SNP data, we used imputation to deduce classical HLA types in each stratum. Significant associations were further evaluated with multivariate logistic regression using SNPTEST and SNP & Variation Suite 7.

Results: Using the above method, we ultimately tested a panel of over 1.6M SNPs for association with sJIA. Using meta-analysis of SNP association data from 9 strata, we identified 2 sJIA-associated regions that exceeded the stringent threshold for genome wide significance (p_{meta}<5E-8). The strongest association was located in the major histocompatibility complex locus, with one SNP nearest to *HLA-DRB1* (**rs112638393**: p_{meta}=1.6E-10, OR 1.5 [1.3, 1.7]) and a second located nearest to *BTNL2* (**rs115945836**: p_{meta}=2.4E-10, OR 2.8 [2.0, 3.9]). Conditioning on the effect of rs112638393 accounted for the majority of the effect around *HLA-DRB1*, while revealing a significant, independent association signal spanning *BTNL2* and *HLA-DRA*. Additionally, meta-analysis of the imputed HLA type associations from 8 strata revealed a strong association between *HLA-DRB1*1101* and sJIA (p_{meta}=1.2E-8, OR 2.1 [1.6, 2.7]). The second strongest regional association, which also exceeded genome wide significance, was located on Chr 1 nearest to *LOC284661* (**rs16838915:** p_{meta}=5.4E-9, OR 2.0 [1.6, 2.5]). Logistic regression analysis demonstrated no residual association signal in this region after conditioning on rs16838915. In total, our study identified 11 loci that were suggestive of association with sJIA (p<5E-5).

Conclusion: We have performed a genome-wide association study of a large collection of sJIA patients. We have identified 2 sJIA susceptibility loci, *HLA-DRB1* and *LOC284661*, both of which have large effect sizes. The association of *HLA-DRB1*1101* with sJIA suggests that antigen presentation and the adaptive immune system are involved in sJIA, an idea that would be further supported by involvement of either *HLA-DRA* or *BTNL2*. The specific roles of each of these loci in the pathogenesis of sJIA remain to be elucidated.

Disclosure of interest: None declared.

A92

PW01-039 – Long-term efficacy of anakinra in SoJIA patients

A Naselli[*], J Tibaldi, A Accogli, A Buoncompagni, S Viola, S Signa, P Picco, A Ravelli, A Martini, M Gattorno
Pediatria 2, IRCCS, Istituto Giannina Gaslini, Genoa, Italy

Pediatric Rheumatology 2013, **11(Suppl 1):**A92

Introduction: Recombinant IL-1 receptor antagonist (anakinra) is an effective treatment in a subgroup of systemic onset JIA (SoJIA). So far no information is available on the long term follow-up of SoJIA patients treated with anakinra.

Objectives: To analyse the long term follow-up of SoJIA patients treated with Anakinra in a single tertiary referral center.

Methods: Since 2005, 34 SoJIA patients (19 M, 15 F) were treated with anakinra at the staring dose of 1-2 mg/kg/die. Complete response was defined as the absence of systemic and articular manifestations and normal acute phase reactants at follow-up, with anakinra as a monotherapy. Other patients were considered as partial responders (still in anakinra with evidence of disease activity) or non-responders (withdrawn of Anakinra due to inefficacy or severe side effects).

Results: At baseline, the mean age was 8.4 year (range 1-17 years) with mean disease duration of 3.05 years (3 months-10.2 years). All patients had active arthritis (mean number of active joints 12.3, range 1-80), 28/34 had fever, 20/34 had skin rash. Failure of anti-TNF treatment or DMARD was observed in 11/34 and 23/34 patients respectively. Ongoing steroid treatment 33/34 patients (mean prednisone mg 0.87/kg/day, range 0.1- 3). The mean follow-up was 4.02 year (range 1.05 - 6.16). At the last follow-up 13 patients (38%) were complete responders, 5 (14%) partial responders and 16 (48%) non responder. Among complete responders, 4 patients withdrawn anakinra without relapses after a mean of 3 years of treatment, 7 are in remission using anakinra as mono-therapy, 2 patients were switched to anti IL-1 monoclonal antibody with a full response. Despite the good control of their disease 11/13 displayed at least one relapse of their disease during the follow-up with a total of 22 relapses (range 1-4 for patient). In 16 non responders patients subsequent treatments were canakinumab (1 patient), tocilizumab (5 patients), or combined immunosuppressive treatment and/or anti-TNF (10 patients), with variable response. Adverse events and complications: 13 Anakinra-treated patients had skin reactions of variable intensity and duration, in 7 patients hitching was also present without an evident skin rash. Six patients (3 responder and 3 non responders) developed a MAS during follow-up. Two non-responders patients died for acute bacterial meningitis and multi-organ failure after an episode of MAS. As previously observed, responders patients confirmed to have an higher number of active and limited joints at baseline (p = 0.006) and higher WBC and neutrhophils count (p = 0.002). These results were confirmed in the newly enrolled patients. In this latter group responder patients displayed a significantly shorter disease duration in respect to non responder patients (p = 0.03).

Conclusion: In responder SoJIA patients Anakinra confirm to be an effective and safe drug in the long term. The use of Anakinra is still able to dissect two distinct populations of SoJIA patients on the basis of the presence or not of a severe joint involvement with a chronic polyarticular course.

Disclosure of interest: None declared.

A93

PW01-040 – Definition of polymorphism C3435T MDR1 gene in JIA

F Rokhlina[*], G Novik
SPbGPMU, St. Petersburg, Russian Federation

Pediatric Rheumatology 2013, **11(Suppl 1):**A93

Introduction: Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic disease in children.

Brug of choice is methotrexate (MTX) - cytostatic drug from the group of antimetabolites, folic acid antagonists. According to various authors, about 70% of patients receiving therapy with MTX are in remission for the disease.
In 1988, MTX was approved FDA (Food and Drug Administration, USA) for the treatment of rheumatoid arthritis. Currently, MTX is the main drug in the treatment of JIA. Preferred therapeutic recommendations on the number of MTX based on a randomized, double-blind, placebo-controlled study conducted in 1992.
MDR1-gene product - the P-glycoprotein (P-gp), the protein acts as a transmembrane pump, and affects the activity of many drugs. Polymorphism in the gene MDR1, may affect the pharmacokinetics of many drugs, including anticancer drugs.
According to the authors of Jinwei Chen (2011) in adult patients with rheumatoid arthritis (RA) in the Chinese population C3435T MDR1 gene polymorphism may be associated with susceptibility to RA, but may influence the effectiveness of antirheumatic therapy of RA, and CC genotype may be associated with immune rheumatoid arthritis (RRA).
Objectives: The aim of our study is to determine the effect on the efficiency of the MDR1 gene therapy JIA.
Methods: Were examined 103 patients diagnosed with juvenile idiopathic arthritis (according to the EULAR), receiving standard treatment with methotrexate at a dose of 15 mg/m2 over 3 months (intramuscular). All children were defined P-glycoprotein (the product of the gene MDR1), and C3435T polymorphism of the gene MDR1. A genetic study was carried out using polymerase chain reaction followed by restriction analysis to determine the C3435T polymorphism of the gene MDR1. Modeling inflammation in vitro was carried out by treating the blood with recombinant human interleukin 2, followed by determination of the level of P-glycoprotein (CD243-PE) on peripheral blood lymphocytes with monoclonal antibodies by flow cytofluorometry.
Results: Among the 103 children included in the study - 65 (63.11%) females and 38 (36.89%) boys with different forms of juvenile idiopathic arthritis: polyarthritis - 46 children (44.66%), oligoarthritis - 25 children (24, 27%), systemic arthritis - 14 children (13.59%), arthritis entezitassociated - 18 patients (17.48%).
Analysis of changes in the level of P-glycoprotein after IL2 stimulation, depending on the genotype of MDR1 gene showed significant differences. When comparing the patients in these groups, it was found that in the group of children with genotype TT, increase P-IL2 protein after stimulation were significantly lower than in children with genotype CT. In patients with genotype TT average sedimentation rate was significantly higher than that of children in the group CT in the acute phase, and in the waning activity of the inflammatory process in the joints. Similar changes were obtained by comparing the genotypes CC + CT and TT.
Conclusion: Given the data, we can assume that children with the TT genotype MDR1 gene (C3435T) is worse respond to standard treatment of JIA.
Disclosure of interest: None declared.

A94

OR5-001 – Characterization of tonsil infiltration in PFAPA

S Chiesa[1*], R Caorsi[1], F Penco[1], A Bertoni[1], S Borghini[2], A Sementa[3], A Omenetti[1], F Bellora[4], R D'Agostino[5], A Martini[1], M Gattorno[1]
[1]Laboratory of Immunology and Rheumatic Diseases, U.O. Pediatria II, G. Gaslini Institute, Genoa, Italy; [2]Laboratory of Molecular Genetics, G. Gaslini Institute, Genoa, Italy; [3]Anatomic Pathology, G. Gaslini Institute, Genoa, Italy; [4]Medicina Sperimentale, University of Genoa, Genoa, Italy; [5]U.O. Otorinolaringoiatria, G. Gaslini Institute, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A94

Introduction: The syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) is the most common periodic fever disease in young children. The etiology of this disorder is still unknown. Palatine tonsils are sites where innate immunity leads to onset of the adaptive immunity, mediated by B and T lymphocytes. Three families of pathogen sensors mediate the recognition of microbes: Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). The interplay of these receptors ensures the efficient coordination of innate immune responses.
Objectives: We aimed to investigate differences in leukocyte subpopulations and innate receptors gene expression of palatine tonsil cells from patients with PFAPA in order to understand the pathogenesis of this inflammatory condition.
Methods: We have collected tonsil tissue from 2 groups of pediatric patients undergoing tonsillectomy: PFAPA patients (n=20) and patients who had indication of recurrent bacterial tonsillitis (control group, CG) (n=16). We have performed staining of subpopulations on tonsil cells and tissues using, respectively, flow cytometry and immunohistochemistry assays . We have analyzed TLRs, NLRs, and RLRs gene expression profiles by quantitative real-time RT-PCR.
Results: Immunohistochemistry analysis has shown preservation of tonsillar architecture without any specific chronic inflammation with respect to GC. FACS analysis has demonstrated a higher number of naïve and a significantly lower percentage of effector memory $CD4^+$ and $CD8^+$ T cells in PFAPA patients compared to CG. Remarkably, we have observed a considerably recruitment of NK cells in tonsils of PFAPA patients with respect to CG. In particular, we have detected a significant expansion of $CD56^+CD16^-$ and $CD56^+CD16^+$ NK cell subsets when compared to CG. A detailed characterization of NK activating receptors and NK cell functions in PFAPA tonsils is still in progress. Finally, in PFAPA patients we have revealed a significant increase in the gene expression of NALP1 and NALP3 when compared to CG.
Conclusion: These results indicate a possible involvement of NK cells and of innate receptors in pathogenesis of PFAPA supporting the crucial role of the innate immunity. Nonetheless, the high numbers of undifferentiated naïve T cells in PFAPA patients suggest that adaptive immune responses might be implicated in these autoinflammatory disorders. Tonsillectomy seems to be an effective treatment for PFAPA syndrome.
Disclosure of interest: None declared.

A95

OR5-002 – In vitro studies in Schnitzler's syndrome

HD De Koning[1*], J Schalkwijk[2], J Jongekrijg[3], IM van Vlijmen-Willems[1], D Rodijk-Olthuis[1], M Stoffels[3], JW van der Meer[3], PL Zeeuwen[1], A Simon[3]
[1]Dermatology, Nijmegen, the Netherlands; [2]Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [3]Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A95

Introduction: Schnitzler's syndrome (SchS) is an autoinflammatory disorder, characterized by chronic urticaria, fever, gammopathy and bone pain. The pathophysiology is unknown, but the effectiveness of interleukin-1 (IL-1) inhibition provides a clue.
Objectives: Our aim was to study the effect of IL-1β inhibition on inflammatory responses *in vivo* and *ex vivo* during a trial of the long-acting anti-IL-1β antibody canakinumab in SchS.
Methods: Eight patients with SchS received monthly injections with 150mg canakinumab s.c. for six months. Blood was drawn at several time points for measurement of inflammation markers and isolation of peripheral blood mononuclear cells (PBMCs), which were stimulated with lipopolysaccharide (LPS). Skin biopsies of urticae and clinically uninvolved skin were taken for mRNA, histology and keratinocyte cultures. Submerged keratinocytes were stimulated with several cytokines and patient and control serum. All data were compared to results of healthy controls.
Results: IL-1β inhibition was highly effective in SchS. IL-6 protein concentration in lysates of freshly isolated PBMCs correlated with disease activity. Stimulation of PBMCs with 0,1 ng/ml LPS induced more IL-6 and IL-1β production in SchS PBMCs than in controls. In lesional epidermis, mRNA and protein expression levels of several antimicrobial proteins were elevated. In primary human keratinocytes, poly:IC, IL-1β, IL-17 and interferon gamma induced mRNA expression levels of several antimicrobial proteins and cytokines to a similar extent in patient and control cells.
Conclusion: Clinical efficacy of IL-1β inhibition in patients with SchS is associated with *in vivo* and *ex vivo* suppression of inflammation. Our data underscore that IL-1β plays a pivotal role in this disease. Also, we show strong upregulation of antimicrobial proteins in the epidermis of these neutrophilic urticaria, and that these proteins can be induced in primary human keratinocytes by IL-1β.
Disclosure of interest: None declared.

A96

OR6-001 - S100A12 as pro-inflammatory TLR4 ligand

C Kessel[1*], H Wittkowski[1], A Lüken[2], T Weinhage[1], G Varga[1], T Vogl[2], T Wirth[1], D Viemann[3], P Björk[4], M van Zoelen[5], F Gohar[1], G Srikrishna[6], J Roth[2], D Foell[1]
[1]Pediatric Rheumatology, Clinical-Translational Innate Immunity Research, WWU Medical Center, Münster, Germany; [2]Institute of Immunology, WWU Medical Center, Münster, Germany; [3]Department of Pediatric Pulmonology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany; [4]Active Biotech, Lund, Sweden; [5]Department of Experimental Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [6]Sanford-Burnham Medical Research Institute, La Jolla, CA, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A96

Introduction: The granulocyte-specific Ca^{2+}-binding protein S100A12 is overexpressed during autoinflammation as well as other inflammatory conditions in humans and has been ascribed to the group of pro-inflammatory Damage Associated Molecular Pattern molecules (DAMPs). S100A12-levels in human serum reveal excellent correlation with patients' inflammation state, which renders S100A12 a powerful biomarker. In order to operate as DAMP S100A12 requires binding to cellular receptors. The protein was originally found to bind RAGE and was in turn entitled extracellular newly identified RAGE-binding protein (EN-RAGE). RAGE ligation by S100A12 is proposed to trigger a pro-inflammatory cascade in microvascular endothelial cells, macrophages and lymphocytes, culminating in NFκ-B activation. This amplifies the inflammatory response by triggering further RAGE expression and thus drives a feed-forward loop that can potentiate inflammation.
Objectives: RAGE-binding by S100A12 can still be discussed controversial and an alternate receptor for S100A12 on mononuclear cells is suggested. Based on the previous discovery of S100A8/A9 signalling through TLR4 we studied the relevance of this particular pathway for S100A12 as alternative to the originally postulated signaling through RAGE.
Methods: The release of human S100A12 from granulocytes as well as the promotion of inflammation by activation of human monocytes after specific receptor-interaction was investigated by a series of *in vitro* experiments on primary cells and receptor-expressing cell lines.
Results: Upon inflammatory challenge S100A12 expression from human granulocytes is rapidly induced *in vitro* and *in vivo*. The protein is both passively released from necrotic cells and secreted via alternative secretory pathways. A global gene expression analysis of S100A12-activated monocytes revealed that human S100A12 induces strong inflammatory responses. These can be abrogated by specifically blocking toll-like receptor 4 (TLR4) on primary human monocytes as well as TLR4-overexpressing HEK-TCM cells. On the contrary, blocking S100A12 binding to RAGE reveals no such pronounced effect on both monocytes and RAGE-overexpressing cell lines. Importantly, as the observed effects on human monocytes appear to be TLR4-dependent, the S100A12-induced gene expressing pattern differed in part significantly from that induced by the primary TLR4 ligand LPS.
Conclusion: We identified human S100A12 as an endogenous TLR4 ligand that induces a unique pro-inflammatory gene expression signature resulting in monocyte activation. Beyond the well-documented implication of S100A12 as inflammatory biomarker, our data shed new light on the role of S100A12 as powerful amplifier of innate immunity during inflammation.
Disclosure of interest: None declared.

A97

OR6-002 – Thromboembolism in autoinflammatory syndromes

PM Vaitla[*], R Weldon, I Tynan, EM McDermott, E Drewe
Immunology, Nottingham University Hospitals NHS Trust, Nottingham, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A97

Introduction: Venous thromboembolism (VTE) has been associated with FMF but risk has not been studied in other auto inflammatory syndromes. We therefore did a retrospective study of incidence of VTE in a cohort of patients under Nottingham University Hospitals.
Objectives: To identify the incidence and site of VTE in patients with auto inflammatory syndrome managed at our centre from 1995 to 2013. In addition we sought to investigate risk factors for VTE present, including laboratory evidence of inflammation.
Methods: The case records of patients with auto inflammatory syndrome were studied to assess history and site of VTE. Subtype of auto inflammatory disease was noted along with risk factors for VTE and therapy for auto inflammatory disease. In addition it was assessed if auto inflammatory disease had been clinically active at the time of VTE and whether inflammatory markers were elevated in the 6 months prior to VTE. Mean and median values for highest CRP in the 6 months preceding the episodes of VTE were calculated for the affected group.
Results: 45 patients with auto inflammatory disease were included in the study (22 with TRAPS, 2 with HIDS, 4 with FMF, 2 with CAPS, 1 with variant Schnitzler, 1 with NOD2 mutations and 13 undefined). 7 subjects (15.5% of cohort) had a total of 11 episodes of VTE. This comprised 3 with TRAPS, 1 with CINCA, 1 variant Schnitzler's syndrome, 1 had auto inflammatory syndrome with NOD2 mutations and granulomas and one undefined under investigation. VTE included deep vein thrombosis (x 6), pulmonary embolism (x 4) and Budd-Chiari syndrome (x 1). In those with multiple VTE, recurrence of VTE occurred 1 to 2 months after discontinuation of anticoagulation.
Apart from underlying auto inflammatory disease no risk factors for VTE were identified. 3 patients had thrombophilia screen which was negative excepting one patient with low protein C presumed due to liver dysfunction. At the time of VTE, all 7 patients were on prednisolone and in addition 2 patients were on methotrexate, 1 on chlorambucil and 1 on anakinra.
All patients reported recurrent flares or continuous auto inflammatory symptoms prior to VTE. 6 out of 7 patients had raised CRP in the 6 months preceding VTE. CRP values in the 6 months preceding VTE were available for 10 episodes. The mean of the highest CRP in the 6 months preceding each VTE episode was 119.8, median was 124 with values ranging from 4 to 251.
3 patients are deceased. The 4 living patients are on lifelong warfarin. Of note pleuritic chest pain was initially attributed to flare of auto inflammatory disease and responded partially to methyl prednisolone delaying diagnosis of pulmonary emboli.
Conclusion: In summary we report a high incidence of VTE in auto inflammatory disease, particularly in the setting of uncontrolled inflammation. VTE may mimic symptoms of auto inflammatory disease and needs to be considered to prevent diagnostic delay. This cohort includes patients managed prior to availability of biological therapies and now early introduction of biologicals to drive down acute phase response may reduce risk of VTE. We also propose whether VTE prophylaxis e.g. aspirin should be initiated for patients with uncontrolled inflammation despite therapy for auto inflammatory disease.
Disclosure of interest: None declared.

A98

OR6-003 – Prospective evaluation of PFAPA patients

I Bosio[1], A Meini[1], P Cancarini[1], M Berlucchi[2], G Savoldi[3], M Cattalini[1*]
[1]Pediatric Clinic, University of Brescia, Brescia, Italy; [2]Department of Pediatric Otorynolaryngology, Spedali Civili di Brescia, Brescia, Italy; [3]Laboratorio di Genetica Pediatrica, Anatomia Patologica, Spedali Civili Brescia, Brescia, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A98

Introduction: PFAPA (Periodic Fever with Aphtous stomatitis, Pharyngitis and cervical Adenitis) is a periodic syndrome described for the first time in 1987 by Marshal et al. In 1999 the diagnostic criteria were formulated by Thomas.
Objectives: all the patients who received a PFAPA diagnosis at our Centre between 1999 and 2012 were prospectively evaluated.
Methods: Sex, age at onset, age at diagnosis, family history, clinical characteristic of the febrile episodes and associated symptoms, prodromes, therapy, therapy response and age at resolution were collected.
Results: In our cohort (148 males and 120 females) fever began at 26.2 ± 24 months of age. 8% of the patients had an onset after the fifth year of life, but all other Thomas criteria were met. A family history was present in 39.6 % of patients. Mean duration of PFAPA episodes was 4 ± 1.6 days, and a mean interval between episodes 27.9 ± 11 days. Most common symptoms with fever were pharyngitis (95.5%), cervical adenitis (63.8%), stomatous aphtosis (38.4%), abdominal pain (32%). Prodromes, such as irritability,

nausea and headache were present in 10% of patients. All patients received treatment with oral steroids, using a single administration of 1 mg/kg of prednisone or prednisone equivalent, the first day of fever. In all patients steroids were effective and only 13 % of them experienced a free-interval shortening, without the perceived need to stop the steroids for this reason. There was no difference in the studied parameters between the population who experienced a free-interval shortening and the population in which this event was not registered.

In 144 children resolution occurred, in 58 % of children spontaneously and in 42 % after tonsillectomy. Mean disease duration was 40 ± 63 months, medium age at resolution 67.7 ± 66 months. Tonsillectomy was efficacious in 60/62 patients. Mutation analysis for FMF, HIDS, and TRAPS in the latter two patients were negative. The tonsillectomy was done after a mean period of 36 months from disease onset.

At multivariate regression analysis disease resolution was independently associated to age onset (β = 1.011 95 %CI 1.000-1.022, p = 0.05) and to tonsillectomy (β = 0.022 95 %CI 0.005-0.092 p = 0.001).

Conclusion: PFAPA is the most common cause of periodic fever in children, however our study confirms that the 5 year of age at disease onset criterion is too strict. Symptoms other than the ones from the classic description, such as abdominal pain, could have clinical relevance. Prodromes are quite common and useful in differentiate the typical PFAPA attack from other episodes of fever. Oral steroids are, in our opinion, the therapy of choice and the free-interval shortening is not perceived as a clinically relevant issue. It is not possible to predict which patients would present this effect. Tonsillectomy is very effective, but should be reserved to a very selected group of patients and with ad adequate period of follow-up before the surgery. Age at onset seems to inversely correlate with disease duration.

Disclosure of interest: None declared.

A99

OR6-004 – MRP8/14 promote MSU-crystal induced inflammation

D Holzinger[1,2*], N Nippe[1], T Vogl[1], K Marketon[1], V Mysore[1], T Weinhage[2], N Dalbeth[3], B Pool[3], T Merriman[4], D Baeten[5], A Ives[6], N Bagnoud[6], N Busso[6], D Foell[2], S Bas[7], C Gabay[7], J Roth[1]

[1]Institute of Immunology, University Hospital Muenster, Muenster, Germany; [2]Department of Paediatric Rheumatology and Immunology, University Children's Hospital Muenster, Muenster, Germany; [3]Department of Medicine, University of Auckland, Auckland, New Zealand; [4]Biochemistry Department, University of Otago, Otago, New Zealand; [5]Clinical Immunology and Rheumatology, University of Amsterdam, Amsterdam, the Netherlands; [6]Laboratoire de Rhumatologie, University of Lausanne, Lausanne, Switzerland; [7]Division of Rheumatology, University of Geneva, Geneva, Switzerland

Pediatric Rheumatology 2013, **11(Suppl 1):**A99

Introduction: Monosodium urate (MSU) crystal induced interleukin-1 (IL-1β) secretion is a critical pathogenic factor in the development of gout and serves as therapeutic target in these patients. Nevertheless, without co-stimulation by a pro-IL-1ß inducing factor, e.g. lipopolysaccharides (LPS), MSU alone cannot induce IL-1β secretion *in vitro*. The endogenous Toll-like receptor 4 (TLR-4) agonists myeloid related protein (MRP) 8 and MRP14 play a significant role in human inflammatory diseases, reflect disease activity and have been shown to be an important pathogenic factor in murine arthritis models.

Objectives: To analyze the co-stimulatory properties of myeloid related protein-8 (MRP8) and MRP14 (endogenous Toll-like receptor 4 (TLR-4) agonists) in MSU crystal induced IL-1β secretion and their relevance in gout.

Methods: The co-stimulatory effects of MRP8 and MRP14 on MSU-induced IL-1ß secretion were tested *in vitro* on primary human monocytes and macrophages as on murine macrophages and were confirmed by ELISA and Western Blot. Furthermore MSU induced release of MRPs from human neutrophils and monocytes were measured by ELISA. Impact of MRP was tested *in vivo* in a crystal-induced peritonitis model.

MRP8 and MRP14 was measured in paired serum and synovial fluid samples (n=15) and was detected in synovial tissue (n=10) of gout patients. Expression of MRPs was further correlated with disease activity in the serum of active and convalescent gout patients (each n=40).

Results: MRP8 and MRP14 are released by MSU activated human neutrophils and monocytes and induce pro-IL-1ß production in monocytes. MSU induced IL-1β secretion is significantly increased by MRP co-stimulation in human and murine cells. Accordingly, targeted deletion of MRP14 in mice led to a significantly reduced response in MSU-induced inflammation *in vivo*. MRPs can be found in the synovia and synovial fluid of active gout patients and levels are significantly elevated compared to osteoarthritis patients. Moreover, the expression level of MRPs in serum of gout patients correlates positively with disease activity (mean ± 95% CI, active: 2020±420 ng/ml, convalescent: 920±70 ng/ml, controls: 430±100 ng/ml).

Conclusion: MRP8 and MRP14 are endogenous enhancers of MSU crystal induced IL-1 β secretion by induction of pro-IL-1ß via TLR-4. The proteins can be found at the site of inflammation in active gout patients and their serum levels reflect disease activity in these patients. These findings indicate a new role of endogenous TLR-4 ligands in the pathogenesis of gout.

Disclosure of interest: None declared.

A100

OR6-005 – Cystine crystals activate inflammasomes

G Prencipe[1*], I Caiello[1], S Petrini[1], L Bracci Laudiero[1], F Emma[2], F De Benedetti[3]

[1]Laboratory of Rheumatology, Bambino Gesù Children's Hospital, Roma, Italy; [2]Department of Nephrology and Urology, Bambino Gesù Children's Hospital, Roma, Italy; [3]Operative Unit of Rheumatology, Bambino Gesù Children's Hospital, Roma, Italy

Pediatric Rheumatology 2013, **11(Suppl 1):**A100

Introduction: Nephropathic cystinosis is a rare autosomal recessive disorder caused by a mutation in the CTNS gene, which encodes for cystinosin. It is characterized by the lysosomal accumulation of cystine, which leads to the formation of cystine crystals within various organs, including kidneys, brain, cornea, intestine and bone marrow. The exact role of intralysosomal cystine crystals accumulation in the pathogenesis of clinical features of cystinosis is still unclear, although it is well known that cystine levels are directly proportional to disease severity.

Objectives: In this study, we investigate whether cystine crystals are able to elicit inflammasome activation.

Methods: Primary human peripheral blood mononuclear cells (PBMCs) were cultured in vitro, pre-incubated with LPS, stimulated with L-cystine crystals in presence or absence of different inhibitors and the IL-1beta (IL-1b) released in the medium was measured by ELISA.

Results: LPS-primed PBMCs stimulated with L-cystine crystals secreted IL-1b in a dose-dependent manner. Similarly to other NLRP3-activating particles, cystine crystal-induced IL-1b secretion was caspase-1-dependent. Indeed, when PBMCs were pre-icubated with the specific CASP-1 inhibitor (Z-YVAD-fmk), a dramatic decrease in IL-1β production was observed, suggesting the involvement of an inflammasome-mediated pathway. By confocal microscopy, we observed that exogenous L-cystine crystals were internalized by monocytic/macrophagic adherent cells. Inhibition of actin polymerization with cytochalasin D effectively blocked cystine crystal-induced IL-1β secretion, showing that phagocytosis is necessary for this effect.

Conclusion: Taken together, these data demonstrate that cystine crystals represent a new endogenous inflammasome activating danger signal, suggesting a new role for cystine crystals in the pathogenesis of nephropathic cystinosis.

Disclosure of interest: None declared.

A101

OR6-006 – IL36RN alleles in skin auto-inflammation

F Capon[1*], N Setta-Kaffetzi[1], D Berki[1], S Mahil[1], A Navarini[1], VM Patel[1], C Siew-Eng[2], AD Burden[3], C Griffiths[4], M Seyger[5], R Trembath[6], C Smith[1], J Barker[1]

[1]King's College London, London, UK; [2]Hospital Sultanah Aminah, Johor Bahru, Malaysia; [3]University of Glasgow, Glasgow, UK; [4]University of Manchester, Manchester, UK; [5]Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [6]Queen Mary University of London, London, UK

Pediatric Rheumatology 2013, **11(Suppl 1):**A101

Introduction: Our group identified recessive mutations of the *IL36RN* gene in patients affected by Generalised Pustular Psoriasis (GPP), a severe skin disorder characterised by acute episodes of pustulation, fever and systemic upset.

Objectives: Here, we sought to characterise the phenotypic spectrum associated with *IL36RN* alleles. We specifically investigated the possibility that *IL36RN* defects may also contribute to chronic forms of pustular psoriasis and to common plaque psoriasis (also known as psoriasis vulgaris or PV).
Methods: We screened a total of 598 patients affected by GPP (n=84), or by localised forms of pustular psoriasis (9 cases of Acrodermatitis Continua of Hallopeau and 139 cases of Palmoplantar Pustulosis) and PV (n=366).
Results: We found *IL36RN* recessive mutations in patients with GPP (7/84) and localised pustular psoriasis (2/9 cases of Acrodermatitis Continua of Hallopeau and 3/139 cases of plamar-plantar pustulosis), but not among PV cases. Of note, we also identified several affected individuals who carried a single heterozygous mutation. In fact, we uncovered a significant enrichment of heterozygous *IL36RN* alleles among patients with pustular psoriasis (frequency in cases 1.4% vs. 0.3% in controls, P=0.004), but not among subjects with PV (0.4% cases vs. 0.3% controls, P=0.77).
Conclusion: We have demonstrated a significant overlap in the genetic basis of acute generalised and chronic localised forms of pustular psoriasis. The recurrence of similar mutations in both disease groups and the observation of affected individual carrying a single recessive allele suggest that other genes may modify the phenotypic expression of *IL36RN* variants. Our findings argue against the notion that *IL36RN* alleles may contribute to PV. In the light of recent data suggesting a pathogenic role of IL-36 in a mouse model of the disease, our results emphasize the importance of genetic studies in the molecular dissection human auto-inflammatory diseases.
Disclosure of interest: None declared.

A102

OR7-001 – By chip pyrin binds the IRF2 promoter

G Wood[1*], Y Kanno[2], J Balow Jr[1], H-W Sun[3], I Aksentijevich[1], DL Kastner[1]
[1]IDS/MGB, NHGRI, Bethesda, MD, USA; [2]LCBS/MIIB, Bethesda, MD, USA; [3]Biodata Mining and Discovery Section/OST, NIAMS, Bethesda, MD, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A102

Introduction: The gene causing familial Mediterranean fever (FMF), *MEFV*, encodes a protein, pyrin, which is expressed at high levels in granulocytes, monocytes, dendritic cells and in some human myeloid leukemia cell lines, such as THP.1. Studies of pyrin localization show a cell-type dependency. In transfection experiments full-length pyrin is cytoplasmic and associates with the cytoskeleton. However, native pyrin is predominantly nuclear in granulocytes, dendritic cells, and synovial fibroblasts, but it is cytoplasmic in monocytes. Recent studies have implicated pyrin in the regulation of IL-1b and NF_KB activation.
Objectives: To provide additional molecular insight into pyrin function.
Methods: RNA interference (RNAi) technique coupled with Affymetrix cDNA microarray analysis was employed to compare gene expression profiles between the human myeloid leukemia cell line, THP.1, expressing endogenous pyrin (scrambled control, SC) and cells in which the gene had been knocked down (si*MEFV*). Western blot and qRT-PCR analysis was used for validation. Promoter analysis was used to investigate the possibility of a common regulator among the subset of genes identified. Chromatin immuno-precipitation (CHiP) followed by quantitative PCR (qPCR) was used to validate binding.
Results: We identified over 300 genes differentially expressed in si*MEFV* treated cells compared to (SC) with 1.4 fold difference and $p < 0.05$. Based on novelty and gene function, 10 down-regulated genes (*CD36*, *LY96*, *S100A8*, *CCR1*, *CD53*, *TIRAP*, *DEDD*, *SGK*, *MyD88*, *CD14*) were identified for further study. Using total RNA and protein from independent siRNA experiments, 7 of 10 genes showed a comparable mRNA and protein alteration consistent with microarray analysis. To investigate if the genes were being regulated by a common transcription factor, promoter analysis was used. We found that these genes showed enrichment of a binding site for interferon regulatory factors (IRFs, p=0.03). Additional screening of the 9 family members identified *IRF2* as the most significantly changed IRF transcription factor with a fold change of -3.4 and a p value of 0.008. Consistent with the computational analysis, si*MEFV* treated cells showed diminution in *IRF2* mRNA and protein compared to SC. Our previous studies identified *MEFV* as an interferon gamma (IFNγ) immediate early gene after IFNγ stimulation in monocytes. Thus, we decided to examine the possibility that pyrin could directly regulate *IRF2*. Using CHiP-qPCR to test this hypothesis, we demonstrated binding of pyrin within the promoter of *IRF2*.
Conclusion: Our findings suggest that in THP1 cells pyrin might regulate expression of innate immune genes by binding to the promoter of the transcription factor *IRF2*. Further research is needed to examine the possibility that pyrin may bind to other DNA using CHiP coupled with next generation sequencing.
Disclosure of interest: None declared.

A103

OR7-002 – Pyrin 577 mutations in dominant autoinflammation

M Stoffels[1,2,3*], A Szperl[4], A Simon[1,2,3], MG Netea[1,2,3], TS Plantinga[1,2], M van Deuren[1,2], S Kamphuis[5], H Lachmann[6], E Cuppen[7], WP Kloosterman[7], J Frenkel[8], CC van Diemen[4], C Wijmenga[4], M van Gijn[7], JW van der Meer[1,2,3]
[1]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [2]Nijmegen Centre for Infection, Inflammation and Immunity (N4i), Nijmegen, the Netherlands; [3]Nijmegen Centre for Molecular Life Sciences, Nijmegen, the Netherlands; [4]Department of Genetics, UMC Groningen, Groningen, the Netherlands; [5]Sophia Children's Hospital, Erasmus UMC, Rotterdam, the Netherlands; [6]Division of Medicine, UK National Amyloidosis Centre, UCLMS, London, UK; [7]Department of Medical Genetics, UMC Utrecht, Utrecht, the Netherlands; [8]Division of Pediatrics, UMC Utrecht, Utrecht, the Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A103

Introduction: Autoinflammatory disorders are disorders of the innate immune system. Standard genetic testing provided no correct diagnosis in a female patient from a non-consanguineous family of British descent with a colchicine-responsive autosomal dominant periodic fever syndrome.
Objectives: We aimed to unravel the genetic cause of the symptoms in this family.
Methods: Whole exome sequencing was used to screen for novel sequence variants, which were validated by direct Sanger sequencing. Ex-vivo stimulations with peripheral blood mononuclear cells were done to study the functional consequences of the mutation. mRNA and cytokine levels were measured by q-PCR and ELISA, respectively.
Results: Whole exome sequencing revealed a novel missense sequence variant, not seen in around 6800 controls, mapping to exon 8 of the *MEFV* gene (c.1730C>A; p.T577N), co-segregating perfectly with disease in this family. Other mutations at the same amino acid (c.1730C>G; p.T577S; c.1729A>T; p.T577S) were found in a family of Turkish descent, with autosomal dominant inheritance of FMF-like phenotype, and a Dutch patient, respectively. Moreover, a mutation (c.1729A>G; p.T577A) was detected in 2 Dutch siblings, suffering from episodes of inflammation of varying severity not resembling FMF. PBMCs from one patient of the index family revealed increased basal IL-1β mRNA levels and cytokine responses after LPS stimulation. Responses normalized under colchicine treatment.
Conclusion: Heterozygous mutations at amino acid position 577 of pyrin can induce an autosomal dominant autoinflammatory syndrome. This suggests that T577, located in front of the C-terminal B30.2/SPRY domain, is crucial for pyrin function.
Disclosure of interest: M. Stoffels: None declared, A. Szperl: None declared, A. Simon Consultant for: Novartis and Swedish Orphan Biovitrum, M. Netea: None declared, T. Plantinga: None declared, M. van Deuren: None declared, S. Kamphuis: None declared, H. Lachmann: None declared, E. Cuppen: None declared, W. Kloosterman: None declared, J. Frenkel Consultant for: Novartis and Swedish Orphan Biovitrum, C. van Diemen: None declared, C. Wijmenga: None declared, M. van Gijn: None declared, J. van der Meer Consultant for: Novartis and Swedish Orphan Biovitrum.

A104

OR7-003 – MEFV genotype, IL1B and role of NLRP3 in FMF

A Omenetti[1,2*], S Carta[3], L Delfino[3], A Martini[1,2], M Gattorno[1], A Rubartelli[3]
[1]UO Pediatria II, G Gaslini IRCCS, Genoa, Italy; [2]University of Genoa, Genoa, Italy; [3]UO Cell Biology, IRCCS AOU San Martino - IST, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A104

Introduction: Familial Mediterranean fever (FMF) is the most common of the hereditary autoinflammatory disorders. FMF is caused by mutations of *MEFV* gene which encodes for pyrin. It has been recently reported that frequency of FMF-like symptoms decreases from patients carrying two high penetrance mutations towards patients with a single low penetrance

mutation. The effectiveness of interleukin (IL)-1b blockers has suggested that IL-1b may play a role in the pathophysiology of the disease. However, evidence of dysregulated IL-1b secretion in FMF patients is so far missing. Moreover, the role of NLRP3 has never been directly examined in FMF patients.
Objectives: To define in patients affected by Familial Mediterranean Fever (FMF), whether or not interleukin (IL)-1β secretion (1) is enhanced, (2) correlates with the type of MEFV mutation and (3) is mediated by NLRP3.
Methods: Freshly isolated monocytes from 20 FMF patients (12 homozygous and 8 heterozygous), 14 MEFV healthy carriers (HC) and 30 healthy donors (HD), unstimulated or after LPS-induced activation, were analyzed for redox state (reactive oxygen species (ROS) production and antioxidant responses), and for IL-1β and IL-1Receptor antagonist (IL-1Ra) secretion. NLRP3 down-modulation was induced by NLRP3 *in vitro* silencing.
Results: LPS-stimulated monocytes from FMF patients displayed enhanced IL-1β secretion which correlated with the number and penetrance of MEFV mutations. Silencing of NLRP3 consistently inhibited IL-1β secretion. As in other autoinflammatory diseases, MEFV mutated monocytes produced more ROS than genetically negative controls. However, contrary to CAPS, they were featured by a conserved and sustained antioxidant response. Consistent with this finding, MEFV mutated monocytes did not exhibit the functional indicators of oxidative stress observed in CAPS, including accelerated IL-1β secretion and deficient IL-1Ra production.
Conclusion: MEFV mutated monocytes display enhanced IL-1β secretion which correlates with the number of high-penetrance mutations and level of endogenous ROS. Unlike NLRP3 mutated cells, monocytes carrying MEFV mutations withstand oxidative stress and preserve IL-1Ra production, thereby limiting inflammation. Finally, in contrast to what found in the animal model, the increased secretion of IL-1β by LPS-stimulated FMF monocytes is NLRP3-dependent.
Disclosure of interest: None declared.

A105

OR7-004 – Validation of AIDAI score

M Piram[1*], I Koné-Paut[1], M Gattorno[2], N Ruperto[2], the AIDAI Validation Study Group
[1]Pediatrics and Pediatric Rheumatology, CHU de Bicêtre, University of Paris Sud, Le Kremlin Bicêtre, France; [2]Pediatria II, Reumatologia, Istituto G Gaslini, PRINTO Coordinating Centre, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A105

Introduction: With the increasing potential for targeted therapies in autoinflammatory diseases, there is the need for validated and standardized assessment tools which can be used to evaluate the level of disease activity and response to therapy.An international collaboration, initiated by Assistance Publique-Hôpitaux de Paris (APHP) in association with the Paediatric Rheumatology International Trials Organization (PRINTO at www.printo.it) and supported by the EUROFEVER and EUROTRAPS networks, has previously designed the content and the preliminary scoring of an Auto-Inflammatory Disease Activity Index (AIDAI)
Objectives: To validate the AIDAI score in the four major hereditary recurrent fever syndromes (HRFs): familial Mediterranean fever (FMF), mevalonate kinase deficiency (MKD), tumor necrosis factor receptor-associated periodic syndrome (TRAPS), and cryopyrin-associated periodic syndromes (CAPS).
Methods: In 2010, an international collaboration established the content of a disease activity tool for HRFs. Patients completed a one-month prospective diary with 12 yes/no (dichotomous) items prior to a clinical appointment during which their physician assessed their disease activity by a questionnaire. Eight international experts in auto-inflammatory diseases evaluated patient's disease activity by a blinded web-evaluation and a nominal group technique consensus conference with their consensus judgment considered as gold standard. Sensitivity/specificity/accuracy measures and the ability of the score to discriminate active versus inactive patients via the best cut-off score were calculated by a receiver operating characteristic (ROC) analysis.
Results: Consensus was achieved for 98/106 (92%) cases (39 FMF, 35 CAPS, 14 TRAPS and 10 MKD) with 26 patients declared as having inactive disease and 72 active disease. The median total AIDAI score was 14 (range = 0-175). An AIDAI cut-off score ≥ 9 discriminated active versus inactive patients, with sensitivity/specificity/accuracy of 89%/92%/90% respectively and an area under the curve of 98% (95%CI=96%>100%).
Conclusion: The AIDAI score is a valid and simple tool for the assessment of disease activity in FMF/MKD/ TRAPS/CAPS This tool is easy to use in clinical practice and has the potential to be used as a standard efficacy measure in future clinical trials.
Disclosure of interest: None declared.

A106

OR7-005 – Canakinumab in childhood colchicine resistant FMF

PJ Hashkes[1*], Y Butbul Aviel[2], S Lubin[3], L Tseng[4], E Ben Dayan[3], T Rachmilewitz[3], R Brik[2]
[1]Shaare Zedek Medical Center, Jerusalem, Israel; [2]Meyer Children's Hospital, Rambam Medical Center, Haifa, Israel; [3]Novartis Pharma Services AG, Petach Tikva, Israel; [4]Novartis Pharmaceuticals, East Hanover, NJ, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A106

Introduction: Familial Mediterranean Fever (FMF) is the most common hereditary autoinflammatory syndrome affecting >10,000 people in Israel. FMF is caused by mutations in the MEFV gene, which encodes for the pyrin protein that is part of the inflammation complex that activates IL-1β. Evidence from case reports/series and one controlled study supports IL-1 blockage as a potential treatment for FMF. Canakinumab (CAN) is a selective fully human monoclonal anti-IL-1β antibody.
Objectives: This study served as a proof of concept to evaluate the role of CAN in the treatment of pediatric colchicine resistant (CR)-FMF.
Methods: This was a 2-center open-label, single-arm study. The population consisted of CR FMF patients (pts) 4-20 years of age, with a history of at least 3 documented FMF attacks in the 3 months prior to enrollment.
Pts entered a 30-day run-in period (RI) during which FMF attacks were documented in a diary. Pts who experienced an investigator-confirmed FMF attack during RI were eligible to enter the treatment phase and receive a SC injection of CAN 2 mg/kg (max 150 mg) every 4 weeks for three times with the 1st dose given during an attack. The dose was doubled to 4 mg/kg (max 300 mg) if an attack occurred between Day 1 and Day 29 visits. Following the end of the treatment period, pts were followed until Day 144 or until an attack occurred, whichever occurred first. Primary outcome was the proportion of pts with ≥50% reduction in FMF attack rate during the treatment vs. pretreatment period.
Results: Fifteen Israeli pts (9 males, 6 females) with CR FMF entered the RI period and 7 (median age 9.5 yrs.; 6.8-14.9 yrs.), advanced to the treatment phase. In total, 6/7 (86%) pts had a ≥50% reduction in their FMF attack rate during the treatment period vs. the pretreatment period. The median attack rate was reduced by 89% from 2.7 per 28 days prior to CAN to 0.3 per 28 days during treatment; 2 pts had their CAN dose up titrated. Elevated median baseline CRP and SAA normalized by Day 8, ESR by Day 28 and all remained normal for remainder of trial. There was no evidence of neutralizing antibody formation. In all, 11 adverse events (AEs) in 4 pts were reported after the first CAN dose; all were mild except for 2 moderate AEs (Strep infection, laceration) assessed as unrelated to study treatment by the study investigator. No AEs led to medication discontinuation.
Conclusion: In this study of pediatric pts with colchicine resistant FMF, canakinumab every 4 weeks substantially reduced the FMF attack rate, consistent with similar findings in adults. AEs were manageable. A larger study is needed to better evaluate the benefit of canakinumab in FMF.
Disclosure of interest: P. Hashkes Grant/Research Support from: Novartis, Consultant for: Novartis, Speaker Bureau of: Novartis, Y. Butbul Aviel: None declared, S. Lubin Employee of: Novartis, L. Tseng Employee of: Novartis, E. Ben Dayan Employee of: Novartis, T. Rachmilewitz Employee of: Novartis, R. Brik Grant / Research Support from: Novartis.

A107

OR7-006 – Autophagy as a player in inflammation in TRAPS

T Bachetti[1*], S Chiesa[2], P Castagnola[3], D Bani[4], E Di Zanni[1], A Omenetti[2], A D'Osualdo[5], A Fraldi[6], A Ballabio[6,7,8,9], R Ravazzolo[1,10], A Martini[11], M Gattorno[11], I Ceccherini[1]
[1]U.O.C. Genetica Medica, Genoa, Italy; [2]Lab Rheumatology, Istituto Giannina Gaslini, Genoa, Italy; [3]San Martino, Genoa, Italy; [4]4Dept. Anatomy, Histology & Forensic Medicine, Università di Firenze, Florence, Italy; [5]Sanford-Burnham

Medical Research Institute, La Jolla, CA, USA; [6]TIGEM, Naples, Italy; [7]Federico II University, Naples, Italy; [8]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [9]Dan Duncan Neurological Research Institute, Texas Children Hospital, Houston, TX, USA; [10]Pediatrics II, University of Genoa, Italy; [11]Pediatrics II, Istituto Giannina Gaslini, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A107

Introduction: Tumor Necrosis Factor Receptor (TNFR) Associated Periodic Syndrome (TRAPS) is a dominant autoinflammatory disorder caused by heterozygous mutations in *TNFRSF1A*, the gene encoding the TNFalpha receptor 1 (TNFR1). *TNFRSF1A* mutations induce aberrant localization and accumulation in aggregates of the mutant TNFR1 proteins, elevated levels of reactive oxygen species (ROS) and excessive inflammatory response. In accordance to the emerging role of autophagy in inflammatory response, we have recently demonstrated that mutant TNFR1 accumulation is due to a defective autophagy function, the main cellular mechanism involved in the elimination of cellular inclusions containing mutant proteins
Objectives: Investigation of the role of autophagy in TRAPS and search for drugs able to counteract mutant TNFR1mutant accumulation by autophagy induction
Methods: To search a link between *TNFRSF1A* mutations and inflammation in TRAPS, by means of both *in vitro* and *ex vivo* systems, represented by HEK293T cells transfected with expression constructs for WT and mutant TNFR1 proteins and by monocytes, derived by TRAPS patients, respectively, we have investigated the cellular response to mutant TNFR1 proteins in terms of autophagy efficiency, NF-kB activity and mutant TNFR1 localization after drugs treatments
Results: We have found that autophagy is the main mechanism involved in mutant TNFR1 elimination and that it is impaired in the presence of misfolded proteins, thus likely accounting for their accumulation. This compellingly accounts for TRAPS associated induction of NF-kB activity, as well as excessive IL-1b secretion and chronic inflammation.
We also show that autophagy inhibition due to TNFR1 mutant proteins can be reverted, as demonstrated by the effects of the antibiotic geldanamycin found to rescue membrane localization of mutant TNFR1 proteins, to reduce their aggregation and to counteract the enhanced inflammation by decreasing IL-1b secretion
Conclusion: Overall, these observations provide a rationale to the apparent paradox that so far the most effective therapy in TRAPS is represented by inhibition of the cascade signaling induced by IL-1b rather than by the use of drugs counteracting the TNFR1-mediated pathway; therefore, we propose autophagy as a novel therapeutic target for TRAPS and other inflammatory diseases
Disclosure of interest: None declared.

A108

P02-001 – A novel TNFRSF1A mutation in periodic fever

E Erken*, F Yıldız, D Arslan Taş, S Dinkci
Rheumatology-Immunology, Çukurova University Faculty of Medicine, Adana, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A108

Introduction: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory disease characterized by periodic fever, accompanying with attacks of abdominal pain, arthralgia, myalgia, erythematous rashes, periorbital edema and conjunctivitis. Mutations in the extracellular domain of the 55-kD tumor necrosis factor receptor (TNFRSF1A) has been shown to be responsible for the TRAPS syndrome.
Case Report: A sixty two years old Turkish female presented with fever, periorbital edema, erythematous skin rash on the face, neck and arms at age 53 on her first admission. She had history of arthritic attacks on wrists, fingers, knees, elbows lasting 10-15 days, once or twice a year, which began at the age of twelve. Since the age of 50 she has experienced febrile attacks accompanying with abdominal pain, lasting 3-4 days in every 3-4 months. After the age of 53 she had attacks of pruritic erythematous rash on the neck, arms, legs and face, and bilateral periorbital edema as well lasting 10-14 days, once or twice a year. She had no family history of periodic fever. At age 55, she was diagnosed as primary Sjögren's syndrome, with the findings of parotitis, dry eyes, positive Schirmer's test and serum ANA and Anti-SSA positivity. Methyl prednisolon (MP) 8 mg/day and hydroxy chloroquine 400 mg/day was started. One year later, at age 56, her erythematous, pruritic skin lesions repeated with periorbital edema. MP was given 32 mg/day. ESR and CRP levels were elevated during attacks (eg. ESR:52 mm/h, CRP:17.4 g/L). Her skin lesions disappeared after commencement of MP. In attack free period, ESR and CRP returned to normal levels (eg. ESR:11 mm/h, CRP<5 g/L). MEFV mutation analysis of Exons 2 and 10 were negative by sequencing. For her periodic fever symptoms such as 39ºC fever accompanying with abdominal pain and skin lesions, TNFRS1 mutations were analyzed. Exon 2,3,4,5,6,7 and intron 2-3, 4-5 and 6-7 mutations were analyzed by polymerase chain reaction/sequence based typing technique. A novel mutation on exon 7 (S168C C>G, p.Ser197Cys) was identified.
Discussion: This patient represents a novel mutation of TNFRSF1A in a Turkish patient with signs and symptoms of TRAPS syndrome, accompanying with primary Sjögren's syndrome. As reported in Japanese cases previously, this patient with a novel mutation, has milder disease and her attacks of fever and rash respond well to glucocorticoid therapy. Coexistence of Sjögren's syndrome may have masked the clinical manifestations of TRAPS in this patient. Without any family history, in this particular patient de novo TNFRSF1A mutation is possible.
Disclosure of interest: None declared.
References

1. Aksentijevich I, Galon J, Soares M, Mansfield E, Hull K, Oh HH, *et al*: **The tumor-necrosis-factor receptor-associated periodic syndrome: new mutations in TNFRSF1A, ancestral origins, genotype-phenotype studies, and evidence for further genetic heterogeneity of periodic fevers.** *Am J Hum Genet* 200, **69(2)**:301-142.
2. Stojanov S, McDermott MF: **The tumour necrosis factor receptor-associated periodic syndrome: current concepts.** *Expert Rev Mol Med* 2005, **7(22)**:1-18.
3. Washio M, Nakano T, Kawaguchi Y, Takagi K, Kiyohara C, Tsukamoto H, *et al*: **Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) in Japan: a review of the literature.** *Mod Rheumatol* 2012, [Epub ahead of print].

A109

P02-002 - IL36RN mutations in patients with DITRA

JI Arostegui[1*], MA Vicente-Villa[2], A Chaves[3], E Gonzalez-Roca[1], E Ruiz-Ortiz[1], J Rius[1], S Plaza[1], MA Gonzalez-Enseñat[2], J Yague[1]
[1]Immunology, Hospital Clinic, Barcelona, Spain; [2]Dermatology, Hospital Sant Joan de Deu, Esplugues, Spain; [3]Dermatology, Hospital Infanta Cristina, Badajoz, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A109

Introduction: Loss-of-function mutations in the *IL36RN* gene define a novel recessively inherited autoinflammatory syndrome named deficiency of IL-36 receptor antagonist (DITRA). This genetically determined deficiency was first described in a subgroup of patients with generalized pustular psoriasis. It is a life-threatening condition characterized by recurrent episodes of severe skin inflammation, with pustule development, associated with fever, malaise, extracutaneous involvement, neutrophilia and a marked acute phase response.
Case report: Methods: The patients' data as well as the outcome of the administered treatments were collected from charts reviews. *IL36RN* analysis was performed by means of Sanger-based sequencing.
Results: We describe two unrelated families with patients diagnosed as suffering from generalized pustular psoriasis. The family 1 is a large consanguineous Algerian family with several affected members living in Algeria and in Spain. The propositus is a 13 year-old child who had suffered from two episodes of severe skin inflammation, with disseminated pustular development and systemic features that required hospital admission. Once DITRA was described in 2011, this diagnosis was suggested for this patient. *IL36RN* mutational analyses revealed a homozygous T-to-C transition in the exon 3 (at c.80 position), which provokes the leucine-to-proline variant at residue 27 (p.L27P) of the protein. This missense variant has been previously identified as a true disease-causing mutation in other Maghrebian (Tunisian) families with DITRA.
The family 2 is an apparent non-consanguineous Spanish family with only one affected individual. The patient is a 15 years-old girl who suffered since 6 months of age from recurrent and severe episodes of generalized pustular psoriasis that required recurrent hospital admissions. She has been treated with different drugs, including methotrexate, acitretin, cyclosporin,

phototherapy, etanercept, infliximab, adalimumab and ustekinumab, with variable and limited efficacy. As a DITRA diagnosis was suggested, *IL36RN* analysis was performed. This study revealed an apparent homozygous 7 bp deletion in the exon 5 (c.420_426del), which should provoke a frameshift of the normal open reading frame. Genetic studies are currently ongoing to elucidate the intrafamilial mutational segregation pattern.

Discussion: We describe two novel families affected by the novel autoinflammatory disease called DITRA. The disease started in these patients during childhood as severe episodes of generalized pustular skin rash and systemic features that required hospital admissions. We identified a novel *IL36RN* mutation in the Spanish family, and the already known missense p.L27P mutation in the Algerian family. This insight probably expands the founder effect of this *IL36RN* mutation to other Maghrebian populations than Tunisian people.

Disclosure of interest: None declared.

References

1. Marrakchi S, *et al*: *N Engl J Med* 2011, **365**:620-8.
2. Onoufriadis A, *et al*: *Am J Hum Genet* 2011, **89**:432-7.
3. Sugiura K, *et al*: *Br J Dermatol* 2012, **67**:699-701.

A110

P02-003 - HIDS in a consanguineous family form Saudi Arabia?

Q Zhou[1*], R Sleiman[2], DL Kastner[1], I Aksentijevich[1]
[1]Inflammatory Disease Section, National Human Genome Research Institute (NHGRI/NIH), Bethesda, MD, USA; [2]Fakeeh Hospital, Jeddah, Saudi Arabia
Pediatric Rheumatology 2013, **11(Suppl 1):**A110

Introduction: The daughter of a first-cousin marriage from Saudi Arabia died at age 7y/o of an unexplained periodic fever illness. At two years of age, the proband presented with recurrent fever attacks associated with febrile seizures, severe anemia, septic arthritis, diarrhea, and severe vomiting causing multiple ICU submissions. Considering her ancestry, she was thought to have an FMF-like disease and was subsequently treated with colchicine with partial response.

Objectives: To identify a disease-causing gene in this family utilizing exome sequencing. Of concern in this family is that the younger siblings have yet to develop the disease.

Methods: We performed exome sequencing in the unaffected parents, the proband, and two younger siblings. Targeted exon enrichment was performed on 3 μg of DNA extracted from peripheral blood using the SureSelect Human All Exon 50 Mb Kit (~24000 genes, Agilent Technologies).

Results: We focused our analysis on missense, nonsense, and splice site variants and coding indels, resulting in 11981 variants on average per exome. After excluding common variants (>2%) the analysis yielded a mean of 1415 variants per individual. Finally, 97.5% of these rare variants were eliminated under an autosomal recessive model for the consanguineous family, leaving 38 candidate variants in total. Based on the protein function and the PolyPhen-2 prediction on protein function we selected 14 potentially pathogenic mutations and we confirmed them by Sanger sequencing. Under the assumption that younger siblings are unaffected, the single genotype that stood out was that the proband was homozygous for the V377I *MVK* mutation, while both siblings and the unaffected parents were heterozygous carriers. There were no other mutations in known PF genes identified in this family. The V377I mutation is the most common HIDS-associated mutation and it is considered mild with reduced penetrance. The patient in our study, however, presented with very severe disease but not inconsistent with HIDS. The complexity of her symptoms suggests a role for other modifying alleles.V377I is known as the Dutch-mutation with estimated carrier frequency 1:65 in the Netherlands, however most HIDS patients are compound heterozygous for V377I and another *MVK* mutation. There are no data available on the frequency of V377I in Arab and other Middle Eastern populations. There are very few sporadic reports of HIDS in non-Caucasian populations and typically many patients are followed for years with the diagnosis of familial Mediterranean fever.

Conclusion: This result should raise awareness for considering HIDS and other uncommon periodic fevers in patients of Middle Eastern ancestry. Alternatively, we may re-analyze the data in the event that one or both of the younger siblings become affected.

Disclosure of interest: None declared.

A111

P02-004 - AID in a registry of children in North America

JS Hausmann[1*], CM Biggs[1], D Goldsmith[2], F Dedeoglu[1], CARRAnet Investigators
[1]Program in Rheumatology, Division of Immunology, Boston Children's Hospital, Boston, MA, USA; [2]Section of Rheumatology, St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A111

Introduction: Autoinflammatory diseases (AIDs) are characterized by recurrent episodes of systemic and organ-specific inflammation. If unrecognized and untreated, they may cause significant morbidity and mortality.

Our understanding and management of these disorders has improved markedly over the last decade. Nevertheless, the majority of children with periodic fevers do not have mutations in known periodic fever syndrome genes. Even in patients with known mutations, the clinical phenotype may vary greatly, likely as a result of the environment. Given the rarity of AIDs, registries of patients with these disorders have been established in some countries.

The Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry is a multicenter, observational database of North American children with rheumatic diseases, including those with AIDs. Patients with defined rheumatic diseases who are 21 years of age or younger and are seen at a CARRA site are eligible to enroll. This study is the first report of patients with AIDs within this registry.

Objectives: To describe the demographic data of patients with AIDs in a North American registry of rheumatic disorders.

Methods: We conducted a cross-sectional study of children and adolescents with AIDs enrolled in the CARRA Registry. Enrollment in the database began in May 2011 and our study included data as of March 1, 2013. Inclusion criteria included diagnoses of specific monogenic and polygenic AIDs, as well as suspected AIDs with undefined diagnoses. Baseline data at enrollment is reported and analyzed.

Results: As of March 1, 2013, 7,931 patients were enrolled in the CARRA database. Fifty-one (0.64%) were diagnosed with AIDs, ranging in ages at enrollment from 2.5 to 20.7 years. There were 28 females (54.9%). Thirty-nine (76.5%) patients were White, 6 (11.8%) were Latino, and there were 2 (3.9%) patients from each of the following ethnic groups: American Indian/Alaskan Native, Black/African American, and Middle Eastern.

Of the 51 patients with AIDs, 20 (39.2%) had CRMO, 7 (13.7%) had PFAPA, 7 (13.7%) had FMF, 4 (7.8%) had TRAPS, 2 (3.9%) had MWS, and 1 (2%) patient each had PAPA, SAPHO, and NOMID. One (2%) patient had both FMF and TRAPS, and 7 (13.7%) patients had undefined AIDs.

Fifteen (29.4%) patients had genetically confirmed disease. The average length of time between the onset of symptoms and the evaluation by a rheumatologist was 1.35 years, ranging from 0.06 to 5 years. At enrollment, 41 (80.4%) patients had an ACR global functional status of Class I.

The age of onset of each disease varied markedly. NOMID was diagnosed at birth, while SAPHO had the latest average age of diagnosis at 13. Average CHAQ scores ranged from 0 in patients with PAPA and FMF/TRAPS, to 2.38 in the patient with NOMID. Physician global score also ranged from 0 for patients with FMF, PAPA, and FMF/TRAPS, to 8 for the patient with NOMID.

Conclusion: This study is the first to examine patients with AIDs in a North American, multiethnic registry. Continued observation of these patients and enrollment of new patients should provide meaningful data on these rare diseases in order to facilitate diagnosis, optimize treatment, and improve prognosis. We hope that this effort will encourage further international collaboration to create a truly international registry.

Disclosure of interest: None declared.

A112

P02-005 - Overlap of FMF and HIDS in one Arabic family

T Moussa[1*], B Aladbe[2], A Aly[1], R Taha[3], H El-Shanti[3], B Fathalla[2]
[1]Pediatrics, Hamad General Hospital, Doha, Qatar; [2]Pediatrics/Rheumatology, Hamad General Hospital, Doha, Qatar; [3]Medical Genetics, Shafallah Medical Genetics Center, Doha, Qatar
Pediatric Rheumatology 2013, **11(Suppl 1):**A112

Introduction: Familial Mediterranean Fever (FMF) is commonly reported in Arabs, whereas Hyper-IgD syndrome (HIDS) is rare. Moreover, the simultaneous presence of *MEFV* and *MVK* mutations segregating in the same family is exceedingly rare. We report here an Arabic family in whom a combination of complex *MEFV* mutations and an *MVK* mutation segregate producing variable clinical phenotypes.
Case report: An 8-year-old female presented with episodes of fever, abdominal pain, vomiting, and arthralgia lasting 3-5 days for 1-year duration suggestive of FMF. Atypical FMF features included longer episodes of fever and partial response to colchicine. Family history revealed HIDS in an 18 years old brother. He presented with episodes of fever, abdominal pain, vomiting, diarrhea, skin rash, lymphadenopathy, and febrile seizures since 1 year of age and was treated as clinical FMF with colchicine for 4 years with poor response. Genetic testing for HIDS done at 7 years of age showed homozygosity of V377I mutation. He was not responsive to Statins but became asymptomatic after puberty. At 17 years of age he developed short episodes of fever and abdominal pain more consistent with FMF. He was responsive to bursts of prednisone during episodes but not compliant with colchicine due to severe diarrhea.
Genetic testing was done for both patients and asymptomatic family members by sequence analysis of entire *MEFV* and *MVK* as well as *TNFRSF1A*, *PSTPIP1*, *IL1RN* and *LPIN2* coding regions and splice sites. Asymptomatic parents are carriers of V377I/- *MVK* mutation. The father is a compound heterozygote for two complex *MEFV* mutations, E148Q/P369S/R408Q and E167D/F479L whereas the mother is a compound heterozygote for M680I and the complex allele E148Q/P369S/R408Q. Both of our patients are homozygous for V377I *MVK* mutation, the girl is compound heterozygote for E148Q/P369S/R408Q and E167D/F479L *MEFV* mutations whereas the boy is compound heterozygote for E148Q/P369S/R408Q and M680I.
Discussion: The presence of concomitant mutations in different genes of monogenic autoinflammatory diseases (AID) could act as potential disease modifiers. Clinical implications to such combinations are not clear but may explain overlap or atypical clinical features. Such combinations have been scarcely reported including *TNFRS1A* and *MEFV* mutations, *MVK* and *TNFRSFA1* mutations, and *CIAS1* and *MEFV* mutations. Utilizing the diagnostic score and proposed diagnostic algorithm for molecular analysis of hereditary AID with periodic fever in children could have possibly resulted in genetic testing for one AID and missed such combinations. Our reported family does suggest that multiple mutations/variants in AID genes can occur in the same patient and could potentially influence the clinical presentation and response to treatment.
Disclosure of interest: None declared.
References

1. El-Shanti H, *et al*: **Familial Mediterranean fever in Arabs.** *Lancet* 2006, **367**:1016-1024.
2. Granel B, *et al*: **Overlap syndrome between FMF and TRAPS in a patient carrying MEFV and TNFRSF1A mutations.** *Clin Exp Rheumatol* 2007, **25(4 Suppl 45)**:S93-5.
3. Van der Hilst JC, *et al*: **International HIDS Study Group. Long-term follow-up, clinical features and quality of life in a series of 103 patients with hyperimmunoglobulinemia D syndrome.** *Medicine (Baltimore)* 2008, **87**:301-310.

A113

P02-006 - A novel PSTPIP1 mutation in PAPA syndrome

B Fathalla[1], M Al-Mutawa[2], F Al-Amri[1], S Al-Dosari[2], M Kambouris[2], H El-Shanti[2*]
[1]Hamad Medical Corporation, Doha, Qatar; [2]Shafallah Medical Genetics Center, Doha, Qatar
Pediatric Rheumatology 2013, **11(Suppl 1):**A113

Introduction: Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome is an autosomal dominant autoinflammatory disease caused by mutations in the proline-serine-threonine phosphatase-interacting protein 1, *PSTPIP1*.
The produced protein is a cytoskeleton-associated adaptor protein that modulates T-cell activation, cytoskeletal organization and IL-1β release.
The only two mutations described so far, A230T and E250Q, have been found in patients and families, and are thought to disrupt the binding of PSTPIP1 with PTP-PEST, a regulatory phosphatase, and increase its avidity for pyrin in the cytosol, thereby dysregulating IL-1β production. PAPA syndrome typically presents with recurrent sterile, erosive arthritis in childhood, resulting in significant joint destruction. By puberty, joint problems tend to subside and cutaneous symptoms increase including pathergy, frequently with abscesses at the sites of injections, severe cystic acne, and recurrent non-healing sterile ulcers, often diagnosed as pyoderma gangrenosum.
Case Report: We describe a a four year old Jordanian male, born to healthy non-consanguineous parent, who presented with cutaneous abscesses at the age of 6 months and then at 18 months at the vaccination injection sites. At the age of 20 months he developed cellulitis. At the age of 23 months, he had acute arthritis of the right ankle. He developed acute arthritis of the left wrist at 24 months, and the right wrist at 27 months and then the right elbow at the age of 45 months. He has two older sisters and the family history is negative for similar conditions. The sequencing of the coding region of *PSTPIP1* and flanking intronic regions revealed a *de novo* variation p.Asp246Asn (p.D246N) in the child. The variant is predicted to be probably damaging by Polyphen and was not found in 360 ethnically-matched control chromosomes.
Discussion: We describe a 4 year old Jordanian boy with a typical clinical presentation of PAPA syndrome, with the exception of the absence of pyoderma gangrenosum and acne. However, both these findings may occur later in the course of the disease, mostly after puberty. We anticipate that this variation is the mutation the explains the symptoms in this child since it falls within the coiled coil domain that harbors all the previously described mutations. Since the E250Q and A230T variants of *PSTPIP1* were shown to severely abrogate binding to PTP-PEST in yeast two hybrid and co-immunoprecipitation experiments, we anticipate this mutation does the same.
Disclosure of interest: None declared.
References

1. Wise CA, Gillum JD, Seidman CE, Lindor NM, Veile R, Bashiardes S, Lovett M: **Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder.** *Hum Mol Genet* 2002, **11**:961-969.
2. Masters SL, Simon A, Aksentijevich I, Kastner DL: **Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease.** *Annu Rev Immunol* 2009, **27**:621-668.
3. Smith EJ, Allantaz F, Bennett L, Zhang D, Gao X, Wood G, Kastner DL, Punaro M, Aksentijevich I, Pascual V, *et al*: **Clinical, Molecular, and Genetic Characteristics of PAPA Syndrome: A Review.** *Current genomics* 2010, **11**:519-527.

A114

P02-007 - Childhood autoinflammatory disorders in Qatar

B Aladbe[1], A Aly[2*], R Taha[3], H Elshanti[3], T Moussa[2], F Al Amry[1], B Fathalla[1]
[1]Pediatrics/Rheumatology, Hamad General Hospital, Doha, Qatar; [2]Pediatrics, Hamad General Hospital, Doha, Qatar; [3]Medical Genetics, Shafallah Medical Genetics Center, Doha, Qatar
Pediatric Rheumatology 2013, **11(Suppl 1):**A114

Introduction: A multi-ethnic background with high rates of consanguinity characterizes the population living in Qatar. Understanding the uniqueness of clinical presentation of autoinflammatory disorders (AID) in this population will enhance our knowledge in regards to the spectrum of clinical phenotype and prevalence of mutations in our region.
Objectives: To report the clinical and genetic profile of children with AID from the only childhood rheumatology center in Qatar over the past 5 years.
Methods: A retrospective review of medical records.
Results: Familial Mediterranean Fever: 30 symptomatic children, 9 asymptomatic carriers, and 21 adult relatives were included. Among symptomatic children, the male to female ratio was 1:1, 19 were Arabic, 8 were Persian, and 3 were Turkish/Arabic. Median age at first symptoms was 5 years (range 1 – 16 years). Most common manifestations included recurrent abdominal pain and fever (n=25), arthralgia (15), chest pain (4), arthritis (3), oral aphthouses (3), erysipelas (1), and recurrent pyogenic arthritis (1). Other features include anemia (4), hypothyroidism or hyperthyroidism (2), and renal failure due to membranoproliferative glomerulonephritis (1). Response to colchicine was good (23) or partial (2); 4 others are not yet started and 1 was lost follow-up. A 23 member four-generation family of Persian ethnicity was followed showing variable

severity of clinical manifestations, severe pustulosis and psoriasis. Out of the expected 34 *MEFV* mutant alleles (17 probands), only 25 were identified while 9 were unidentified. Of the 25 MEFV mutations M694V (12), E148Q (5), E167D/F479L (2), V726A (2), M694I (2), N599D (1), and M680I (1).

Hyper-IgD syndrome group includes one Arabic family: parents and 3 siblings are carriers of V377I/- *MVK* mutation. Two symptomatic siblings are homozygote for V377I *MVK* mutation. All members have complex *MEFV* mutations. Detailed clinical and genotype characteristics are reported separately due to exceptionality of such combination.

One boy with Pyogenic Arthritis, Pyoderma Gangrenosum and Acne syndrome presented at 6 months of age and diagnosed at 4.5 years. He had recurrent pyogenic arthritis and skin abscesses and had a de novo and novel D246N mutation of *PSTPIP1*. He responded well to courses of prednisone.

Chronic Recurrent Multifocal Osteomyelitis (CRMO) group inlcuded 5 Arabic patients (2 males and 3 females) with a median age of disease onset of 7 years presenting with recurrent arthralgia (5), arthritis (3), abnormal gait (4) and back pain (2). One had compression fractures of the spine with kyphosis within 6 months of presentation. Other features included anemia (5) and psoriasis (1). All had elevated acute phase reactants, a diagnostic bone biopsy (3), bone scans (5), and MRI studies (5). Genetic testing results are pending in 2 whereas 2 had no *LPIN2* mutations but one had Q219H/- *PSTPIP1* variant. Treatments include naproxen (5), infliximab (3), pamidronate (2), and canakinumab (1).

Conclusion: We report an expanding cohort of children with AID in Qatar. Clinical manifestations were variable for similar mutations even among the same family. Concomitant mutations in different AID genes can be present. Clinical phenotype of CRMO in our cohort was more severe then typically reported in the literature.

Disclosure of interest: B. Aladbe: None declared, A. Aly: None declared, R. Taha: None declared, H. Elshanti: None declared, T. Moussa: None declared, F. AlAmry: None declared, B. Fathalla Grant / Research Support from: Medical Reserach Committee/ Hamad General Hospital.

A115

P02-008 - Dramatic response to canakinumab in MKD

B Makay*, E Ünsal
Pediatric Rheumatology, Dokuz Eylül University Hospital, İzmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A115

Introduction: Mevalonate kinase deficiency-associated periodic fever syndrome (MKD) is a systemic autoinflammatory disease caused by mutations in the mevalonate kinase gene (MVK), previously named "hyper-IgD syndrome" due to its characteristic increase in serum IgD level. The patients suffer recurrent fever attacks every 2-8 weeks beginning from infancy, often precipitated by immunizations, infections or emotional stress. Fever lasts 2-7 days and can be accompanied by malaise, headache, diarrhea, abdominal pain, vomiting, skin rashes, arthralgia, arthritis, tender lymphadenopathy and hepatosplenomegaly.

Fever attacks usually respond to the administration of steroids. However, increasing frequency of fever episodes with steroid use and the natural chronic disease course may require a continuous long-term treatment. Colchicine, cyclosporine, thalidomide and statins are not effective. A TNF-α blocking agent etanercept and an IL-1 blocking agent anakinra have been demonstrated to reduce the frequency of fever attacks in MKD. Canakinumab is a human monoclonal antibody targeted at interleukin-1 beta. Here, we report a 6-year-old boy with MKD who had a dramatic response to canakinumab.

Case Report: The patient was diagnosed as MKD when he was 2-year-old based on two mutations in MVK gene (Exon 8. c.803T>C (p.268I>T), and Exon 10 c.1129 G>A (p.377V>I). Colchicine, simvastatin and etanercept failed to reduce the attacks. He could not tolerate daily anakinra injections after a trial of two months, though a favourable response. Canakinumab, another IL-1 blocker, was started at a dose of 4 mg/kg every 28 days, which had a dramatic response, and he has never had attack since then.

Discussion: Canacinumab may be a therapeutic option in mevalonate kinase deficiency-associated periodic fever syndrome.

Disclosure of interest: None declared.

A116

P02-009 - Candle syndrome: expanding spectrum

I Kone-Paut[1*], A Kinoshita[2], H Ida[3], D Roybet[4], C Leaute-Labreze[5], P Pillet[6]
[1]Paediatric Rheumatology, CeReMAI, Bicetre Hospital, University of Paris Sud, Le Kremlin Bicetre, France; [2]Human Genetics, Nagasaki University Hospital, Nagasaki, Japan; [3]Respirology, Neurology, Rheumatology, Kurume University School of Medicine, Kurume, Japan; [4]Pediatrics, CH de Hyères, Hyeres, France; [5]Dermatology, CHU de Bordeaux, Bordeaux, France; [6]Pediatric Rheumatology, CHU de Bordeaux, Bordeaux, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A116

Introduction: CANDLE syndrome is an exceptional inflammatory condition starting within the first months of life, and comprising elevated fever, panniculitis with lipoatrophy, purplish and swollen eyelids, arthralgia, and developmental retardation. Most patients carry homozygous mutations in the *PSMB8* gene that impair the assembly of the immunoproteasome (iP) and lead to interferon g deregulation. Since now, 39 published case reports under various acronyms have shown clinical and genetic heterogeneity, suggestive of various mechanisms underlying this very severe condition. We present two new cases enlarging the spectrum of CANDLE phenotype

Case Report: 1: A Sicilian girl, born at term in 1986 with microcephaly, skin panniculitic rash since 2 weeks, hepatosplenomegaly, microcytic anemia, leucopenia with extreme lymphopenia, myelemia, thrombopenia, elevated ESR, liver enzymes and ANA, first suspected with neonatal lupus. She developped recurrent otitis media and pneumonitis. Following years, recurrent fever, severe growth and mental retardation (cerebral calcification, seizures), purplish swollen eyelids, rashes mimicking dermatomyositis, joint contracture, severe lipoatrophy, diabetes requiring insulin therapy, dyslipidemia and finally severe hypertension and giant aortic aneurysm that killed her at the age of 16y. Proteasome exons PMSB1-10, PSME1-3, PSMA1-6 were screened and did not reveal any mutation. No other biologic material is available for IFNy investigation. **2:** A French girl, born in 2009, onset at 2 months, hepatosplenomegaly, seizures, panniculitis, fever with peaks, swollen eyelids and sometimes hands and feet, joint contracture and lipoatrophy increasing with time. She also has marked leucopenia, microcytic anemia and elevated liver enzymes. Extensive work up to rule out other condition not contributive. *PMSB8* gene analyses are ongoing

Discussion: Case 1 could be considered as an extreme severity phenotype of CANDLE, as the developmental retardation was intriguing (final height 87cm), immunodeficiency and autoimmunity were both present with also important metabolic disturbances, finally she died early at 18y. Case 2 shares many features of CANDLE, even the skin biopsy was not typical (immature white cells not retrieved). She may have mild developmental retardation. These 2 patients confirm that other causes outside the proteasome may cause CANDLE phenotype.

Disclosure of interest: None declared.

References

1. Kanazawa N, *et al*: *Procced Nat Ac Sci USA* 2011, **108**:14914-9.
2. Liu y, *et al*: *Arthritis Rheum* 2012, **64(3)**:895-907.

A117

P02-010 - A novel 24 nucleotide deletion in the TNFRSF1A

DM Rowczenio*, H Trojer, G Wang, PN Hawkins, HJ Lachmann, NM Stewart
Medicine, University College London, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A117

Introduction: TNF receptor associated periodic syndrome (TRAPS) is a rare autosomal dominant disease characterized by episodes of fever usually lasting two to three weeks, associated with severe abdominal pain, arthralgia, skin rash and red and swollen eyes. The age of onset varies from early childhood to adulthood, and the disease affects both sexes equally. Although first cases were described in individuals of Irish-Scottish descent, TRAPS has since been reported world-wide.

Case report: A 41 year old British man, with no family history, presented in early adolescence with episodes of severe abdominal pain for which he had an appendectomy at the age of 12. He had 10 to 12 attacks per year each lasting almost exactly two weeks accompanied by a headache, abdominal pain, arthralgia, myalgia, night sweats, generalised erythema and unilateral non-painful cervical lymphadenopathy. He had occasional red eyes but no

periorbital oedema or periorbital pain. His puberty was relatively late and he is significantly shorter than his siblings. During attacks his inflammatory markers, serum amyloid A protein (SAA) and C-reactive protein (CRP), were elevated to median values of 258 mg/L (range 60 – 679) and 46 mg/L (range 30 – 96) respectively. He is in full time employment and remains physically extremely fit.

He underwent screening of the four genes associated with periodic fever syndrome: *MEFV* (the gene associated with FMF); *TNFRSF1A* (the gene associated with TRAPS) *NLRP3* (the gene associated with CAPS) and *MVK* (the gene associated with MKD).

A novel in frame deletion of 24 nucleotides (c.255_278del) in exon 3 of the *TNFRSF1A* gene was identified by PCR and Sanger sequencing and subsequently confirmed with allele-specific PCR by using primers complementary to the sequence of a mutant DNA. Screening of parental DNA showed no evidence of nucleotide alternation in their *TNFRSF1A* gene suggesting that the deletion identified in our patient was a de novo mutation.

He commenced treatment with anakinra, to which had a dramatic response with a rapid resolution of symptoms and normalization in SAA and CRP to healthy levels of 8.1 and 4 mg/L.

Discussion: Genetic aberrations in tumor necrosis factor receptor superfamily 1A gene (*TNFRSF1A*) located on chromosome 12p13 are the cause of TRAPS. To date, 89 variants have been reported to be associated with clinical TRAPS, of which 95% are single nucleotide substitutions.

The novel mutation identified in our patient results in a deletion of eight amino acids p.Ser86_Glu93del (Infever description S57_E64del) in the second domain of the tumor necrosis factor receptor superfamily member 1A protein (TNFR1).

Based on the crystal structure of TNFR1 proposed by Banner et al, residues from Ser57 to Phe60 are structurally conserved between the second and third extracellular domains. Additionally Phe60 residue is crucial for proper domain 2 folding and its side chain further stabilizes the structure by interacting with both the second disulfide bridge and the Ser-74-Asp-93 hydrogen bond bridge. It is therefore very likely that the novel variant we have identified produces profound changes to the three dimensional shape of the TNFR1 impairing its folding and binding with TNF.

Disclosure of interest: None declared.

A118

P02-011 - TRAPS syndrome debuted as systemic JIA

T Sleptsova*, E Alexeeva, E Mitenko, S Valieva, T Bzarova, R Denisova, K Isaeva
Department of Rheumatology, Scientific Centre of Children's Health, Moscow, Russian Federation

Pediatric Rheumatology 2013, **11(Suppl 1):**A118

Introduction: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is characterized by periodic fever, cutaneous rash, conjunctivitis, lymphadenopathy, abdominal pain, myalgia, and arthralgia. It is a rare autosomal dominant disease and strongly associated with heterozygous mutations in the tumor necrosis factor (TNF) receptor super family 1A (TNFRSF1A) gene. The great diversity of manifestations and the difficulties in genetic analyses make the diagnosing of this disease a challenge. Our aim was to report on case of autoinflammatory syndromes that is considered to be rare entity.

Case report: A 16-year-old Caucasian boy presented at the age of 15 years with fever (39 ºC), weakness, lymphadenopathy, splenomegaly and arthralgia. The laboratory tests revealed anemia, leukocytosis, thrombocytosis, ESR of 80 mm, CRP of 90 mg/l (normal < 0.5). A diagnosis of systemic juvenile idiopathic arthritis (JIA) was made and the patient was treated with corticosteroids, NAIDs and gamma globulin. Over the next 6 months, he presented skin rash regarded as reaction to drugs. No abdominal pain or conjunctivitis was noted. Pulse therapy with methylprednisolone and constant administration NAIDs were needed to control fever and pain. After extensive work-up of infectious etiology, an oncological disease with negative results he underwent diagnostic laparoscopic surgery for lymph node biopsy because of abdominal lymphadenopathy, without findings. Questioned further autoinflammatory syndrome was suspected. DNA analysis showed a mutation present in exons 9 of the TNFRSF1A gene (deletion c.792delT), thus resulting in a diagnosis of TRAPS. He had partial clinical response to corticosteroids. However, the treatment response to TNF-α inhibitor infliximab was dramatic. At the present he still presents rash but no fever and lymphadenopathy.

Discussion: Here, we report a patient with TRAPS who recovered from steroid dependency by infliximab and kept remission with infliximab. The patient was thought to have had systemic JIA. Failure to respond to therapy, good response only to very high doses of corticosteroids led us to suspect autoinflammatory syndrome. Clinical features are important for the diagnosis, but confirmation is obtained through genetic analysis. Autoinflammatory syndromes should be considered in patients with fever of unknown origin and the clinicians must be aware of the diversity of manifestations and diagnostics of these conditions.

Disclosure of interest: None declared.

References

1. Monogenic autoinflammatory diseases: new insights into clinical aspects and pathogenesis.
2. Autoinflammatory syndromes: report on three. *Sao Paulo Med J* 2009, 127(5):314.
3. Manki A, Nishikomori R, Nakata-Hizume M, Kunitomi T, Takei S, Urakami T, Morishima T: Tumor necrosis factor receptor-associated periodic syndrome mimicking systemic juvenile idiopathic arthritis. 2006, 55(3):337-41.

A119

P02-012 - HAIDS in practice of Russian rheumatologist

E Fedorov*, S Salugina, N Kuzmina
Pediatric Department, Research Institute of Rheumatology under the RAMS, Moscow, Russian Federation

Pediatric Rheumatology 2013, **11(Suppl 1):**A119

Introduction: Human autoinflammatory diseases (HAIDS) are defined as illnesses caused by primary disfunction of the innate immune system. This diseases had different prevalence in different ethnic groups.

Objectives: To determine the spectrum of HAIDS in the practice of pediatric rheumatologist on the results of appealability to the Federal rheumatologic center.

Methods: This study included patients who applied to the Federal Rheumatologic Center in 2009 - 2012 years for diagnosis adjustment because of fever of unknown origin. All patients were submitted to routine rheumatology examination and HLA-I-typing, and molecular genetic testing.

Results: 42 children with HAIDS in age between 2 and 17 years were identified in 3 years. Age of onset ranged from 0 to 16 years, on an average 5.1 years old. The average age at diagnosis was 8.2 years old. The following diseases were identified: Behcet's disease (BD) - 16 children (male/female (M/F) - 12/4); Cryopyrin-Associated Periodic Syndromes (CAPS): Muckle-Wells syndrome - 4 (M/F - 0/4); CINCA/NOMID syndrome - 2 (M/F - 2/0); Familial Mediterranean fever (periodical disease) (FMF) - 7 (M/F - 1/6); PFAPA syndrome - 4 (M/F - 2/2), chronic recurrent multifocal (nonbacterial) osteomyelitis (CRMO) - 3 (M/F - 0/3), hyper-IgD syndrome/mevalonate kinase deficiency - 2 (M/F -0/2), undifferentiated HAIDS - 4 (M/F = 2/2). Patient's ethnicity with BD: peoples of the North Caucasus - 3, Tatars - 4, Uzbeks - 1, Azerbaijanians - 1, Russians - 5, Ukrainians- 1, ethnic Germans- 1. Among the patients with FMF: Armenians- 4, Azerbaijanians- 2. Russian patients dominated for other nosologies. HLA-B51 antigen was detected in 8 (50%) patients with BD. For all patients with FMF, CAPS and for one patient with hyper-IgD syndrome the diagnoses were confirmed by genotyping.

Conclusion: The pediatric rheumatologist can meet in his practice patients with various HAIDS. The most frequent in our Russian Federal rheumatologic center were BD and FMF. For these diseases the prevalence of certain ethnic groups was identified. The patients with CAPS are the most similar in clinical laboratory performance to rheumatic diseases and they need differential diagnosis of systemic juvenile arthritis and early prescription of targeted therapy (IL-1 inhibitors) that significantly changes the initial poor prognosis.

Disclosure of interest: None declared.

A120

P02-013 - TH17 cells and regulatory T cells in TRAPS

PM Vaitla[1*], E Drewe[1], EM McDermott[1], I Todd[2], L Fairclough[2]
[1]Immunology, Nottingham University Hospitals NHS Trust, Nottingham, UK; [2]Immunology, University of Nottingham, Nottingham, UK

Pediatric Rheumatology 2013, **11(Suppl 1):**A120

Introduction: The immunopathogenesis of TRAPS is thought to centre on activation of the innate immune system resulting in episodic inflammation. The adaptive immune system, Tregs and Th17 T cells, has not been studied in TRAPS. Different anti-TNF agents have different clinical effects on TRAPS i.e. etanercept has benefits but adalimumab and infliximab may trigger flares of TRAPS. It has been shown that different anti-TNF agents have differential effects on regulatory T cells in rheumatoid arthritis i.e. adalimumab but not etanercept induces peripheral Tregs which have the ability to suppress Th17 cells. We considered whether Tregs and Th17 cells in TRAPS could underpin differential response to biologicals.
Objectives: To investigate whether there are differences in the numbers of regulatory T cells and Th17 cells in TRAPS patients compared to controls.
Methods: Regulatory T cells from 5 patients with C33Y mutation TRAPS and 5 healthy controls were analysed by flow cytometric analysis on fresh blood. Lymphocytes which were CD4+/CD25 high were regarded as regulatory T cells. Th17 cells were also analysed by flow cytometric method involving cytoplasmic staining for IL17. Cells which were CD3+/CD8-/IL17+ were regarded as Th17 cells.
Results: The TRAPS patients included 2 patients on anakinra, 1 on etanercept, 1 on tocilizumab and 1 on canakinumab. The mean number of CD4 +/CD25high cells was 21.4 cells/μl in TRAPS patients compared to 13.2 cells/μl in controls. Although TRAPS patients had higher numbers of CD25 high T cells, this did not reach statistical significance with a P value of 0.107. The mean percentage of Th17 cells in TRAPS patients was 1.92% compared to 1.72% in controls; p value of 0.649. Due to small numbers, it was not possible to comment on any differences between different biological therapies.
Conclusion: Although CD4+/CD25high regulatory T cells appeared to be higher in the TRAPS patients, this did not reach statistical significance which could reflect small numbers studied. There were no significant differences in Th17 cells between the 2 groups. We suggest a further study of a larger group of patients using intracellular FoxP3 staining to further investigate the increased regulatory T cells apparent in TRAPS.
Disclosure of interest: None declared.

A121

P02-014 - Consequences of Arginine 92 mutations in TNFR1

I Jéru[1,2,3*], S Charmion[4], E Cochet[1], B Copin[3], G Le Borgne[2,3], P Cathebras[4], J Gaillat[5], P Duquesnoy[3], S Karabina[2,3], V Hentgen[6], S Amselem[1,2,3]
[1]Service de Génétique, APHP, Hôpital Trousseau, France; [2]Université Pierre et Marie Curie-Paris6, France; [3]UMR_S933, INSERM, Paris, France; [4]Service de Médecine Interne, Centre Hospitalier Universitaire Saint-Etienne, Saint-Etienne, France; [5]Service des Maladies Infectieuses, Centre Hospitalier de la Région d'Annecy, Pringy, France; [6]Centre de Référence des Maladies AutoInflammatoires , Centre Hospitalier de Versailles, Le Chesnay, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A121

Introduction: *TNFRSF1A* is involved in a Mendelian autosomal dominant autoinflammatory disorder called *TNFR*-associated periodic syndrome (TRAPS). Most *TNFRSF1A* mutations are missense changes and, apart from those affecting conserved cysteines, their deleterious effect remains often questionable. This is especially true for the frequent R92Q mutation, which might not be responsible for TRAPS per se but represents a susceptibility factor to multifactorial inflammatory disorders.
Objectives: This study investigates TRAPS pathophysiology in a family exceptional by its size (13 members).
Methods: *TNFRSF1A* screening was performed by PCR-sequencing. Comparison of the 3-dimensional structure and electrostatic properties of wild-type and mutated TNFR1 proteins was performed by *in silico* homology modeling. TNFR1 expression was assessed by western blotting and ELISA in lysates and supernatants of HEK293T cells transfected with plasmids encoding wild-type and mutated TNFR1.
Results: A *TNFRSF1A* heterozygous missense mutation, R92W (c.361C>T) perfectly segregated with typical TRAPS manifestations within the family ($p<5.10^{-4}$), and was associated with very high disease penetrance (0.9). Prediction of its impact on protein structure revealed local conformational changes and alterations of electrostatic properties. In addition, R92W leads to abrogation of the receptor shedding, whereas TNFR1-R92Q behaves like the wild-type receptor.
Conclusion: These data demonstrate the pathogenicity of a mutation affecting arginine 92, a residue whose involvement in inflammatory disorders is deeply debated. Combined with previous data on arginine 92 mutations, this study discloses an unusual situation in which different amino acid substitutions at the same position in the protein are associated with a clinical spectrum bridging Mendelian to multifactorial conditions.
Disclosure of interest: None declared.

A122

P02-015 - A novel MVK mutation in a child with AA amyloid

A Kolsky[1], D Rowczenio[2], H Lachmann[2], Z Vernerova[1], F Votava[1], P Dolezalova[3*]
[1]University Hospital Kralovske Vinohrady, Prague, Czech Republic; [2]National Amyloidosis Centre, UCL Medical School, London, UK; [3]General University Hospital, Prague, Czech Republic
Pediatric Rheumatology 2013, **11(Suppl 1):**A122

Introduction: AA amyloidosis may develop as a consequence of chronic inflammatory conditions including inherited periodic fever syndromes. Mevalonate-kinase (MVK) deficiency (MKD) appears to be the least frequent underlying condition after FMF, TRAPS and CAPS. Moreover, amyloidosis rarely manifests during childhood. We report a case of a small child in whom renal biopsy performed because of the cortico-resistant nephrotic syndrome revealed AA amyloidosis.
Case Report: A 4-years old Caucasian girl with negative family history presented with features of nephrotic syndrome in 1/2011. Over the previous 2 years she had been suffering with recurrent episodes of unexplained fever with pharyngitis and lymphadenitis lasting 3 days in 2-4 weekly intervals and received a putative diagnosis of PFAPA (periodic fever, adenitis, pharyngitis, aphtae) syndrome. Despite the increasing frequency of febrile episodes over the last year investigations aimed at excluding monogenic fevers were not performed. In early 2011 her IgD was normal, IgA mildly increased, serum amyoid A (SAA) fluctuated from normal to 200 mg/l. After the standard corticosteroid (CS) treatment of nephrotic syndrome had failed to induce remission after 6 wks, a renal biopsy revealed AA amyloidosis with focal segmental glomerular and vascular involvement. While genetic analysis was pending, Colchicine was added to the CS treatment followed by daily anakinra injections with good response. After a laborious genetic screening to exclude mutations causing FMF, TRAPS and CAPS, following variants in MVK gene were identified: Mutation V377I and a novel deletion in exon 5 C152WfsX6(c.450_453delGGTG). The latter terminates the protein six amino acids after the deletion occurs, effectively making the protein shorter. Within 6 months of the treatment her proteinuria stabilised at protein/creatinine ratio around 23 mg/mmol with normal GFR. So far there have been no other signs of organ involvement. Despite ongoing anakinra therapy she continues to have occasional febrile episodes with temporary increase of proteinuria and SAA which remains below 10 mg/l during afebrile intervals.
Discussion: MKD/HyperIgD-syndrome has been so far reported in only a few cases of AA-amyloidosis. Our patient has been the youngest one to develop this severe complication. The only other child reported so far was also a compound heterozygote carrying the genotype G326R/V377I. The long-term follow-up with careful SAA serial measurements will tell us more about the prognostic significance of the newly described MVK gene mutation. This case report also underlines the importance of careful differential diagnostic re-evaluation of children presenting with PFAPA-like disease in whom febrile episodes do not show a typical prolongation of afebrile intervals over the time.
Disclosure of interest: None declared.
References

1. Lachmann HJ, *et al*: AA Amyloidosis complicating hyperimmunoglobulinemia D with periodic fever syndrome. A report of two cases. *Arthritis Rheum* 2006, 54(6):2010-14.
2. Lane T, *et al*: AA amyloidosis complicating the inherited periodic fever syndromes. *Arthritis Rheum* 2012, doi: 10.1002/art.37827.
3. Yel S, *et al*: Amyloidosis an a child with hyperimmunoglobulinemia D syndrome. *IJKD* 2013, 7:70-2.

A123

P02-016 - A novel PSMB8 mutation causing candle syndrome

V Messia, M Pardeo, R Nicolai, C Bracaglia, F De Benedetti, A Insalaco*
Division of Rheumatology, Department of Pediatric Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A123

Introduction: Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome is a newly described autoinflammatory disease, which had been recently reported in 9 patients. It is characterized by onset during the first year of life of recurrent fevers, purpuric skin lesions, arthralgia, progressive lipodystrophy, hypochromic or normocytic anemia, delayed physical development and increased levels of acute phase reactants.
Case Report: A 10 year-old young girl presented at 10 months of age with recurrent fevers, hepato-splenomegaly and nodular erythematous skin lesions of trunk and limbs; subsequently she progressively developed lypodistrophy, arthralgia and arthritis and edema of eyelids. She started steroids and, then, cyclosporine with partial benefit and with recurrence of symptoms following tapering and/or discontinuation. Her weight and height were below the 5th percentiles with partial growth hormone defect. Skin biopsy showed typical features of lobular panniculitis. Laboratory tests showed persistent elevated acute phase reactants and Serum amyloid A levels persistent chronic anemia, mild recurrent leucopenia (minimum neutrophil count 1040), thrombocytopenia (minimum 94.000) and decreased IgA, IgG and IgM levels.Immunological and cytogenetic studies performed on bone marrow were normal. Response to hydroxychlorochine or colchicine was unsatisfactory. Subsequently, the patient developed severe proteinuria. Renal biopsy revealed a minimal change glomerulopathy; she was started on a standard nephrotic syndrome high-dose steroid protocol with remission of proteinuria.Complete sequencing of TNFRSF1A and MVK genes showed no mutations. Molecular analysis of PSMB8 (proteasome subunit β type 8) gene revealed the presence of c.208A>T p.(Thr70Ser) variant in heterozygotic status that has never been reported before. Because of a persistent inflammatory state, she was started on daily therapy with Anakinra (2 mg/Kg/die), discontinued after 10 days for absence of response. She is currently managed with chronic low dose glucocorticoids.
Discussion: The similarities in the clinical phenotype of this case with those described by Liu et al support the conclusion that this novel variant Thr70Ser in the PSMB8 gene is a causative mutation. Minimal change glomerulopathy has not been reported in CANDLE patients. It may be a casual association: however, one of the 9 original patients is described as having nephrotic syndrome. Our patient also did not respond to Anakinra. A better understanding of the pathophysiology of the disease is needed to improve its management.
Disclosure of interest: None declared.
References

1. Torrelo A, *et al*: **Chronic atypical neutrophilic dermatosis with lipodistrophy and elevated temperature (CANDLE) syndrome.** *Journal of American Academy of Dermatology* 2010, **62(3)**:489-495.
2. Ramot Y, *et al*: **Chronic Atypical Neutrophilic Dermatosis with Lipodistrophy and elevated temperature Syndrome: A case report.** *Pediatric Dermatology* 2011, **28(5)**:538-541.
3. Liu Y, *et al*: **Mutations in Proteasome Subunit β Type 8 Cause Chronic Atypical Neutrophilic Dermatosis with Lipodistrophy and Elevated Temperature with evidence of Genetic and Phenotypic Heterogeneity.** *Arthritis and Rheumatism* 2012, **64(3)**:895-907.

A124

P02-017 - Periodic fever syndrome masquerading as eczema…

TV Nguyen, J Sugarman, K Leslie*
Dermatology, University of California, San Francisco, San Francisco, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A124

Introduction: "Periodic Fever Syndrome Masquerading as Eczematous Dermatitis: Report of a D12E Mutation in *TNFRSF1A*".
Case Report: A 22 year-old woman presented with a six-year history of periodic fevers, skin and joint disease. She presented with an intermittent, intensely pruritic red rash on the torso and extremities, associated with recurrent non-axial joint pain and swelling, and periodic fevers lasting for days to weeks. These symptoms were triggered by exposure to heat, cold weather, dry air, and sweat. She denied eye or gastrointestinal involvement. Physical exam revealed generalized, poorly demarcated, excoriated, erythematous macules and patches. Skin histology showed minimal psoriasiform hyperplasia and mixed perivascular infiltrates. Serum C-reactive protein, erythrocyte sedimentation rate, and immunoglobulin D levels were within normal range. Antinuclear antibody and rheumatoid factor tests were negative. Genetic testing revealed a heterozygous nucleotide substitution in the *TNFRSF1A* gene causing Tumor Necrosis Factor-Associated Periodic Syndrome (TRAPS). This mutation, namely D12E, results in substitution of an Aspartic acid with a Glutamic acid. Her mother is an asymptomatic heterozygote. After failing high-potency topical steroids and antihistamines, she was started on etanercept 100 mg weekly. Initial improvement of symptoms was dramatic, but the effects waned after four months of therapy. She was then switched to adalimumab 40 mg every other week and reported marked reduction of her skin manifestation and fevers. However, her condition relapsed after six months of using adalimumab. At that point, adalimumab was replaced with anakinra, an interleukin 1 (IL-1) receptor antagonist, and the patient has been reporting significant improvement in the past four months.
Discussion: Patients with *TNFRSF1A* mutations affecting the cysteine residues are predisposed to a more severe phenotype, with fevers lasting for 23 days on average and many inflammatory symptoms.[1] Thus far only three cases of D12E carriers have been described: one with short-lived fevers and almost no other manifestations,[1] one with TRAPS phenotype, [2] and one also harboring a homozygous mutation for and presenting with the Familial Mediterranean Fever syndrome phenotype. [3] Our patient's disease is likely related to aberrant innate immunity. The fact that she and her mother carry the same mutation, yet only one is symptomatic, suggests incomplete penetrance of the allele. Response to inhibition of IL-1 signaling is reassuring.
Disclosure of interest: None declared.
References

1. D'Osualdo A, Ferlito F, Prigione I, Obici L, Meini A, Zulian F, *et al*: **Neutrophils from patients with TNFRSF1A mutations display resistance to tumor necrosis factor-induced apoptosis: pathogenetic and clinical implications.** *Arthritis Rheum* 2006, **54**:998-1008.
2. Havla J, Hohlfeld R, Lohse P, Kumpfel T: **MS-like disease after anti-TNF therapy in a patient with severe rheumatoid arthritis carrying a TNFRSF1A D12E mutation (Abstract).** *20th Meeting of the European Neurological Society* 2010.
3. Gattorno M, Sormani MP, D'Osualdo A, Pelagatti MA, Caroli F, Federici S, *et al*: **A diagnostic score for molecular analysis of hereditary autoinflammatory syndromes with periodic fever in children.** *Arthritis Rheum* 2008, **58**:1823-32.

A125

P02-018 - PSTPIP1 gene mutations in periodic fever patients

K Takabe*, Y Adachi, H Saito, T Yamashita, Y Wakai, K Saito, Y Shinohara
Internal Medicine, Tsuchiura Kyodo Hospital, Tsuchiura, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A125

Introduction: Familial Mediterranean Fever (FMF) is considered a rare disease in Japan.
Our institution began screening for MEFV gene mutations in patients with periodic fever in 2005. Among the 18 patients screened, we have identified 11 (56.5%) FMF patients with heterozygous M694I/E148Q mutations. Among the other 7 patients, no pathogenic mutations were detected by the direct sequencing of all exons and the promoter region of the MEFV gene. PSTPIP1, the protein responsible for PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum, and acne), has recently been found to bind with pyrin and to allow pyrin to interact with ASC. In this study we investigated whether PSTPIP1 mutations could be found in the 7 periodic fever patients without pathogenic MEFV mutations.
Objectives: The patients were 3 males and 4 females with a mean age of 40.1 years (range: 5~74 years). The mean age at onset was 37.6 years (range: 2~72 years). The pattern of fever was consistent with the Tel-Hashomer criteria. The symptoms other than fever were headache (3 patients), pharyngeal pain (2), arthralgia (2), and skin rash (2). None of the patients experienced abdominal or chest pain during their fevers.
Methods: We extracted DNA from peripheral leukocytes and performed sequence analyses of all exons and their flanking sequences of the PSTPIP1 gene. We also sequenced the MVK gene (exons 2 to 11), NFLP3 gene (exon 3), and TNFRSF1 gene (exons 2 to 4 and 6 to 7).
Results: MEFV mutations identified in these patients were as follows; three heterozygous L110P/E148Q mutations, one heterozygous E148Q/R202Q and one P115R mutation. No mutations were detected in the MVK, NLRP3,

or TNFRSF1 gene. One novel missense mutation (c.5 C>T, p.Met2Thr) was identified in exon 2. Single nucleotide variants in intronic regions were frequently found. A 14 bps deletion in intron 14 was detected in 3 patients and a 3 bps deletion was detected in the 3' downstream region in 4 patients. The longer tandem repeats of (CCTG)8 in the promoter region were detected in 3 patients. None of the 43 healthy controls were positive for the p.Met2Thr mutation. A 14 bps deletion was detected in 3 of 42 healthy controls and a 3 bps deletion was detected in 20 of 42 controls. Six control cases were positive for the L110P/E148Q mutation, but none of them possessed a 14 bps deletion. In contrast, the three periodic fever patients with L110P/E148Q mutations also possessed 14 bps deletions. The frequency of 14 bps deletions in the L110P/E148Q mutation-positive cases with periodic fever was significantly higher than that in the L110P/E148Q mutation-positive cases without fever (healthy controls, COPD patients, and pulmonary fibrosis patients) (3/3 vs. 0/20, p=0.0006, Fishers' exact test). RT-PCR analyses of exons 14 and 15 were performed using RNA extracted from two periodic fever patients with 14 bps deletions, but no splicing variants of exon 15 were identified.
Conclusion: The p.Met2Thr variant was absent in the healthy controls, but its pathogenic role in the fever patients is unknown. The significance of the co-existence of L110P/E148Q mutation and 14 bps deletions also remains to be determined. Further investigations to elucidate how these sequence variants impact the pyrin/ASC interaction are awaited.
Disclosure of interest: None declared.

A126

P02-019 - Detection of risk factors for AA-amyloidosis

S Németh[1*], L Obici[2], S Grandemange[3], H Lachmann[4], C Oberkanins[1]
[1]ViennaLab Labordiagnostika GmbH, Vienna, Austria; [2]Biotechnology Research Laboratories, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [3]Unité médicale des maladies auto-inflammatoires, Hôpital Arnaud de Villeneuve, Montpellier, France; [4]National Amyloidosis Centre, UCL Medical School, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A126

Introduction: Systemic reactive (AA) amyloidosis represents the most important complication within TNF receptor associated periodic syndrome (TRAPS), familial Mediterranean fever (FMF) and other autoinflammatory syndromes, progressively leading to endstage renal failure. The homozygous condition of the serum amyloid A (SAA) variant *SAA1.1* is significantly associated with the occurrence of AA amyloidosis in TRAPS patients. Likewise in FMF patients the *MEFV* mutation c.2080A>G (M694V) correlates with amyloidosis and the *SAA1.1/SAA1.1* genotype increases clinical severity (age at disease onset, amyloidosis, arthritis).
Objectives: To develop a reverse-hybridization assay (Amyloidosis StripAssay) for detection of (1) genetic markers modulating the risk of developing AA amyloidosis in TRAPS and FMF [c.209C>T, c.224T>C (*SAA1*); c.2080A>G (*MEFV*)], as well as (2) mannose-binding lectin 2 (*MBL2*) and *TNFRSF1A* variants, which are suspected to be modulating factors in TRAPS [g.4447 C>G, g.4776 C>G, g.5000 C>T, c.154C>T, c.161G>A, c.170G>A (*MBL2*); c.473-33C>T (*TNFRSF1A*)].
Methods: The Amyloidosis StripAssay is based on a multiplex PCR and hybridization of biotinylated amplicons under exactly defined stringency to a teststrip presenting a parallel array of allele-specific oligonucleotide probes. Specifically bound PCR products are detected using enzymatic colour reaction.
Results: The specificity of the StripAssay was validated by hybridizing teststrips against PCR products obtained from plasmid clones, as well as reference DNA samples. All genetic variants covered by the StripAssay could be unambiguously identified.
Conclusion: The Amyloidosis StripAssay proved to be a fast and reliable method for detection of genetic factors modulating the risk of developing AA amyloidosis in FMF and TRAPS. An association between *MBL2* alleles and amyloidosis is not conclusively established, but *MBL2* is of considerable diagnostic interest in closely related fields, including rheumatoid arthritis or innate immunity. Also in case of the *TNFRSF1A* intron 4 polymorphism c.473-33C>T the StripAssay could be a useful tool: the c.473-33C>T variant is involved in *TNFRSF1A* expression and an implication on the TRAPS phenotype still needs to be investigated.
Disclosure of interest: None declared.

A127

P02-020 - CAPS in Turkish children: treatment with ANTI IL1

FK Kara Eroglu*, N Besbas, F Ozaltin, Y Bilginer, A Duzova, S Ozen
Pediatric Rheumatology Department, Hacettepe University Faculty of Medicine, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A127

Introduction: Criyopyrin associated periodic syndrome (CAPS) has a heterogenous presentation and in a number patients mutations can not be found. Here we present our initial results with CAPS patients.
Case Report: Results: All of our patients had symptoms within the first 3 months of life. All had fever, urticaria and persistant labaratory inflammation. All except one patient had failure to thrive. Except for the one patient with Muckle Wells syndrome all had neurological features ranging from headache to convulsions, hydrocephalus, cognitive dysfunction.Two patients, one without a mutation, had hearing impairment. Two patients have diarrhea during attacks. All were started on anti IL1 , one patient who did not respond to anakinra was started to canakinumab and on the fourth dose he developed MAS. After MAS was subsided canakinumab was re-started and he continues the drug without further problems. Presently three patients are on anakinra and three are on canakinumab, all with normal acute phases and improved quality of life. One patient has associated Duchenne muscular dystrophy.
Discussion: Conclusions: Anti IL1 treatment is efficacious in CAPS patients. Somatic mutations may enlighten the mutation negative patients. Until then classification criteria are needed to guide pediatricians in diagnosis and treatment.
Disclosure of interest: None declared.
Reference

1. Sibley Cailin H, Plass Nikki, Snow Joseph, *et al*: **Sustained Response and Prevention of Damage Progression in Patients With Neonatal-Onset Multisystem Inflammatory Disease Treated With Anakinra. A Cohort Study to Determine Three- and Five-Year Outcomes.** *ARTHRITIS & RHEUMATISM* 2012, **64(7)**:2375-2386.

A128

P02-021 - Atypical CAPS consequence of novel NLPR3 mutations

E Gonzalez-Roca[1*], G Espinosa[2], J Bartra[3], E Ruiz-Ortiz[1], J Rius[1], S Plaza[1], J Yague[1], JI Arostegui[1]
[1]Immunology Service, Hospital Clinic, Barcelona, Spain; [2]Autoimmune Department, Hospital Clinic, Barcelona, Spain; [3]Allergy Department, Hospital Clinic, Barcelona, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A128

Introduction: Cryopyrin-associated periodic syndromes (CAPS) are a group of dominantly inherited disorders caused by gain-of-function *NLRP3* mutations. These disorders represent different degrees of severity of a same disease being familial cold autoinflammatory syndrome the milder form, Muckle-Wells syndrome an intermediate form and chronic infantile neurologic cutaneous and articular syndrome the severest form. Overlapping phenotypes among these diseases have been also reported. Here we describe two different Spanish families with atypical presentation for CAPS. The initial *NLRP3* screening, which only included the analysis of exon 3, was negative. However, the complete gene analysis revealed two novel missense mutations in exon 1 and 8, respectively.
Case Report: The patient 1 is a 36 year-old woman who suffered from recurrent episodes of uveitis since 2011 and from progressive neurosensorial hypoacusia. She did not refer other symptoms related to CAPS. There was familial history of hypoacusia in her sister, mother and maternal grandmother, suggesting a dominant inheritance pattern for this trait. The *NLRP3* analyses revealed a heterozygous A-to-G transition in the exon 1 of *NLRP3* (at c.146 position), which provokes the missense histidine-to-arginine variant at residue 49 (p.H49R) of cryopyrin .
The propositus of second family is an adult man who referred a pharmacological allergy, and a skin rash in hands, arms and auricular pavilions that apparently appeared in winter and after cold exposure. These data suggested to perform a *NLRP3* analysis that revealed a heterozygous point C-to-T transition at c.2885 nucleotide position, on the exon 8, that provokes the threonine to metionine amino acid change in residue 952 (p.T952M) of cryopyrin.

Discussion: Most of disease-causing *NLRP3* mutations are located on the exon 3. Here we describe two novel *NLRP3* mutations located on exons 1 and 8, that were identified in patients with atypical presentations for CAPS. In the first family, the detected missense p.H49R mutation represents the first detected mutation identified in the exon 1 of the gene, affecting the PYD domain of cryopyrin. Interestingly, the patient with this mutation was affected by recurrent episodes of uveitis and neurosensorial hypoacusia that appeared during the adulthood, with no other clinical features of CAPS. In the second family, the p.T952M mutation was detected on the exon 8 of the *NLRP3* gene and represents the first and unique mutation identified to date in this exon. These cases highlight the relevance to perform a complete *NLRP3* analyses in potential candidates, including those patients that could refer atypical presentations for CAPS in terms of age at disease-onset or clinical features.
Disclosure of interest: None declared.

A129

P02-022 - Atypical cryopirin associated periodic syndrome

S Bujan Rivas[1*], JI Arostegui Gorospe[2], A Sellas Fernandez[3], CP Simeon Aznar[1], J Aguilar Company[1], J Ordi Ros[1], M Vilardell Tarres[1]
[1]Internal Medicine, Hospital Vall Hebron, Barcelona, Spain; [2]Immunology, Hospital Clinic I Provincial, Barcelona, Spain; [3]Rheumatology, Hospital Vall Hebron, Barcelona, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A129

Introduction: Cryopyrin-associated periodic syndromes (CAPS) are dominantly inherited autoinflammatory diseases (AD) caused by NLRP3 mutations. They include different phenotypes (FCAS, Muckle-Wells syndrome, and CINCA/NOMID) with different severity, usually as childhood onset fever and urticarial-like rash. In the last years, the clinical picture of CAPS is growing with other manifestations than fever, urticaria, or sensorineural deafness.
Objectives: To communicate the clinical picture, genetic mutation, therapeutic approach and follow-up of five patients from three generations of the same pedigree with late symptoms onset (at fourth decade of life) diagnosed from the study of urinary bladder AA-amyloidosis of the oldest member.
Methods: Review of clinical file of the 5 involved patients from diagnosis until January 2013. Data studied included: onset and diagnosis age, past medical history, clinical, laboratory, pathologic and genetic data, treatment onset, clinical and laboratory evolution and adverse effects related to therapy.
Results: 71 yo. male was referred to Internal Medicine Service of Vall Hebron Hospital in June 2010 due to nephrotic syndrome and haemorraghic cystitis. A urinary bladder and rectal biopsies showed Congo-red deposits stained with antiamyloid A antibody. Patient's medical history revealed self-limited episodic palindromic monoarthritis, and sensorineural hypoacusia since mid-thirties without recurrent fever or skin rash. ESR, CRP and serum A-amyloid protein (SAA) were elevated. Genetic study for AD identified a p.Ala-439-Thr NLRP3 mutation. Four family members, -3 adults (2 ♂ /1♀) and 1 child (12 yo ♀)-, shared this mutation. All 3 adults presented adult-onset sensorineural deafness and SAA elevation; the two males also refered recurrent pericarditis and monoarthritis from mid-twenties on. Off-label treatment with rIL-1RA anakinra for symptomatic patients was considered a cost-effective option to canakinumab, the approved drug for CAPS. Early clinical remission, normalization of SAA, ESR and CRP and deafness stability was achieved. Index case 24-h proteinuria 20 months after anakinra was <500 mg. Anakinra adverse effects were minor local reaction at injection site (all patients) and transient alopecia in 1 adult female patient. After 24 months of anakinra, patients are asymptomatic. The child also remains asymptomatic since diagnosis.
Conclusion: CAPS spectrum is still in evolution. These patients' phenotype was remarkable by late disease onset, reactive AA amyloidosis as haemorraghic cystitis and recurrent palindromic arthritis and pericarditis without urticarial-like skin rash or fever. Otherwise, anakinra proved to be an effective treatment in this CAPS serie. CAPS must be kept in mind for familiar sensorineural loss with AA amyloidosis and/or relapsing pericarditis or arthritis, even in adults.
Disclosure of interest: None declared.

A130

P02-023 - NLRP3 mosaicism as a cause of late-onset CAPS

Q Zhou*, AK Ombrello, D Chin, DL Kastner, I Aksentijevich
Inflammatory Disease Section, National Human Genome Research Institute (NHGRI/NIH), Bethesda, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A130

Introduction: The dominantly inherited cryopyrin-associated periodic syndromes (CAPS) are caused by heterozygous missense gain-of-function mutations in the *NLRP3* (*CIAS1*) gene encoding NLRP3 (also known as cryopyrin). Most patients present at a young age with a variety of clinical symptoms including fevers, urticaria-like skin rash, arthropathy, and CNS inflammation. A subset of patients followed at the National Institute of Health's autoinflammatory disease clinic has adult-onset fevers and urticarial rash but conventional genetic testing has been unremarkable for any mutations in *NLRP3*. We analyzed one such "mutation-negative" patient. She is a 63y/o female of Irish ancestry who developed a gradually worsening stress-induced urticarial rash in her 40s. Additional clinical history is remarkable for severe arthralgia, myalgia, chills, and occasional conjunctivitis. Initially started on anakinra 100mg/day in 2003, she had dramatic improvement in symptoms; however, her anakinra dose has required periodic adjustments since that time to control her symptoms.
Objectives: To identify the cause of disease in a patient who presented with late-onset CAPS and who was mutation-negative in *NLRP3* based on the conventional Sanger sequencing.
Methods: We performed whole exome sequencing in the DNA sample extracted from peripheral blood. A total of 192 subclones were randomly selected and subjected to Sanger sequencing to validate the *NLRP3* somatic mutation. Targeted deep sequencing on *NLRP3* will be done using the DNA from blood and buccal cells.
Results: Our established exome data analysis pipeline failed to identify any plausible candidate gene in this patient. In order to increase the sensitivity of variant calling, we used a different combination of parameters including calling SNPs/Indels with extreme strand bias, which resulted in a rapid increase in false positive rate but also raised the possibility of identifying somatic mosaicism mutations. Based on this new analytic approach, we identified a possible mutation p.Tyr570Cys (c.1709A>G) in *NLRP3*. Exome data showed that there are 12 reads carrying the mutant G allele and 64 reads carrying the wild type A allele (15%). This mutation has been previously reported in a NOMID/CINCA patient from Australia. The putative mosaic p.Tyr570Cys mutation was validated by subcloning a 718bp fragment from the patient's leukocyte DNA and subsequent Sanger sequencing of 192 clones. We found 20 clones carrying the p.Tyr570Cys mutation, which established the level of somatic mosaicism at 10.4%. We are in the process of evaluating this mosaic mutation in other tissues from this patient.
Conclusion: We report *NLRP3* somatic mosaicism in a patient presenting with a late onset CAPS. To our knowledge this is the first time that a *NLRP3* mutation has been identified in patients developing symptoms in adulthood. Other possible causal genes have been ruled out based on exome sequencing analysis. Our result contributes significantly to understanding the pathogenesis of disease in patients with adult-onset autoinflammatory diseases.
Disclosure of interest: None declared.

A131

P02-024 - Clinical impact of V198M mutation in NLRP3 gene

R Caorsi[1*], A Insalaco[2], L Obici[3], L Cantarini[4], A Meini[5], M Alessio[6], L Lepore[7], F Zulian[8], F Caroli[9], I Ceccherini[9], A Martini[1], M Gattorno[1]
[1]2nd Division Of Pediatrics, Istituto Gaslini, University of Genoa, Genova, Italy; [2]Department of Pediatrics, Ospedale Pediatrico Bambino Gesù, Roma, Italy; [3]Amyloidosis Research and Treatment Center, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [4]Department of Rheumatology, Policlinico Le Scotte, University of Siena, Siena, Italy; [5]Department of Pediatrics, Spedali Civili, Brescia, Italy; [6]Department of Pediatrics, Federico II Hospital, Napoli, Italy; [7]Department of Pediatrics, IRCCS Burlo Garofalo, University of Trieste, Trieste, Italy; [8]Department of Pediatrics, University of Padua, Padova; [9]Department of Genetics, Istituto Gaslini, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A131

Introduction: The V198M mutation is described as a possible hypomorphic variant of the NLPR3 gene. However the impact of this mutation is still largely unknown.
Objectives: To analyse the prevalence of V198M mutation in patients with a clinical history suggestive for CAPS and to describe the clinical and laboratory findings of patients carrying this mutation.
Methods: From 2002 the molecular analysis of the NPLR3 gene was performed in 524 patients with a clinical history suggestive for CAPS. In order to estimate the pravalence of the mutation of this gene in the healty population 98 healthy individuals were also analyzed for the same mutation.
Results: The V198M mutation was found in 13 screened patients: 10 were heterozygous for the mutation only. In one patient with a typical MWS phenotype the V198M variant was associated with the Q703K and the D303N mutation of the same gene. In a patient a low-penetrance mutation of TNFRSF1A gene (P46L) was also found, while another one carried the A91V mutation of Pfr1 gene.
Out of the 10 patients heterozygous for the V198M mutation, five displayed a story of periodic fever associated with urticarial rash, arthralgia and transient arthritis, associated with elevation of acute phase reactants and responding to steroid treatment on demand or to treatment with IL-1 blockers. In two patients the clinical picture was mild and uniquely characterized by urticarial rash and arthralgia, often induced by cold, but not associated with elevation of acute phase reactants. The other three patients presented episodes of fever with an inconstant elevation of acute phase reactants and not associated to other symptoms suggestive of CAPS; however one of this patients developed renal amiloidosis. The patients carrying the P46L mutation of TNFRSF1A gene presented periodic fever with athralgia and headache, not associated with elevation of acute phase reactants. The patient carrying the A91V mutation of Pfr1 gene presented some clinical characteristics suggestive of CINCA syndrome associated to not typical ones; this patient died at the age of 4.7 years with a clinical pictures consistent of MAS.
3 patients were treated with IL-1 blockers (anakinra at the staring dosage of 1 mg/kg and canakinumab at the starting dosage of 2 mg/kg every 8 weeks), two heterozygous for V198M and one compound heterozygous for V198M, D303D and Q703 K, with a rapid a complete control of the clinical manifestations.
None of the healthy individuals screened for the V198M mutation turned out to be positive.
Conclusion: This study confirms the low-penetrance of the V198M mutation of the NLPR3 gene. However a minority of these patients may present a clinical phenotype consisting with a CAPS, thus requiring treatment with IL-1 blockers.
Disclosure of interest: None declared.

A132

P02-025 - Homozygous Q705K sequence variant in NLRP3

S Berg[1,2*], M Sundqvist[3], K Christenson[3], A Welin[3], H Björnsdottir[3], J Bylund[3], P Wekell[4,5], P Söderkvist[6], A Karlsson[3]
[1]The Queen Silvia Childrens Hospital, Sahlgrenska University Hospital, Göteborg, Sweden; [2]Pediatrics, Gothenburg University, Gothenburg, Sweden; [3]Department of Rheumatology and Inflammation Research, Gothenburg University, Gothenburg, Sweden; [4]The Queen Silvia Childrens Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden; [5]Pediatrics, NU-Hospital Organization, Uddevalla; [6]Division of Cellbiology, Linköping University, Linköping, Sweden
Pediatric Rheumatology 2013, **11(Suppl 1):**A132

Introduction: The *NLRP3* sequence variant Q705K (Q703K) might have an increased frequency in autoinflammatory conditions including PFAPA and atypical cases of CAPS. It has also been discussed as a modifier of inflammation in other inflammatory conditions. We report the clinical picture and laboratory findings in a patient with the homozygous sequence variant Q705K in *NLRP3*.
Case Report: The patient, a 12-year-old boy with healthy parents, experienced his first long febrile episode, associated with abdominal pain, aseptic meningitis, spleenitis and increased inflammatory markers, at the age of 2.5 years. The patient was given corticosteroids, and responded well. After the age of three years, the patient developed recurrent febrile episodes (approximately twice a year) with abdominal pain of a duration exceeding 2 weeks, without, skin rash and conjunctivitis. Corticosteroid treatment was necessary to terminate the episodes. As steroids were given early during the episodes, the full clinical picture is not known, except for that the patient developed another aseptic meningitis. The severity of the episodes increased and today the patient depends on continuous steroid treatment. Anakinra and colchicine has been used without satisfactory response.
During febrile episodes, inflammatory markers including SAA were elevated. Also, IL18 was increased while IgD and IgA were normal.
The genes *TNFSR1A* and *NLRP3* were screened and a homozygous sequence variant, Q705K, was found in *NLRP3*.
We have investigated cellular and molecular features after the patient had developed fever, abdominal pain and increased inflammatory markers, due to partial withdrawal of corticosteroids. Analysing secretion of cytokines by isolated monocytes incubated for 20 h, in the absence and presence of LPS, we found that the patient produced less IL10 as compared to parents/controls and that the production of IP10 was decreased in both the patient and the parents as compared to controls. The production of IL1β and the monocyte caspase-1 activity were similar between the patient and parents/controls. Also, the production of IL6, IL8, IL12, IL17, GMCSF, INFγ and TNFα were similar. Neutrophil functions, including apoptosis, degranulation and ROS production, were normal.
Discussion: The sequence variant Q705K in *NLRP3* has been considered a polymorphism in its heterozygous form but might also be a low-penetrance mutation. To our knowledge this is the first description of a patient with a severe inflammatory condition and a homozygote sequence variant Q705K. The patient did not respond to IL1β blockade in a moderate dose and IL1β mediation could not be confirmed in our laboratory investigation. However, the clinical unresponsiveness to IL1β blockade could be related to the dose administered and the corticosteroids may have influenced the laboratory results. It is probable that the homozygous Q705K variant contributes to this severe inflammatory condition in a multifactorial fashion, as the phenotype otherwise would be much more common than clinically found in Sweden.
Disclosure of interest: None declared.

A133

P02-026 - Model-based characterization of the PKPD relationship for canakinumab in CAPS: a step towards personalized

A Gautier, P Lowe, A Skerjanec, P McKernan, O Luttringer, M Fink*
Novartis Pharma AG, Basel, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A133

Introduction: Canakinumab is a high-affinity fully human monoclonal antibody of the IgG1/k isotype, designed to bind and functionally neutralize the bioactivity of IL-1β, which is recognized as one of the principal pro-inflammatory cytokines in cryopyrin associated periodic syndromes (CAPS).
Objectives: The objectives of the study were to describe the kinetics of canakinumab and dynamics of binding IL-1β in CAPS patients; to determine if these are different in 2- and 3-year-old children versus older children and adults; and to explore the impact of CAPS phenotype (Muckle-Wells Syndrome [MWS], Familial Cold Autoinflammatory Syndrome [FCAS], Neonatal-Onset Multisystem Inflammatory Disease [NOMID]) on the kinetics of canakinumab and dynamics of binding to IL-1β.
Methods: A pharmacokinetics (PK)-binding model was used to describe the kinetic and binding parameters of canakinumab and IL-1β in CAPS patients, and in other populations relative to CAPS. The subgroup of 7 CAPS patients who were 2 and 3 years of age at baseline was also compared to the overall CAPS population.
Results: The 7 CAPS patients did not show any difference in terms of PK. However, they showed a higher IL-1β turnover including IL-1β clearance and production. IL-1β levels were linked with the severity of the CAPS phenotype. In the pediatric population, MWS and especially NOMID patients had higher concentrations of the inert canakinumab/IL-1β complexes after administration of canakinumab, indicating more cytokine in the body to be captured.
Conclusion: Correlation with clinical responses suggested that these increased levels of IL-1β may explain why younger and NOMID phenotype patients require higher doses or escalation to higher doses.

Disclosure of interest: A. Gautier Shareholder of: Novatis Pharma AG, Employee of: Novatis Pharma AG, P. Lowe Shareholder of: Novartis Pharma AG, Employee of: Novartis Pharma AG, A. Skerjanec Employee of: Novartis Pharma AG, P. McKernan Shareholder of: Novartis Pharma AG, Employee of: Novartis Pharma AG, O. Luttringer Shareholder of: Novartis Pharma AG, Employee of: Novartis Pharma AG, M. Fink: None Declared.

A134

P02-027 - Quality of life in CAPS treated by Canakinumab

C Marsaud[1], I Marie[2], I Koné-Paut[2*]
[1]Pediatrie générale, Bicêtre hospital, France; [2]CEREMAI, Le kremlin-Bicêtre, Bicêtre hospital, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A134

Introduction: The impact of cryopyrin associated periodic syndrome (CAPS) on quality of life (QoL) is very high. Constitutional symptoms, extreme fatigue, chronic pain and physical limitation lead to severe restriction of daily activities and social life. In addition neurological and sensory impairments may aggravate social exclusion of these patients.
Objectives: To evaluate the quality of life, the social and professional impact of a small cohort of CAPS patients after a long-term treatment with Canakinumab, a selective anti interleukine1β (IL-1β) monoclonal antibody.
Methods: Patients were those first included in the pivotal (D2304) and in the extension (D2306) Canakinumab/CAPS study [1] and followed in the reference center for autoinflammatory diseases at Bicêtre Hospital (Paris). All carried a heterozygous mutation in the NLPR3 gene and had received Canakinumab (dose of 150 mg every 8 weeks or 2mg/kg for patient weight under 40kg). All patients had been followed prospectively and their quality of life had been evaluated regularly with generic questionnaires: Functional Assessment of Chronic Illness Therapy-fatigue (FACIT-F), 36-item Short Form health survey (SF-36) and Health Assessment Questionnaire (HAQ) for adults. Furthermore supplementary questions regarding their social activities and how they deal with their treatment were added.
Results: 7 patients were analyzed (3males/4females, age 24 to 63-years-old). The mean time of follow-up from initial treatment to last visit was 4.8 +/- 0.8 years. All patients were in remission defined as a physician global assessment of disease activity that was minimal or absent, with skin rash that was minimal or absent, and serum values of CRP and/or SAA in the normal range. A significant and sustained improvement in physical SF-36 (37.7 from baseline vs 49,7 at end of D2306 study and 48.6 points at last visit, $p<0.05$) and FACIT-F (25.1 vs 42,6 at end of D2306 study and 42.3 points at last visit $p<0.05$) scores was found, with scores approaching scores in American general population. They notified modifications in their social lives: 1 patient restarted professional activity, 4 patients restarted with sportive activity and 1 patient stopped smoking. Concerning their affective lives, 1 single man patient lives actually in a relationship, and 1 patient divorced. No local reaction after injection was observed during the follow-up. Only 2 patients realized subcutaneous injections by their own, the 5 others needed a nurse for the injection. Doses or intervals of injections were modified in 4 patients because of incomplete remission during follow-up (1 received 300 mg instead 150mg, and 3 received injections at 6 or 7 interval weeks).
Conclusion: Long-term treatment with Canakinumab in patients with CAPS allows a sustained and significant improvement of quality of life with the generic questionnaire FACIT-F and SF-36. Patients show important modification in their social and professional lives.
Disclosure of interest: None declared.
Reference

1. Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, *et al*: **Use of canakinumab in the cryopyrin-associated periodic syndrome.** *N. Engl. J. Med* 2009, **360(23)**:2416-25.

A135

P02-028 - Muckle-Wells syndrome and renal transplantation

B Kortus-Götze*, J Hoyer
Nephrology, Internal Medicine, Nephrology, Marburg, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A135

Introduction: The Muckle-Wells syndrome (MWS) is a rare inherited disease and belongs to the group of cryopyrin-associated periodic syndromes (CAPS). Recurrent fever attacks, myalgia, arthralgia, urticarial rash, headache, conjunctivitis, sensorineural deafness and a severe fatigue syndrome are the typical symptoms of MWS. Due to an unregulated production of IL1 a continuous formation of serum amyloid leads ultimately to the development of AA-amyloidosis, which is life-threatening and in some cases the fatal complication of MWS.
Case Report: Here we report the three years follow up on a 34-year old female patient with Muckle-Wells syndrome and biopsy proven systemic AA amyloidosis and end stage renal disease. After renal transplantation therapy with canakinumab subcutaneously in a dosage of 150 mg every eight weeks was continued in combination with the immunosuppressive therapy.
Discussion: Before and after renal transplantation the patient had a very good response to canakinumab with low activity in inflammation markers with an improved quality of life. Over the period of three years the triple immunosuppressive therapy (CSA, MMF, Prednisone) in combination with canakinumab has had no negative effect on activity of MWS and no pharmacological interactions between medication were observed. Even 3 years after renal transplantation, the patient remains an excellent kidney function without proteinuria. There are no signs of recurrence of AA-amyloidosis in the transplanted kidney.
Disclosure of interest: None declared.
Reference

1. According to our data, treatment with different immunomodulators in patients with Muckle-Wells syndrome and renal transplantation is safe, feasible and without severe side effects also over a longer time.

A136

P02-029 - CAPS or SJIA

E Alekseeva, R Denisova*, S Valieva, T Bzarova, T Sleptsova, E Mitenko, K Isayeva
Rheumatology, Scientific Center Of Children's Health of RAMS, Moscow, Russian Federation
Pediatric Rheumatology 2013, **11(Suppl 1):**A136

Introduction: Systemic Juvenile Idiopathic Arthritis (sJIA) is chronic disease. Some patients are resistant to standard immunosupressivetherapy and anti IL6 treatment. Some of these patients have autoinflammatory disease.
Case Report: The patient became ill when she was 3 years old. She had fever, rash and artralgia and pericarditis. After examination in hospital she was diagnosed sJIA. She took glucocorticosteroids 1 mg/kg daily per os, methylprednisolone 10 mg/kg IV, №3, and MTX 10mg/m2/week with positive results. She took that therapy during 2 years. When the dose of GC decreased she had flares (fever, rash, arthritis). In 2 years after beginning she was examined for TRAPS. She took infliximab without improving. The analysis for TRAPS was negative. When she took GC 0,5 mg/kg/day she had sever flare and tocilizumab treatment was initiated. The fever disappeared and CRP was normal but rash was persisted. She took tocilizumab 10 months and dose of GC was decreased to 0,05 mg/kg/day. After 10 months of tocilizumab treatment she had toxic allergic reaction and tocilizumab was canceled. She was examined for CAPS and mutation in gene **NLRP3** - c.2113C>A in geterozygota. In that time there was no anti IL-1 medicines in Russia so she was given cyclosporine and MTX was continued. When she was 7 years old for flare canacinumab therapy was initiated in dose 4 mg/kg. The fever, rash, arthritis were disappeared and CRP and ESR became normal. After 3 months GC was cancelled. After 4 canacinumab injection she had flare and puls therapy of GC IV was performed with positive results.
Discussion: So it is still question if she has CAPS or sJIA. Because She is not in remission on anti IL 1 therapy and also GC therapy is effective for this patient and on the other hand she has mutation in the cryopirin gene.
Disclosure of interest: None declared.

A137

P02-030 - Unusual CNS manifestation

E Schuh[1*], P Lohse[2], I Meinl[1], T Kümpfel[1]
[1]Neurology, Institute of Clinical Neuroimmunology, Munich; [2]Laboratoriumsmedizin, Labor Blessing, Singen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A137

Introduction: Cryopyrin-associated periodic syndrome (CAPS) is a rare systemic, monogenetic inherited autoinflammatory condition caused by NLRP3/CIAS 1 gene mutations encoding for cryopyrin, a major component

of the inflammasome, leading to an excessive production of interleukin-1beta (IL-1ß). The clinical picture of genetic variations of the NLRP3 inflammasome is characterized by recurrent episodes of systemic inflammation involving skin, joints, eyes and the central nervous system and shows variable penetrance regarding disease severity and symptoms.
Case Report: Here, we report an unusual case of a 43 year-old woman who presented to us with diagnosis of CRION (chronic relapsing inflammatory optic neuropathy) due to recurrent episodes of optic neuritis on both eyes leading to atrophy of the left optical nerve with blindness. Repeated cerebral MRI scan showed an unusual excessive contrast enhancement involving both optic nerves with massive swelling as well as contrast enhancement in the oculomotor nerve and the pituitary gland. Additionally a few unspecific hyperintense white matter lesions were seen. Examination of the cerebrospinal fluid (CSF) revealed a mild pleocytosis and oligoclonal bands. Extensive laboratory investigations for other inflammatory CNS diseases as well as rheumatological diseases remained all unremarkable. Treatment with high dose i.v. steroids improved visual acuity, but worsened again after cessation of steroid therapy.
Furthermore the patient reported about intermittent tendinitis and tension type headache. Laboratory examinations revealed recurrent increased levels of Serum Amyloid A (SAA) and C-reactive protein (CRP). Molecular genetic testing showed a homozygous Q703K mutation in Exon 3 of the NLRP3/CIAS 1 gene. Diagnosis of CAPS was set and high dose anti-Interleukin 1 therapy initiated which led so far to moderate improvement of the CNS inflammation as well as reduction of SAA levels. The patient is currently followed up.
Discussion: This is an unusual and severe CNS manifestation in a patient homozygous for the Q703K variant in exon 3 of the NLRP3/CIAS 1 gene which has been considered so far as a low-penetrance mutation with a mild phenotype. Ocular manifestations including optic nerve atrophy usually occur in classical CICNA/NOMID patients. This case illustrates that CAPS should be also considered in adult patients with chronic recurrent optic neuritis and additional symptoms suggestive of an autoinflammatory syndrome.
Disclosure of interest: E. Schuh: None Declared, P. Lohse: None Declared, I. Meinl: None Declared, T. Kümpfel Grant/Research Support from: Novartis.
References

1. Vitale A, Lucherini OM: **Long-term clinical course of patients carrying the Q703K mutation in the *NLRP3* gene: a case series.** *Clinical and Experimental Rheumatology* 2012.
2. Tarabishy Ahmad Bakir, Hise Amy G, Traboulsi Elias I: **Ocular manifestations of the autoinflammatory syndromes.** *Ophtalmic genetics* 2012.
3. Kidd , Burton B, Plant GT, Graham EM: **Chronic relapsing inflammatory optic neuropathy (CRION).** *Brain* 2003.

A138

P02-031 - Phenotype of V198M and Q703K NLRP3 variants

V Messia, M Pardeo, R Nicolai, C Bracaglia, F De Benedetti, A Insalaco*
Division of Rheumatology, Department of Pediatric Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A138

Introduction: The term CAPS (cryopyrin-associated periodic syndromes) identifies a spectrum of autoinflammatory diseases caused by heterozygous mutations of the CIAS1/NLRP3. Affected individuals may present three different phenotypes: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and CINCA syndrome, the most severe form of the clinical spectrum. Clinical manifestations include urticaria-like rash, recurrent fever, arthralgia, conjunctivitis; chronic aseptic meningitis, cerebral atrophy and bone malformations in the severe cases.
Objectives: To describe the long-term clinical course of a cohort of patients carrying two different low-penetrance NLRP3 mutations (V198M and Q703K).
Methods: Six patients were identified carrying the NLRP3 V198M mutation (mean age 10,35±4,73 years, 4 males and 2 females), and 5 patients were identified carrying the *NLRP3* Q703K (mean age 9,72±4,55 years,3 males and 2 female). All were Caucasians.
Results: In the V198M cohort the mean age at disease onset was 5,85±4,08 years. All patients had symptoms consistent with recurrent inflammatory syndrome: 6/6 presented recurrent episodes of skin lesions and arthralgia, 4/6 of fever attacks, 3/6 of arthritis, 2/6 of headache and subcutaneous edema. One patient showed fatigue, conjunctivitis and recurrent abdominal pain. Half of the patients had a positive family history for recurrent inflammatory episodes. In 3 out of 6 patients the severity of phenotype and the persistence of elevated acute phase reactants, led to initiation of anti IL-1 therapy with immediate benefit . In the cohort of patients with Q703K variant the mean age at disease onset was 3,73±3,33 years. All patients had skin rash, 4/5 patients presented recurrent fever, 3/5 arthralgia and myalgia, 2/5 subcutaneous edema, pharyngitis and lymphadenitis; 1 patient had mild arthritis, headache and abdominal pain. Only in 1 case, symptoms were triggered or worsened by cold exposure. None of our patients had a family history relevant for autoinflammatory symptoms. Laboratory test showed no increase in acute phase reactants, with one exception. This patient presented also with recurrent fevers, treatment resistant epilepsy and carries an heterozygous MEFV mutation. She failed colchicine and anti IL-1 therapy was started.
Conclusion: The pathogenic significance of these NLRP3 mutations is still discussed [2]. In our experience patients carrying Q703K mutation appear to have a milder and self-limited phenotype than those with V198M variant in which therapy with IL-1 inhibitor drugs is often necessary. The factors that affect the pathogenic consequences of these variants are still to be established.
Disclosure of interest: None declared.

A139

P02-032 - CAPS: a novel mutation and an unusual phenotype

A Insalaco[1*], PS Buonuomo[1], C Bracaglia[1], M Pardeo[1], I Ceccherini[2], R Nicolai[1], F De Benedetti[1]
[1]Division of Rheumatology, Department of Pediatric Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy; [2]Molecular Genetic Laboratory, Istituto Giannina Gaslini, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A139

Introduction: Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory syndrome caused by heterozygous mutations of CIAS1/NLRP3 gene. Affected patients may present with three different phenotypes: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome and CINCA syndrome. Common symptoms include sporadic or cold-induced non pruritic urticarial rash and fever. Severe cases suffer from deafness, meningitis, articular contracture and secondary amyloidosis.
Case Report: We describe a 13-year-old female who complained, starting at 12 years of age, of recurrent episodes of high fever, pericarditis, arthralgia, arthritis of the knees, abdominal pain. These episodes were associated with marked increase in inflammatory markers and in serum amyloid level (5 episodes in the first 5 months of disease). Symptoms were poorly responsive to NSAIDs and colchicine but responded to steroid therapy. Molecular analysis of the MEFV, TNFR and MVK genes did not show any pathogenic mutations. In the subsequent months she developed recurrent (up to daily) episodes of chest pain, skin rash and swelling of the subcutaneous tissue of limbs, trunk, joints and lips, in the absence of fever, with spontaneous resolution.
Molecular analysis of the CIAS1 gene revealed the presence of a c.1105C>A mutation in the heterozygous state, that predicts a L369M amino acid substitution. To the best of our knowledge this variant has never been reported. One hundred chromosomes were examined and the variant was not found. In order to verify the potential pathogenic effects of the L369M amino acid substitution, daily therapy with anakinra (2 mg/kg/day) was started with rapid disappearance of clinical symptoms and normalization of CRP levels in 24 hours. Since the pathogenic significance of the mutation observed is not known, prediction of the effect of this mutation on the protein function has been attempted, in silico, by subjecting the p. L369M substitution to the Variant Effect Predictor tool. The mutation was predicted to significantly affect protein structure (scoring as “dangerous” and “deleterious”).
Discussion: The fast response to IL-1 inhibition suggests that the disease of this patient is driven by IL-1 and support the conclusion that this novel mutation is pathogenic and may be associated with a new CAPS phenotype.
Disclosure of interest: None declared.
References

1. ter Haar Nienke, Paediatric Rheumatology International Trials Organisation (PRINTO) and the Eurofever/Eurotraps Projects, *et al*: **Treatment of autoinflammatory diseases: results from the Eurofever Registry and a literature review.** *Ann Rheum Dis* 2012, Online First published on as 10.1136/annrheumdis-2011-201268.

2. Kone-Paut I, Piram M: **Targeting interleukin-1β in CAPS (cryopyrin-associated periodic) syndromes: What did we learn?** *Autoimmunity Reviews* 2012, **12(1)**:77-80.

A140

P02-033 - CAPS diagnosis and treatment in an Israeli family

Y Shinar[1*], G Breuer[2,3], A Livneh[1,4], P Hashkes[5]
[1]Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer, Israel; [2]Rheumatology Unit, Shaare Zedek Medical Center, Israel; [3]Hebrew University School of Medicine, Hebrew University, Jerusalem, Israel; [4]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; [5]Pediatric Rheumatology Unit, Shaare Zedek Medical Center, Jerusalem, Israel
Pediatric Rheumatology 2013, **11(Suppl 1):**A140

Introduction: Only one family in Israel, from Ethiopian Jewish origin has been diagnosed with the familial cold autoinflammatory syndrome phenotype of the cryopyrin associated periodic syndromes (CAPS)[1].
Case Report: We confirmed the Muckle-Wells syndrome phenotype of CAPS by *NLRP3* genetic testing in a three generation family of Turkish Jewish origin, previously diagnosed with familial Behcet disease due to the presence of mucosal ulcers in several family members with the finding of the HLA-B51 antigen in at least one family member. Eight family members including a deceased grandfather, 4 of his daughters and three grandchildren had brief episodes of fever and chills, accompanied by headache, myalgia, arthralgia, and an urticarial skin rash. Most family members had substantial hearing loss. None developed amyloidosis. Four family members tested for a *NLRP3* pathogenic variant had the known NM_001243313.1:c.1043C>T, p.Thr348Met variant[2]. Following initiation of treatment with canakinumab (150 mg every 8 weeks) and colchicine for mucosal ulcers all disease symptoms resolved and acute phase reactants normalized except for persistent headaches in one grandchild and tinitus in another. The health-related quality of life of the treated grandchildren markedly improved.
Discussion: *NLRP3* genetic testing was instrumental in the diagnosis of CAPS in this family, particularly as some family members presented with atypical features suggestive of Behcet disease, which is much more common in Israel. Although CAPS is a rare disease, additional cases with other *NLRP3* variants may exist in Israel.
Disclosure of interest: Y. Shinar: None Declared, G. Breuer: None Declared, A. Livneh Grant / Research Support from: Novartis, P. Hashkes Grant / Research Support from: Novartis, Consultant for: Novartis, Speaker bureau of: Novartis

References

1. Shalev SA, Sprecher E, Indelman M, Hujirat Y, Bergman R, Rottem M: **A novel missense mutation in CIAS1 encoding the pyrin-like protein, cryopyrin, causes familial cold autoinflammatory syndrome in a family of ethiopian origin.** *Int Arch Allergy Immunol* 2007, **143**:190-3.
2. Dodé C, Le Dû N, Cuisset L, *et al*: **New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes.** *Am J Hum Genet* 2002, **70**:1498-506.

A141

PW02-001 - Exome sequencing for autoinflammatory disorders

SE Boyden*, Q Zhou, I Aksentijevich, DL Kastner
Inflammatory Disease Section, National Human Genome Research Institute, NIH, Bethesda, Maryland, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A141

Introduction: Exome sequencing is the process by which exonic portions of the genome are selectively enriched from genomic DNA samples and sequenced using next generation methodologies. It has been used extensively since 2009 to identify the pathogenic variants underlying Mendelian disorders.
Objectives: The Inflammatory Disease Section in NHGRI has conducted exome sequencing on 162 subjects from families with a variety of unexplained autoinflammatory, autoimmune, and allergic diseases, to determine the disease etiologies, inform treatments plans, or provide a molecular diagnosis to patients.
Methods: Samples were prepared at the NIH Intramural Sequencing Center using one of four different exome capture kits, and libraries were sequenced on the Illumina HiSeq 2000 platform using 2x100 bp paired-end reads, to an average depth of coverage in the target intervals of 68X across all samples, and with an average of 89% of target bases producing high-confidence calls. The raw data are analyzed according to the following pipeline: per-sample alignment of reads to the human reference genome with Novoalign and removal of PCR duplicate reads with Picard, followed by multi-sample re-alignment around small insertions and deletions, re-calibration of per-base quality scores, variant calling, and re-calibration of variant quality scores using the Genome Analysis Tool Kit (GATK), and finally variant annotation with Annovar. These steps are performed using the high-performance Biowulf Linux compute cluster at NIH. Generally, annotated variants are filtered to include only those that are nonsynonymous or in splice sites, within linkage intervals (if available), absent from dbSNP v132, have less than 0.1% frequency in 1094 genomes from the 1000 Genomes Project, 6503 exomes from the Exome Sequencing Project, and 938 exomes from the NHGRI ClinSeq project, and co-segregate with the phenotype among all sequenced family members. Putative candidates are then individually examined in the Integrated Genome Viewer (IGV) to eliminate probable false-positives arising from low coverage or mis-aligned reads, and variants passing this check are validated by Sanger sequencing and tested for co-segregation in all available family members.
Results: We have compared two popular alignment programs, BWA and Novoalign, as well as two popular variant calling tools, SamTools and GATK, and determined that the combination of Novoalign and GATK usually provides the best compromise between specificity and sensitivity for the purposes of Mendelian disease gene identification. Our lab has identified or is currently pursuing the genetic causes of several disorders using the above methodology.
Conclusion: This approach has been most successful for recessive families with consanguinity or multiple affected individuals, dominant families large enough to produce at least suggestive LOD scores in linkage scans, or families with transmitted *de novo* mutations. For small families and single cases the major challenge is that filtered variant lists contain tens or hundreds of candidates, in which case additional family members or new families with the same phenotype must be collected in order to implicate a single candidate gene.
Disclosure of interest: None declared.

A142

PW02-002 - Single MVK mutation and recurrent fevers

K Barron[1*], A Ombrello[1], D Goldsmith[2], I Aksentijevich[1], A Jones[1], D Kastner[1]
[1]National Institutes of Health, Bethesda, PA, USA; [2]Drexel University Coll Med, PA, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A142

Introduction: HyperIgD syndrome is an autoinflammatory disorder caused by mutations in the *MVK* gene. While mutations in most patients follow autosomal recessive inheritance, we have identified a cohort of patients with recurrent fevers and only 1 mutation in the *MVK* gene.
Objectives: To compare clinical features in those with 1 vs. 2 *MVK* mutations and to report therapeutic responses in all.
Patients were evaluated at the NIH. Clinical and laboratory information were collected at each visit.
Methods: Patients were evaluated at the NIH. Clinical and laboratory information were collected at each visit.
Results: 31 pts with mutations in *MVK* were evaluated: 22 had 2 mutations (21 with V377I and 1 other mutation; 1 with V203A/H380R), 9 had only 1 mutation after testing the whole gene (8 with V377I, 1 with I268V). The carrier frequency of V377I in our control Caucasian population is 0.3% (2/739). In contrast, in 344 independent cases of recurrent fever submitted for *MVK* testing, 8 bore a single copy of V377I for a frequency of 2.3%.
Clinical or laboratory presentation at the time of a flare was compared between the 2 groups. There was no significant difference with regard to age of onset, duration of flares, frequency of flares, flares after immunizations, GI symptoms, oral ulcers, sore throat, arthralgia, or adenopathy associated with flares. Rash was more common in pts with 2 mutations, 20/22 compared to 4/9 in those with one mutation (p=.01). While there was no difference in level of IgG, IgA was increased in those with 2 mutations (452 ± 230 mg/dl) compared to those with 1 mutation (230 ± 175) (p=.01), as well as level of IgD, (95 ± 95, 2 mutations, vs. 8.3 ± 7.4, 1 mutation, p=.01).

Since there was no significant difference in clinical presentation, other than presence of rash and levels of IgA and IgD, pts were considered together to evaluate their therapeutic responses. Of 8 pts treated with colchicine, 7 reported no response, 1 reported some improvement. Of 27 pts treated with prednisone at the time of a flare, 18 noted some improvement; 7 reported either none or shortening of the interval before next flare. Of 15 pts receiving montelukast, 4 reported some improvement; 11 reported none. Of 19 pts receiving intermittent anakinra at the time of a flare, 13 reported some improvement, 3 too early to assess efficacy, and 3 no improvement including one who developed acute renal failure. 5 pts received daily anakinra, with 4 reporting some improvement and 1 too early to assess. Of 9 pts receiving etanercept, 4 reported improvement, 5 report none.

Conclusion: Aside for the presence of rash and higher IgA and IgD levels in those children with 2 *MVK* mutations, there are no significant clinical differences between these groups. There are no clear trends that allow identification or predictability of the disease course in children with either 1 or 2 mutations. Given the higher frequency of V377I heterozygotes in our patient cohort compared to the general population, our data suggest that under some circumstances this may be associated with recurrent fevers. Therapeutic options for children with *MVK* mutations include intermittent prednisone or anakinra, either given intermittently or daily; however, not all patients respond to therapy and there are associated adverse events in some patients.

Disclosure of interest: None declared.

A143

PW02-003 - Efficacy of anakinra in etanercept-resistant TRAPS

AK Ombrello[1*], PM Hoffmann[1], A Jones[1], KS Barron[2], DL Kastner[1]
[1]NHGRI, National Institutes of Health, Bethesda, USA; [2]NIAID, National Institutes of Health, Bethesda, USA

Pediatric Rheumatology 2013, **11(Suppl 1):**A143

Introduction: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autoinflammatory disease inherited in an autosomal dominant fashion. TRAPS develops secondary to mutations in *TNFRSF1A*. Associated symptoms include periodic attacks of peritonitis, constipation, arthritis in large joints, arthralgia, migratory rash with underlying myalgia, periorbital edema, conjunctivitis, splenomegaly, and increased risk for AA amyloidosis. Typically, attacks last from days to weeks. The common treatment modalities are corticosteroids, the p75 TNFR:Fc fusion protein, etanercept, and IL-1 antagonists. Recent studies suggest that multiple cytokines are involved in the pathogenesis of TRAPS. To date, there are limited data comparing the efficacy of etanercept and IL-1 inhibitors in TRAPS.

Objectives: To explore the efficacy of anakinra in nine TRAPS patients who had modest response while on etanercept.

Methods: CRP and ESR were measured serially in nine patients with TRAPS (eight adults and one child) who had been initially treated with etanercept and were subsequently switched to anakinra. Patient records were evaluated for clinical and laboratory associations. Patients with the R92Q and P46L variants were excluded from our analysis.

Results: Eight adult patients and one child with TRAPS were switched from etanercept to anakinra treatment due to poor symptom control and persistent elevation in inflammatory markers. Among all nine patients, the range of ESR before starting anakinra (no patients were actively flaring at the time labs were drawn) was 37-91 mm/hr and after was 5-18 mm/hr. The range of CRP before starting anakinra was 18.10-186 mg/L and after was <0.5-8.85 mg/L. The etanercept doses ranged from 50 mg weekly to 75 mg weekly. The anakinra doses ranged from 100 mg daily to 300 mg daily. One patient with AA amyloidosis had normalization of proteinuria and stabilization of creatinine within 16 months of starting anakinra. Patients reported fewer flares, shorter duration of flares, and decreased necessity for additional medications during flares (corticosteroids and narcotics).

Conclusion: Our findings indicate that in some patients, anakinra is superior to etanercept for the treatment of TRAPS. Of the nine patients, all of them experienced clinically significant decreases in inflammatory markers including CRP and ESR as well as clinical improvement in symptoms related to TRAPS upon the initiation of anakinra.

Disclosure of interest: None declared.

A144

PW02-004 - Autoinflammatory syndromes: a clinical review

CM Biggs[*], JS Hausmann, S Kim, E Janssen, P Nigrovic, R Fuhlbrigge, R Sundel, F Dedeoglu
Program in Rheumatology, Division of Immunology, Boston Children's Hospital, Boston, MA, USA

Pediatric Rheumatology 2013, **11(Suppl 1):**A144

Introduction: Autoinflammatory syndromes are a group of rare conditions that cause intermittent episodes of fever and organ system inflammation. The majority of these conditions have been linked to single gene mutations that are involved in the acute inflammatory response. Many of these monogenic disorders, such as Familial Mediterranean Fever (FMF) and Hyper IgD syndrome (HIDS), are more prevalent in certain geographic locations such as Europe and the Mediterranean basin. It is therefore not surprising that the literature describing these conditions has largely originated from these regions. Research on these conditions so far suggests variable presentations due to a variety of modifiers such as genetic polymorphisms and/or environment. There is currently a paucity of studies from mixed populations, such as those found in urban North American centers.

Objectives: To describe the clinical and laboratory features of children with autoinflammatory syndromes as seen in a large, North American pediatric rheumatology clinic.

Methods: We conducted a retrospective chart review of children evaluated for periodic fevers from 2002-2012 at the Rheumatology Clinic in Boston Children's Hospital. Charts were first identified by searching for key words or billing codes related to autoinflammatory syndromes. A manual chart review was then performed to confirm appropriate case identification. Diagnosis of autoinflammatory syndromes was based on expert opinion as well as on genetic testing if available.

Results: One hundred and seventy-four patients were found by the initial search, and 84 patients were excluded after Periodic Fever, Aphthous Stomatitis, Pharyngitis and Adenitis (PFAPA) was identified as the most likely diagnosis. FMF was identified as the most likely diagnosis in 37 patients. Mutations in the MEFV gene were found in 63% of the 27 patients with suspected FMF who underwent genetic testing; 65% had a single heterozygous mutation, while 35% had either 2 homozygous or 2 heterozygous mutations. Thirty-two FMF patients were treated with colchicine and had subsequent follow up, with 100% showing at least a partial response. None of the patients in the FMF group were diagnosed with amyloidosis during an average of 5.3 years of follow-up. Four patients were diagnosed with TNF Receptor Associated Periodic Syndrome (TRAPS) or TRAPS variant, all carrying the R92Q mutation in the TNFRSF1A gene. HIDS was identified in 4 patients, all with confirmed mutations in the MVK gene. Three patients were diagnosed with Cryopyrin Associated Periodic Syndrome (CAPS), with one patient having an identified mutation in the CIAS1 gene. Thirty-nine patients were diagnosed with an undefined autoinflammatory syndrome. Mutations in genes associated with autoinflammatory syndromes were found in 26% of the 19 patients in the undefined group who underwent genetic testing. Eighty-three percent of the 23 patients in the undefined group who were treated with colchicine had at least a partial response.

Conclusion: This study demonstrates the genetic and phenotypic diversity seen in a mixed North American population. More research is needed to further characterize the clinical features of autoinflammatory syndromes in this population.

Disclosure of interest: None declared.

A145

PW02-005 - A web registry of genotype-phenotype correlation

M Doglio[1*], R Papa[1], R Caorsi[1], S Federici[1], M Finetti[1], A Naselli[1], N Ruperto[1], A Martini[1], I Ceccherini[2], M Gattorno[1]
[1]Pediatria 2, IRCCS Istituto Giannina Gaslini, Genoa, Italy; [2]Laboratorio di Genetica Molecolare, IRCCS Istituto Giannina Gaslini, Genoa, Italy

Pediatric Rheumatology 2013, **11(Suppl 1):**A145

Introduction: The possible range of clinical manifestations associated to the different mutations associated to autoinflammatory disorders is still largely unknown. A registry of hereditary auto-inflammatory disorders

mutations is available on the web (Infevers, http://fmf.igh.cnrs.fr/ISSAID/infevers/). This registry gathers updated information on all mutations responsible for hereditary inflammatory disorders. The clinical phenotype associated with the single mutation in the first reported case is also available.
Objectives: To provide a web page with the description of the genotype-phenotype correlation found in all the patients enrolled in an international registry for Autoinflammatory diseases (EUROFEVER).
Methods: For each disease, we created a table describing the correlation between genotype and phenotype in all the patients enrolled in the EUROFEVER registry. In autosomal dominant diseases (CAPS, TRAPS and PAPA syndrome) all mutations were analyzed individually. In autosomal recessive diseases (FMF and MKD), the clinical phenotype of homozygous patients was described. For patients with compound heterozygosis the description of all possible combinations is given. For each mutation, the following items are shown: i) number of patients described, ii) mean age of onset; iii) disease course (recurrent or chronic); iv) prevalent clinical manifestations and duration of fever episodes; v) atypical manifestations; vi) response to treatment; vii) complications.
Results: We analyzed the genotype-phenotype correlation of 666 patients (313 FMF, 108 CAPS, 72 MKD, 158 TRAPS and 15 PAPA) enrolled in the registry and validated. A summary of the main clinical features associated to 48 variants of *TNFRFS1A*, 33 variants of *MVK* (with 35 combinations for compound heterozygous); 25 variants of *NLRP3*, 16 variants of *MEFV* (with 42 combinations for compound heterozygous) and 6 variants of *PSPTPI1* was performed. For each disease a table with all variable described in method section has been established. A dedicated webpage is on construction in the EUROFEVER web-site (http://www.printo.it/eurofever).
Conclusion: We provide a useful tool for all the clinicians, creating a web page for the consultation of the correlation between genotype and phenotype in autoinflammatory diseases based on the patients enrolled in the Eurofever registry. This tool is complementary to Infevers database and will be implemented in parallel with the registry.
Disclosure of interest: None declared.

A146

PW02-006 - PAPA syndrome clinical spectrum and IL1B release

A Omenetti[1,2*], R Caorsi[1,2], S Carta[3], L Delfino[3], A Martini[1,2], A Rubartelli[3], M Gattorno[1]
[1]UO Pediatria II, G Gaslini IRCCS, , AOU San Martino - IST, Genoa, Italy; [2]University of Genoa, AOU San Martino - IST, Genoa, Italy; [3]Cell Biology, IRCCS, AOU San Martino - IST, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A146

Introduction: Pyogenic sterile Arthritis Pyoderma gangrenosum and Acne (PAPA) syndrome is a rare autosomal dominant inherited autoinflammatory disease caused by mutations in Proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1). In childhood, the syndrome is featured by recurrent sterile, erosive arthritis, potentially leading to joint destruction. By puberty, cutaneous symptoms become predominant, with recurrent onset of pathergy, abscesses, severe cystic acne, and pyoderma gangrenosum. Typically, both articular and cutaneous outcomes occur following a minor trauma. PSTPIP1 may interact with NLRP3 and caspase-1 but a clear involvement of IL-1β is still controversial. While anti-IL1 treatment seems to be effective on joint manifestations, IL inhibition does not display the same effectiveness in the management of skin lesions.
Objectives: To investigate in our PAPA cohort whether 1) PSTPIP1 mutated monocytes display enhanced IL1β secretion; 2) different PSTPIP1 mutations and/or clinical manifestations and disease activity correlate with degree in IL1β pathway activation; 3) IL1β release is mediated by NLRP3.
Methods: Fourteen PAPA patients were examined. Thirteen genetically confirmed patients (2 children and 11 adults) carrying different PSTPIP1 mutations (N=11 E250Q, N=1 E250K, N=1 E256G) and 1 pediatric patient genetically negative for common PSTPIPI1 variants, were analyzed and compared to 30 healthy donors (HD). Peripheral blood primary human monocytes were freshly isolated and studied at baseline and after 3-6-18 hours (h) of LPS-induced *in vitro* activation, and pattern of IL-1β secretion was assessed by ELISA. The involvement of NLRP3 was investigated by *in vitro* silencing.
Results: Monocytes isolated from PAPA patients tend to secrete higher levels of IL1β but variability occur even in the presence of the same PSTPIP1 variant. IL1 β secretion is higher in patients displaying prevalent articular vs skin manifestations, and increases in the presence of acute phase reactants elevation and/or joint/skin lesions. The blockage of NLRP3 activity leads to IL1 release inhibition in both PAPA and HD monocytes.
Conclusion: IL1 β secretion is higher in PAPA patients displaying prevalent articular vs skin manifestations, correlates with disease activity and is mediated by NLRP3.
Disclosure of interest: None declared.

A147

PW02-007 - The Eurofever registry: 3 years of enrollment

S Federici[*], J Frenkel, S Ozen, A Jordi, JI Arostegui, F De Benedetti, P Dolezalova, H Girschick, V Hentgen, M Hofer, H Lachmann, I Koné-Paut, J Kuemmerle-Deschner, B Neven, H Ozdogan, C Rose, A Simon, S Stojanov, N Toplak, I Touitou, R Vesely, P Woo, C Wouters, N Ruperto, A Martini, M Gattorno
for PRINTO and Eurofever Project, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A147

Introduction: The main limitation to a better knowledge of Autoinflammatory diseases is related to the extreme fragmentation of the diagnosed cases that are spread over different centers and countries. The general aim of the Eurofever Project (agreement n 2007332, EAHC) is to build an international registry on Autoinflammatory diseases.
Objectives: To evaluate the number of patients enrolled in the registry in the first 36 months after starting the enrolment.
Methods: A web-based registry collecting baseline and cross-sectional clinical information on Autoinflammatory diseases is available in the member area of the PRINTO web-site (www.printo.it). The registry is open to all pediatric and adult Centers with a specific interest in Autoinflammatory diseases. The following monogenic autoinflammatory diseases were considered: Familial Mediterranean Fever (FMF), Cryopyrin-associated periodic syndromes (CAPS), TNF receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), Blau syndrome, pyogenic arthritis, pioderma and acne (PAPA) syndrome, deficiency of IL-1 receptor antagonist (DIRA), NLRP12-mediated periodic fever. Information on CRMO, Behçet's disease, PFAPA and undefined periodic fevers were also collected.
Results: 2721 patients, from 221 centers in 56 countries, have been enrolled in the registry during the first 36 months. Baseline demographic data (country of residence, disease onset, disease duration, mutations, family history ect) from all patients are now available. In 2213 (81%) complete information on clinical manifestations and responses to treatments is also available. The disease distribution of enrolled patients is: FMF 787 (621 with complete clinical data); TRAPS 237 (211 with with complete clinical data); CAPS 207 (186 with complete clinical data); MKD 153 (133 with complete clinical data); Blau syndrome 62 (21 with complete clinical data); PAPA 19 (18 with complete clinical data); NLRP-12 mediated periodic fever 8 (6 with complete clinical data); DIRA and Majeed 3 and 2 patients, respectively (all with complete clinical data).Among multifactorial autoinflammatory diseases: PFAPA 564 (402 with complete clinical data); CRMO 392 (370 with complete clinical data); pediatric Behçet disease 84 (68 with complete clinical data) and 205 patients with undefined periodic fever (174 with complete clinical data).
Conclusion: A large registry of patients with Autoinflammatory diseases is available and the enrolment is still ongoing. In the next months novel inherited autoinflammatory diseases (CANDLE, DITRA) will be included and data collection will become longitudinal.
Disclosure of interest: None declared.

A148

PW02-008 - TRAPS in the real world: an international registry

HJ Lachmann[*], R Papa, K Minden, L Obici, L Cantarini, J Frenkel, J Anton, I Koné-Paut, M Cattalini, B Bader-Meunier, N Ruperto, A Martini, I Touitou, P Woo, M Gattorno
for PRINTO, Eurotraps/Eurofever Projects, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A148

Introduction: TRAPS is an autosomal dominant disease due to mutations in the TNFRSF1A gene.
Objectives: To analyze the clinical features of TRAPS and genotype/phenotype correlations of TRAPS patients enrolled in the Eurofever/Eurotraps registry.

Methods: The Eurofever Project (agreement n 2007332, EAHC) is aimed to build a common web-based registry for all Autoinflammatory diseases in collaboration with the Eurotraps Project (FP7, HEALTH-F2-2008-200923). A web-based registry collecting demographic and clinical information on Autoinflammatory diseases is available in the member area of the PRINTO web-site.
Results: 158 TRAPS pts were enrolled from November 2009 to June 2012. analyzed. The majority of pts were adults (105, 67%). The median age at disease onset and at disease diagnosis were 4.1 (range 0.2-63 years) and 25.9 (0-77) years, respectively. The median delay of diagnosis was 10.3 years, with a rate of delay 10 times higher in adult patients. Patients were enrolled by 18 centers in 11 countries, with 18 different countries of origin. 145 (91%) were European Caucasians, 4 Arabs, 3 black Africans, 2 Asiatics, 2 Ashkenazi Jewish, 2 had a mixed origin. 58 pts (36.7%) carried high-penetrant mutations (T50M or involving cysteine residues), 30 pts (19%) other missense mutations previously associated to TRAPS phenotype. Low-penetrant or hypomorphic variants, such as R92Q and P46L, were found in 53 and 5 pts, respectively. 12 pts carried mutations with uncertain association with TRAPS phenotype. High penetrant mutations were observed in 50/123 (40%) pts with pediatric onset and in 8/35 pts (22.9%) with an adult onset ($p < 0.001$). The prevalence of patients carrying low-penetrant or hypomorphyc mutations were two time higher in the adult onset (48%) compared with pediatric onset (25.2%) ($p < 0.001$).
Mean duration of fever episodes was 10.6 days with mean number of 6.6 episodes/year. In one third of the cohort a mean duration of fever episodes was shorter than 1 week. The most frequent symptoms accompanying attacks were fever, limb pain (arthalgia, myalgia), abdominal pain and a variety of skin rashes. The prevalence of a number of clinical manifestations significantly differ among the age of onset. Pediatric patients displayed an increased prevalence of enlarged cervical lymph nodes abdominal pain and periorbital edema in respect to patients with an adult onset. Adult onset was characterized by an higher prevalence of respiratory symptoms (chest pain, persistent cough), serositis and polyarthritis. The most common long term complication was amyloidosis, that was observed in 16 (10%) adult patients. The mean age at onset of amyloidosis was 43 years (range 20-77). Patients who developed AA amyloidosis had significantly longer disease duration than those who have not (39 years versus 19.4 years; p=0.001).
Conclusion: TRAPS has a wide geographical distribution and can be observed in different ethnicities. Short episodes (less than 1 week) are observed in 30% of patients. The presentation of the disease in adulthood significantly differs from that observed in pediatric age.
Disclosure of interest: None declared.

A149

PW02-009 - PAPA syndrome: results from the Euroefever registry

R Caorsi[1*], A Insalaco[2], D Marotto[3], J Frenkel[4], A Martini[1], F De Benedetti[2], M Gattorno[1],
Paediatric Rheumatology International Trial Organization (PRINTO) and the Eurofever Project
[1]2nd Division Of Pediatrics, Istituto Gaslini, Genoa, Genova, Italy; [2]Department of Pediatrics, Ospedale Pediatrico Bambino Gesù, Rome, Italy; [3]Department of Rheumathology, Ospedale di Tempio Pausania, Tempio Pausania (OT), Italy; [4]Department of Pediatrics, University Medical Center, Utrecht, The Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A149

Introduction: PAPA syndrome is a very rare autoinflammatory condition. Few data are nowadays available about the clinical characteristic, the response to treatment and the outcome of this disease.
Objectives: to analyse the data of the PAPA patients enrolled to the Eurofever registry.
Methods: The data analysed in the study were extracted from the Eurofever registry, which is hosted in the PRINTO website (www.printo.it). The patients were included in the study in the presence of clinical manifestations consistent with PAPA syndrome and mutations in the PSTPIP1 gene. Demographic data, clinical manifestations and response to treatment were analysed.
Results: In February 2013 baseline and clinical information were available of 2567 patients from 88 centers in the Eurofever registry. Of these 16 patients PAPA patients (M:F = 8:8), from 3 different centers, fulfilled the inclusion criteria and were therefore analysed: 10 were of the same family, in 3 patients the disease was caused by a *de novo* mutation while in 3 cases the mutation was found in one parent (not yet included in the registry). The mean age at enrolment was 26,22 years (4 paediatric and 12 adult patients). The mean age at disease onset was 5,7 years (range birth – 18 years). The mean age at diagnosis was 24,5 years (range 1,8 – 57,5), with a mean delay of 18,8 years (range 2 months – 50 years). The mutations found in the PSTPIP1 gene were V344I (1pt), E250K (1 pt), E257G (1 pt), A230T (2 pts), and E250Q (11 pts).
The disease course was recurrent in 8 patients, while the other 8 presented a chronic disease course with periodic recrudesces. 15 patients presented an articular involvement during their disease course, while 11 patients presented clinical manifestations affecting the skin (folliculitis in 8, pyoderma gangrenosum in 3, skin abscess 8 patients) and 2 patients complained with suppurative hidradenitis. 7 out of the 16 patients presented clinical manifestations not typical of PAPA syndrome (psoriasis, osteolytic bone lesions, chronic renal failure, muscular abscesses, anaemia and hepatosplenomegaly). Response to treatment with NAIDS was as partial or absent in 8 and 2 patients respectively, while the steroids caused a complete or partial control of disease manifestations in 5 and 6 patients respectively. Two patients were treated with methotrexate with partial response. Etanercept was used in one patient with complete response, adalimumab in 3 patients (2 partial and 1 complete responders) and anakinra in 4 patients (2 partial and 2 complete responders).
Conclusion: The study analyses the largest series of PAPA syndrome patients described so far. The wide clinical heterogeneity and the usual presentation with a single manifestation might be responsible for under-recognition of the syndrome.
Disclosure of interest: None declared.

A150

PW02-010 - The diagnostic challenge of bone lesions in AID

R Caorsi[*], P Picco, A Buoncompagni, S Signa, F Minoia, S Federici, A Martini, M Gattorno
2Nd Division of pediatrics, Istituto Gaslini, Genoa, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A150

Introduction: Osteolytic lesions are the hallmark of a number of inherited (DIRA and Majeed syndrome) and multifactorial (CRMO and SAPHO) autoinflammatory diseases. We report a clinical case in which bone lesions are part of the clinical picture of an additional inherited AID.
Case Report: A 33 months old girl is admitted in our hospital for recurrent episodes of arthritis and bone lesions. At the age of 18 months, she presented arthritis of the left knee associated to low-grade fever, leukocytosis and increased inflammatory markers; the arthrocentesis revealed a small amount of corpuscular liquid with a negative cultural test. The girl was treated with i.v. antibiotic therapy with a partial improvement. In the following months she presented a worsening of the pain and swelling of the left knee, associated to stiffness, and started to complain of pain in the right knee and ankles; the treatment with on demand NSAIDS was poorly effective.
Due to the persistence of these symptoms, at the age of 29 months the child was admitted to another Hospital, where an X-ray of the lower limbs revealed the presence of an osteolytic lesion of about 2.3x0.7 cm, with indefinite margins and periosteal apposition, in the distal diaphysis of the left tibia. The bone scintigraphy showed a metabolic hyperactivity in the same area only. A biopsy of the lesion revealed a pattern consistent with a chronic osteomyelitis.
In the following months the girl complained of pain in the pelvis, legs and hands, with marked morning stiffness and lameness; the blood tests revealed a slight increase of the inflammatory markers. Bone marrow aspiration was negative for malignancies. A diagnosis of CRMO was pointed out. The girl was treated with steroids with a prompt good response but recurrence of the symptoms after discontinuation.
The girl was then admitted to our center. The blood test revealed a slight neutrophilic leukocytosis with elevation of acute phase reactants (ERS 39 mm/1h, CRP 4,28 mg/dl); the X-ray of the left limb confirmed the presence of the osteolytic lesion with periostitis and the whole-body stir-MRI revealed a bilateral alteration of signal in the diaphysis and distal metaphysis of the tibia and in the left heel. The girl was discharged in treatment with NSAIDS with partial control of the bone pain. In the following months she developed arthritis of the right knee associated with low-grade fever. The right knee

ultrasound, X-ray and MRI revealed the presence of intra-articular effusion with synovial vegetations; the arthrocentesis revealed corpuscular liquid with a high rate of polimorfonuclear cells and a negative cultural test. The family history revealed the presence of severe acne in the father and of recurrent episodes of arthritis in the grandmother. In light of that and of the symptoms complained, the PSTPIP1 gene was tested, revealing an E250Q mutation.
Discussion: This case enlightens a clinical overlap between different autoinflammatory diseases that has to be considered in the differential diagnosis. In fact the presence of osteolytic lesions has never been reported in PAPA syndrome, while this clinical manifestation is typical of CRMO, SAPHO, Majeed and DIRA syndrome, in which periostitis isInizio modulo also frequently observed.
Disclosure of interest: None declared.

A151

PW02-011 - Favorable response to anakinra in aisle patients

H Ozdogan[1], M Gattorno[2], S Ugurlu[1], M Di Rocco[2], G Hatemi[1], D Ustek[3], A Gul[4*]
[1]Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; [2]EULAR Centre of Excellence in Rheumatology 2008-13, "G. Gaslini" Scientific Institute, Genova, Italy; [3]Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey; [4]Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A151

Introduction: We previously described a new autosomal recessively inherited autoinflammatory syndrome in two Turkish patients, who were second-degree cousins. Their clinical features included recurrent inflammatory attacks lasting 3-10 days since the first year of life, which are characterized by fever, erythematous or urticarial rash with hyperesthesia, serositis and edema on the face and extremities. Both patients eventually developed a lymphedema symmetrically affecting lower extremities and genitalia. Using a homozygosity mapping approach and targeted capture array based sequencing, we identified a homozygous insertion mutation in the exon 3 of the MyoD family inhibitor domain containing gene (MDFIC), causing a frameshift and inhibiting the translation of its functional cysteine-rich C-terminal domain. We herein report another patient of Italian origin and also their response to anakinra treatment.
Case report: Italian patient, a 11 year-old girl healthy unrelated parents, had a history of caesarean section at 33th week of pregnancy, because of fetal subcutaneous tissue edema and pleural effusion. Within the first 24 h, she developed respiratory distress, and pleural effusion was drained the intensive care unit. She required ventilatory support, antibiotics and diuretics for 2 months. At the age of 9 months hearing loss due to mutation 35delG of GJB2 gene was detected and cochlear implantation was subsequently done. At the age of 2 yr, she started to have attacks of fever, limb edema, severe pleural and pericardial effusions requiring urgent pleural and pericardial fluid drainage. Laboratory investigations showed a severe anemia and elevated acute phase response, and she only responded to high dose methylprednisolone. At the age of 8 yr, she had recurrent attacks during tapering of steroids. Anakinra was started at the dosage of 2 mg/kg/day with a dramatic control of inflammatory manifestations and acute phase response. After 6 months of continuous treatment anakinra was tapered to every other day injections. Sequencing of the MDFIC gene of this patient revealed the same insertion mutation causing a frameshift.
Similarly, two Turkish cousins first tried canakinumab 150 mg every 4 weeks, but they continued to experience attacks on this treatment up to 3 to 4 months. Their treatment switched to anakinra during the last 4 months, and a favorable response (complete in the elder, and partial in the younger) was observed in both clinical and laboratory findings.
Discussion: Identification of the same MDFIC gene mutation in an Italian patient with similar manifestations confirms the association of this variation with AISLE. Differential response to anakinra in those patients may suggest the importance of IL-1 alpha in its pathogenesis.
Disclosure of interest: None declared.

A152

PW02-012 - First clinical description of an infant with DITRA

L Rossi-Semerano[1*], M Piram[1], C Chiaverini[2], D De Ricaud[3], A Smahi[4], I Koné-Paut[5]
[1]Department of Paediatrics and Paediatric Rheumatology, Bicêtre Hospital, National Reference Centre for Auto-inflammatory Diseases, Le Kremlin-Bicêtre, France; [2]Service de dermatologie et centre de référence des épidermolyses bulleuses héréditaires Hôpital Archet 2 CHU de Nice, France; [3]Service de pédiatrie, GCS CHU-Lenval, Nice, France; [4]U781 INSERM, Hôpital Necker Enfants Malades, Paris, France; [5]Department of Paediatrics and Paediatric Rheumatology, Bicêtre Hospital, National Reference Centre for Auto-inflammatory Diseases, Le Kremlin Bicêtre, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A152

Introduction: Interleukin-36-receptor antagonist deficiency (DITRA) is a recently described auto-inflammatory disease[1], characterized by repeated flares of generalized pustular psoriasis, high fever, asthenia and systemic inflammation. This condition is caused by homozygous missense mutation in the *IL36RN* gene, encoding the interleukin-36-receptor antagonist (IL-36Ra), an anti-inflammatory cytokine. We report herein the first exhaustive clinical description of an infant with DITRA, who was successfully treated with anakinra.
Case report: Y.M. is the first son of Tunisian consanguineous parents who developed, at two weeks of life, an erythematous and scaly eruption, with subsequent rapid evolution toward generalized pustular psoriasis. Afterwards, cutaneous flares of diffuse erythematous rash and pustules involving the whole body appeared, with a once weekly periodicity. Intense irritability was present during flares without fever. Moreover, since 1 month of age the infant presented diarrhea, dysphagia and reduced feeding rate, with failure to thrive. Laboratory tests during acute flares showed marked leukocytosis, thrombocytosis and anemia without C-reactive protein elevation. Skin biopsy and clinical presentation were consistent with pustular psoriasis, nevertheless, the patient did not respond to high-potency topical corticosteroids and retinoid acid.
As the patient presented repeated skin flares early after birth, as well as serious constitutional distress with failure to thrive, an auto-inflammatory syndrome like DIRA (interleukine-1 receptor antagonist deficiency) [2] or DITRA was considered. The hypothesis was reinforced by parental consanguinity, and absence of skin lesions improvement under standard topical treatment. Genetic analyses showed a homozygous mutation in the *IL36RN* gene (L27P) which represents the same mutation recently described in DITRA patients[1,3]. At 6 months we started treatment with the recombinant IL-1 receptor antagonist anakinra with efficacy both on constitutional symptoms and skin involvement.
Discussion: To the best of our knowledge, we report the first detailed clinical description of an infant with DITRA. Even if neonatal onset has been already reported[1], no detailed clinical description was provided, probably due to late diagnosis. Our clinical report brings new clinical characteristics and educational iconography.We even report, for the first time, a favorable clinical response of this disease to anakinra treatment.
Disclosure of interest: L. Rossi-Semerano: None Declared, M. Piram: None Declared, C. Chiaverini: None Declared, D. De Ricaud: None Declared, A. Smahi: None Declared, I. Koné-Paut Grant / Research Support from: Educational and research grant from Swedish Orphan Biovitrum, Consultant for: Consultant fee from Novartis.

References

1. Marrakchi S, Guigue P, Renshaw BR, *et al*: **Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis.** *N Engl J Med* 2011, **365(7)**:620-8.
2. Aksentijevich I, Masters SL, Ferguson PJ, *et al*: **An Autoinflammatory Disease with Deficiency of the Interleukin-1–Receptor Antagonist.** *N Engl J Med* 2009, **360(23)**:2426-2437, doi:10.1056/NEJMoa0807865.
3. Onoufriadis A, Simpson MA, Pink AE, *et al*: **Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis.** *Am J Hum Genet* 2011, **89(3)**:432-437.

A153

PW02-013 - The role of IL6 and LPS in pathogenesis of TRAPS

S Savic[1*], L Ouboussad[2], LJ Dickie[2], CH Wong[2], R Doble[3], W Miriam[4], MF McDermott[2]
[1]Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK; [2]Experimental rheumatology, NIHR-Leeds musculoskeletal biomedical research unit, Leeds, UK; [3]University of Leeds, Leeds, UK; [4]Dermato-rheumatology research group, NIHR-Leeds musculoskeletal biomedical research unit, Leeds, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A153

Introduction: Tumour necrosis factor (TNF) receptor– associated periodic syndrome (TRAPS) is a hereditary autoinflammatory condition resulting

from a range of mutations in the TNFRSF1A gene. It is characterised in part by recurrent episodes of inflammation, affecting connective tissues, and manifesting as migratory erysipelas, myalgia and serositis. A number of aberrant inflammatory responses have been described in this condition, including hyperresponsiveness to lipopolysaccharide (LPS). The microRNA mir155 has been implicated in mediating downstream pro-inflammatory response to LPS, and it has has also been implicated in the pathogenesis of rheumatoid arthritis (RA). Tocilizumab (anti-IL6 receptor antibody) has been reported to be an effective treatment in at least one published case of TRAPS.

Objectives: To investigate the effects of LPS challenge on TRAPS fibroblasts and the roles of IL-6 and mir155 in this process.

Methods: A patient with a T50M mutation who had previously failed to respond to etanercept and anakinra was consented to treatment with tocilizumab. Skin fibroblasts were obtained by biopsy and were propagated ex-vivo. The effects of LPS, IL-1 and TNF on IL-6 production were compared between patient's fibroblasts and fibroblasts obtained from a healthy control (HC). TRAPS and HC fibroblasts, as well as synovial fibroblasts (SFs) from RA and osteoarthritis (OA) patients were then subjected to a graded LPS challenge. IL-6 levels were quantified from media supernatants using ELISA kits (BD Biosciences). mir155 levels were determined using qPCR from total RNA.

Results: The patient's fibroblasts showed greater production of IL-6 in response to LPS and IL-1 but not TNF when compared to HC. In the case of RA and OA SFs there was a dose dependent increase in mir155 levels, whilst, in the case of TRAPS fibroblasts, the response was not dose dependent but maximum levels were achieved even at the minimal dose of LPS (dose range 0.1 ng/l-100ng/l). The patient had a good clinical response to tocilizumab with a reduction in the symptoms, decreased steroid use and resolution of the biochemical markers of inflammation.

Conclusion: Skin fibroblasts are capable of responding to LPS with production of mir-155 and IL-6. This response appears to be particularly enhanced in TRAPS fibroblasts, which may be one of the reasons why a number of clinical manifestations localise to the connective tissue. The ex-vivo experiments, showing hyperresponsiveness to LPS and the effective use tocilizumab in this patient would support a role for IL-6 in the pathogenesis of TRAPS.

Disclosure of interest: None declared.

A154

PW02-014 - Long term outcome and quality of life in TRAPS

T Lane*, A Bybee, NM Stewart, DM Rowczenio, JD Gillmore, R Al-Nackkash, H Trojer, AD Wechalekar, PN Hawkins, HJ Lachmann
National Amyloidosis Centre, University College London, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A154

Introduction: TRAPS is a rare autosomal dominant disease. The long term outcome in adults has been relatively little studied.

Objectives: We describe a cohort of 80 patients with sequence variants in *TNFRSF1A* who attended our centre for diagnosis and management.

Methods: We examined medical and laboratory records for investigation and test results including genotype, phenotype, treatments and treatment responses, and organ function.

Results: Between 2000 and 2012, 80 individuals with sequence variants and clinical disease were identified: 26 (33%) with one of the pathogenic variants of undetermined significance, R92Q or P46L; and 54 (67%) with one of a variety of other known or novel pathogenic variants. 32 (40%) were male, and 42 (53%) had a positive family history.

Of the 54 patients with a pathogenic sequence variant, 21 (39%) were not on long term treatment: 15 did not have persistent symptoms and so were treated with intermittent steroids (5 with intronic/splice junction mutations, 2 C33Y, and 1 each having H66L, D12E, C55Y, H22R, C43G, V83M, C88Y, C96Y); 1 patient (T50M) was refractory or intolerant of other treatments; 5 patients elected not to have treatment (1 each with C29F, C33Y, H22Q, D42del, T50M).

33 (61%) patients were on long term treatment for persistent and/or disabling disease symptoms: 29 (88%) on anti-IL-1 agents, 3 (9%) on anti-TNF and 1 (3%) on daily steroid therapy. 19 were treated with anakinra (4 C33Y, 3 T50M, 2 H22R and D42del, and 1 each of R92P, I57S, C55Y, H22Q, C30Y, T37I, F60L, and 1 with a novel in-frame deletion of 24 nucleotides [c.255_278del] in exon 3); all but 1 experienced a complete remission (CR) of disease. 10 patients were treated with canakinumab (2 C33Y, 2 T50M, and 1 each with C30R, Y38S, C43G, D42del, T37I, F60L); 9 have had a CR and 1 patient was not evaluable. 3 were treated with etanercept, 1 (C33Y) with CR and 2 (1 with T50M and the other with intronic substitution c.626-32G>T in intron 6) having intermittent steroids as well with only partial remission (PR, defined as good but incomplete resolution of symptoms or serum inflammatory markers). 1 patient (C33Y) with severe disease proving refractory to other therapies was treated with steroids 30mg daily, achieving PR.

Health-related quality of life (QoL) was surveyed in 29 patients with a pathogenic sequence variant, 10 who were treated only with intermittent steroids as their phenotype was not severe enough to warrant use of biologics, and 8 were surveyed before and after commencement of biologic therapy. In the latter group, post-treatment scores in physical, role and social functioning and bodily pain, general health and vitality were all greatly improved from prior to treatment. Interestingly, those who were only treated with intermittent steroids showed scores comparable with the post-treatment scores of the group treated with biologics, showing the impact of the untreated, more severe phenotype on QoL.

Conclusion: This cohort shows the great variability in genotype and phenotype associated with sequence variants in *TNFRSF1A*. Those with a less severe phenotype may enjoy a good quality of life, and this may also be achieved by those with more severe disease provided they are being treated effectively.

Disclosure of interest: None declared.

A155

PW02-015 - Eight years HPFS experience in a single UK centre

DM Rowczenio*, H Trojer, G Wang, PN Hawkins, HJ Lachmann, A Baginska, T Russell, R Al-Nackkash, A Bybee
Medicine, University College London, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A155

Introduction: In 2004 we established a formal clinical service for patients with hereditary periodic fever syndromes (HPFS). Patients are either referred directly for clinical evaluation, or undergo initial genetic screening on receipt of blood or DNA sample and clinical details via our secure online request procedure: http://www.ucl.ac.uk/medicine/amyloidosis/nac/genetic_testing

Objectives: To evaluate eight years experience of a dedicated fever clinic and associated laboratory service at the National Amyloidosis Centre.

Methods: Between 2004 and 2009 the basic genetic screen encompassed FMF gene *MEFV* exons 2 and 10; TRAPS gene *TNFRSF1A* exons 2-6; MKD gene *MVK* exons 9 and 11 and CAPS gene *NLRP3/CIAS1* exon 3. When deemed appropriate, these tests were extended to additional exons on a case by case basis. From 2010 additional genes were added to our repertoire: *NOD2* (exons 2 and 4) associated with Crohn's Disease and Blau Syndrome; *NLRP12* (exons 2 and 3) associated with familial cold autoinflammatory syndrome 2 and *IL36RN* (exons 2-5) associated with DITRA.

In each case the specific analyses were determined by the NAC physicians after clinical assessment or on the review of information provided by the external clinician.

Results: Since January 2005, 3063 patients have undergone genetic screening at the NAC; 996 were assessed directly at the fever clinic (33%), and blood or DNA were received on a further 2067 (67%). *MEFV* was most frequently requested (75%), followed by *TNFRSF1A* (58%), *MVK* (42%) and *NLRP3* (29%), other genes accounted for 6%.

Genetic variants were identified in 1048 patients (34%): 627 (60%) had an amino acid variation in *MEFV* (56% had a single variant including 34% who had E148Q, 33% were compound heterozygotes and 11% homozygotes). *TNFRSF1A* variants were found in 133 cases (13%); *MVK* in 74 (7%) (in 58% we were unable to detect a second variant despite screening of all exons); *NLRP3* in 103 (16%) and *NOD2* in 46 (4%). In 19 patients we identified genetic aberrations in more that one HPFS gene. 28 novel variants were discovered: 9 in *NLRP3*; 6 in *TNFRSF1A*; 7 in *MVK*; 5 in *MEFV and* 1 in *NOD.*

Conclusion: Since creation of the HPFS clinical service in 2004, demand for genetic testing has grown substantially, including a 125% increase in referrals during the past year. In many cases two or more HPFS genes were screened. We found 1234 genetic variants in 1048 screened cases, of which nearly 40% accounted for low penetrance sequence variants of undetermined significance including *MEFV* E148Q, *TNFRSF1A* R92Q and P46L, *MVK* S52N and *NLRP3* V198M and Q703K. Interestingly we found 19 patients with variations in more than one HPFS gene. In 16 of these we were able to make a clinical diagnosis: 7 had CAPS; 5 MKD; 3 TRAPS and 1 FMF; the remaining 3 had atypical autoinflammatory phenotypes.

An overlap in clinical features between different HPFS highlights the importance of genetic testing in providing accurate diagnosis, leading to appropriate treatment and improvement of quality of life.
Disclosure of interest: None declared.

A156

PW02-016 - 41 cases of TRAPS, a rare autoinflammatory disease

M-C Chastang[1*], M Desjonqueres[1], V Hentgen[2], P Quartier Dit Maire[3], G Grateau[4], I Kone-Paut[5], B Bader-Meunier[3], C Wouters[6], I Lemelle[7], P Cochat[1]
[1]Hopital Femme Mère Enfant CHU Lyon, Lyon, France; [2]CH Versaille, Le Chesnay, France; [3]Hopital Necker Enfants Malades, Paris; [4]CH Tenon, Paris; [5]Bicetre, Le Kremlin Bicetre, France; [6]University Hospital Leuven, Leuven, Belgium; [7]Brabois Enfant, Nancy, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A156

Introduction: TRAPS (TNF receptor associated periodic syndrome) is a rare autoinflammatory disease that can affect children and adults. It is caused by the mutation of TNFRSF1A encoding for the TNF receptor. The main complication is amyloidosis.
Objectives: The aim is to increase knowledge about the disease in order to make the diagnostic easier. Another purpose is to analyse the biotherapy treatment in TRAPS.
Methods: It consists in a retrospective descriptive multicentre study in French and Belgian hospitals. Data has been directly collected thanks to patient files.
Inclusion criteria are: presence of TNFRAF1A mutation, recurrent symptoms.
Results: We have included 25 children and 16 adults (isolated cases and 9 families), coming from France (45%), south of Europe (22%), north of Europe (10%), Maghreb (9%), east of Europe (6%).
19,5% of the patients have had an appendectomy. 26 patients have recurrent fever in their family, among which 22 have TRAPS. Two kids have homozygous mutation for MEFV and one heterozygous.
The disease starts mainly before the age of 5 years (61,1%) but for 13,5%, it begins in adulthood. The average time of diagnosis (delay between first symptoms and diagnosis) is 12,9 years.
51% of R92Q heterozygous mutation, 10% of T50M, 7% de L67P, 5% C29S, 5% C43S have been encountered. 2% of the patients have R92Q homozygous, 2% Q82R and R92Q heterozygous.
The seizures occur 9,7 times a year on average (<1 to 48 times a year), last 10,8 days on average (1 to 49 days). A trigger exists in 43.9% of the cases.
78% have rheumatologic symptoms, 70,7% arthralgia (mainly knees, spine, elbows), 22% arthritis (small and big joints). 24,4% have chest pain, 7,3%serositis. Dermatological symptoms (70,7%) are frequent (56,1% rash). Lots of patients have abdominal pain (70,7%), myalgia (65,7%), asthenia (48,8%). Headache is present in 39% of this population. Only 3 patients have periorbital oedema.
Between the seizures there is no symptomatology, but in 24% of the cases inflammatory syndrome persists. We note the interest to dose the Serum Amyloid A to detect the activity of disease between the attacks.
The screening of proteinuria was positive in 29% of the cases but no amyloidis has been reported.
No interesting correlation was found between genotype and phenotype.
Corticosteroids were used for treatment of seizures. Only 9 patients were treated by biotherapy. Etanercept was efficient in a first time, but not always in the long term. Anakinra always allowed remission.
Conclusion: 77%of this population of TRAPS has 3 symptoms among arthralgia, rash, abdominal pain, myalgia, asthenia and headache. Etanercept is not always efficient and Anakinra is probably a good alternative for the treatment. The inscription of the patients in autoinflammatory disease registers would allow a better knowledge of TRAPS.
Disclosure of interest: None declared.

A157

PW02-017 - New tools for the ISSAID society website

I Touitou[*], F Milhavet
Laboratoire de génétique des maladies rares et auto-inflammatoires, CHRU Montpellier, Montpellier Cedex 5, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A157

Introduction: The **ISSAID** (International Society for Systemic Auto-Inflammatory Diseases) born in November 2005, is supported by a website portal.
Objectives: The purpose of the website is to:
• gather resources related to the systemic auto-inflammatory diseases in order to facilitate contacts between interested physicians and researchers.
• provide support to share and rapidly disseminate information, thoughts, feelings and experiences to improve the quality of life of patients and families affected by systemic auto-inflammatory diseases, and promote advances in the search for causes and cures.
Methods: Several existing items have been improved to provide extensive. Others have been developed. As decided at the last **ISSAID** council meeting in Amsterdam, access to specific sections of the **ISSAID** website is subjected to the payment of annual membership dues.
Results: Improved items:
1. basic information
2. society missions and membership
3. previous and future meetings content
4. mutation and patient registries
5. image library showing characteristic physical features from patients suffering from AID
6. information related to laboratories such as quality control schemes for molecular diagnosis
7. useful links

New items: We recently implemented a new "**expert views**" area with the possibility to
1. share professional experience
2. request expert advice
3. read updates on a specific topic, and research highlights
4. call for collaboration

Conclusion: We do believe that this relooked **ISSAID** website will further help disseminate information, promote advances in the search for the causes of AID, and improve the quality of life and cure of patients affected by AID.
Disclosure of interest: None declared.

A158

PW02-018 - Impact of PSTPIP1 mutaions on clinical phenotype

D Holzinger[1,2*], P Lohse[3], S Faßl[1], J Austermann[1], T Vogl[1], W de Jager[4], S Holland[5], M Gattorno[6], C Rodriguez-Gallego[7], J Arostegui[8], S Fessatou[9], B Isidor[10], K Ito[11], H-J Epple[12], J Bernstein[13], M Jeng[13], G Lionetti[13], P Ong[14], C Hinze[15], B Sampson[16], C Sunderkoetter[17], D Foell[2], J Chae[18], A Ombrello[18], J Brady[18], I Aksentijevich[18], J Roth[1]
[1]Institute of Immunology, University Hospital Muenster, Germany; [2]Department of Paediatric Rheumatology and Immunology, University Children´s Hospital Muenster, Muenster, Germany; [3]Department of Clinical Chemistry Großhadern, University Munich, Munich, Germany; [4]Department of Pediatric Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands; [5]National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, USA; [6]2nd Division of Pediatrics , "G. Gaslini" Scientific Institute, Genova, Italy; [7]Department of Immunology, Dr. Negrín University Hospital, Las Palmas de Gran Canaria, Spain; [8]Immunology Department-CDB/IDIBAPS, Hospital Clinic, Barcelona, Spain; [9]3rd Department of Pediatrics , Athens University Medical Faculty, Athens, Greece; [10]Service de Génétique Médicale, Centre Hospitalo-Universitaire, Nantes, France; [11]Department of Pediatrics and Neonatology, Nagoya City University, Nagoya, Japan; [12]Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité University Hospital Berlin, Berlin, Germany; [13]Department of Pediatrics, Stanford University Medical Center, Stanford, LA, USA; [14]Division of Clinical Immunology and Allergy, Children's Hospital Los Angeles, LA, USA; [15]Deutsches Zentrum für Kinder- und Jugendrheumatologie, Garmisch-Patenkirchen, Germany; [16]Department of Clinical Chemistry, Charing Cross Hospital, London, UK; [17]Department of Dermatology, University Hospital Muenster, Muenster, Germany; [18]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Health, Bethesda, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A158

Introduction: Hyperzincaemia and hypercalprotectinaemia (Hz/Hc), a rare condition within the spectrum of autoinflammatory diseases, is associated

with hepatosplenomegaly, arthritis, anemia, cutaneous inflammation, and failure to thrive. So far, no genetic cause has been identified. While the clinical appearance is heterogeneous, all affected individuals present with extremely elevated MRP8/MRP14 (calprotectin) serum concentrations (0.9-12.0 g/l (normal range < 0.001 g/l)).
Objectives: The clinical phenotype of 12 patients was characterized and compared to 11 patients with classical PAPA syndrome. Screening of candidate genes was performed to identify disease-causing mutations.
Methods: Serum concentrations of MRP8/14 complex were analyzed in 12 patients with Hz/Hc and compared to 11 PAPA patients. Candidate exons of these patients were sequenced. Cytokine profile of 12 patients with *PSTPIP1* mutations was analyzed by mulitplex ELISA. MRP8/14 secretion from patient´s PBMCs was measured and activity of patient`s sera on monocytes evaluated. The clinical phenotype of all enrolled patients was characterized and compared.
Results: Ten of twelve patients were heterozygous carriers of a glutamic acid 250 (GAG)→lysine (AAG)/p.Glu250Lys/E250K substitution and 1 patient of a glutamic acid 257 (GAG)→lysine (AAG)/p.Glu250Lys/E257K substitution in exon 11 of the *PSTPIP1* gene. MRP8/MRP14 concentrations were extremely elevated in these patients (0.9-12 g/l) compared to eleven patients presenting with classical PAPA symptoms (0.02-0.35 g/l), whose levels again were significantly higher compared to normal controls. Cytokine profiling confirmed the heterogeneity of *PSTPIP1* mutations with a distinct profile for the Hz/Hc phenotype. MRP8/14 hypersecretion was found in PBMCs of patients with *PSTPIP1* mutations and the serum of patients with active disease showed co-stimulatory properties on monocytes activated with TLR-1 ligands.
Conclusion: The novel *PSTPIP1* E250K and E275K mutations cause an autoinflammatory disorder known as hyperzincaemia and hypercalprotectinaemia. The disease causes a heterogeneous spectrum of symptoms that only partially overlaps with the presentation of the classical PAPA syndrome. Elevated MRP8/14 levels are a common hallmark and biomarker of disorders caused by mutations in the *PSTPIP1* gene.
Disclosure of interest: None declared.

A159

PW02-019 - Inflammatory pathways activation in TRAPS patients

O Negm[1*], EM McDermott[2], E Drewe[2], R Powell[1], F Lucy[1], T Ian[1], P Tighe[1]
[1]Immunology, School of Molecular Medical Sciences, University of Nottingham, UK; [2]Nottingham University Hospitals, National Health Service Trust, Nottingham, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A159

Introduction: Mutations in TNFRSF1A can result in the autosomal dominant TNF receptor-associated periodic syndrome
(TRAPS): a complex and heterogeneous systemic autoinflammatory disorder. Misfolding, intracellular aggregation and ligand-independent signalling by mutant TNFR1 play central roles in disease pathophysiology.
Objectives: This work was conducted to study the intracellular signalling pathway activation elicited by mutant TNFR1.
Methods: To understand the complexity of intracellular signalling pathway perturbation in TRAPS, a prototypic mutant TNFR1 (C33Y), or wild-type TNFR1 (WT), were expressed at near physiological levels in an SK-Hep-1 cell model system. TNFR1-associated signalling pathway intermediates were examined under a range of conditions, employing reverse-phase protein microarray. Peripheral blood mononuclear cells (PBMC) from C33Y TRAPS patients and matched healthy controls were similarly examined.
Results: In comparison to cells expressing WT TNFR1 alone, expression of C33Y-TNFR1 in SK-Hep–1 cells and TRAPS patients' PBMCs revealed a subtle up-regulation of a wide spectrum of signalling intermediates and their phosphorylated forms. These were associated with a proinflammatory/anti-apoptotic phenotype, including NF-**k** B, p38, MEK/ERK and JNK MAP kinase pathways, Phosphoinositide 3 kinase, STAT3, JAK2/c-Src, Gsk-3β and transcription factors (including ATF, Elk, Jun). Increased activated Jak2/STAT3 may contribute to an "IL6 amplifier" positive feedback loop that promotes and sustains a proinflammatory state.
Conclusion: The study thus reveals the pleiotropic effect of a TRAPS-associated mutant form of TNFR1 on multiple inflammatory signalling pathways.
Disclosure of interest: None declared.

A160

PW02-020 - Colitis revealing mevalonate kinase deficiency

L Michael[1*], J Camille[2], BM Brigitte[3]
[1]Hopital Robert Debré, Paris, France; [2]CHI Créteil, Créteil, France; [3]Rhumatology, Hôpital Necker Enfants Malades, Paris, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A160

Introduction: Hyperimmunoglobulinemia D (HIDS) is the less severe form of mevalonate kinase deficiency (MKD) caused by recessive inherited mutation in the mevalonate kinase gene (*MVK*). HIDS is characterized by febrile attacks, often associated with transient digestive manifestations, such as abdominal pain, diarrhea and vomiting.
Case report: Here we report for the first time two patients with MKD revealed by a severe neonatal colitis. Both patients had chronic bloody diarrhea and failure to thrive, one patient since the age of one month and the other twelve days. Total parenteral nutrition was required. A marked elevation of acute phase reactants was present, and no evidence of infection was found. In patient 1, ileocolonoscopy revealed an ulcerative colitis at the age of 5 months. Patient 2 suffered from enterocolitis and shock, associated with multiple bowel adhesions at age 5 weeks; the rectosigmoidoscopy showed aphtoid lesions of the sigmoid colon. Pathological findings of colonic biopsies revealed a dense polymorph inflammatory infiltrate associated with deep ulcerations. Febrile attacks occurred 2 months after the onset of digestive symptoms in patient 1, and at onset of disease in patient 2. Genomic sequencing of the *MVK* gene revealed compound heterozygous mutations in both patients. Anti-interleukin 1 (Anti-IL1) agent (anakinra) produced long-term remission of all digestive features and laboratory parameters.
Discussion: This report emphasizes that MKD may be the cause of severe early-onset inflammatory colitis, and must be considered by physicians, even in the absence of fever, after ruling out infections. Anti-IL1 therapy may result in a dramatic improvement of MKD-related inflammatory bowel disease.
Disclosure of interest: None declared.
References

1. van der Hilst JC, Bodar EJ, Barron KS, Frenkel J, Drenth JP, van der Meer JW, *et al*: **Long-term follow-up, clinical features, and quality of life in a series of 103 patients with hyperimmunoglobulinemia D syndrome.** *Medicine* 2008, **87(6)**:301-310.
2. Bader-Meunier B, Florkin B, Sibilia J, Acquaviva C, Hachulla E, Grateau G, *et al*: **Mevalonate kinase deficiency: a survey of 50 patients.** *Pediatrics* 2011, **128(1)**:e152-159.
3. Touitou I, Lesage S, McDermott M, Cuisset L, Hoffman H, Dode C, *et al*: **Infevers: an evolving mutation database for auto-inflammatory syndromes.** *Human mutation* 2004, **24(3)**:194-198.
4. Cailliez M, Garaix F, Rousset-Rouviere C, Bruno D, Kone-Paut I, Sarles J, *et al*: **Anakinra is safe and effective in controlling hyperimmunoglobulinaemia D syndrome-associated febrile crisis.** *Journal of inherited metabolic disease* 2006, **29(6)**:763.
5. Nimubona L, Laloum D, Rolland MO, Read MH, Guillois B, Duhamel JF: **An intestinal obstruction in an eight-month-old child suffering from mevalonic aciduria.** *Acta paediatrica* 2002, **91(6)**:714-716.
6. Ruemmele FM, El Khoury MG, Talbotec C, Maurage C, Mougenot JF, Schmitz J, *et al*: **Characteristics of inflammatory bowel disease with onset during the first year of life.** *Journal of pediatric gastroenterology and nutrition* 2006, **43(5)**:603-609.

A161

PW02-021 - SAA1 is the strongest predictor of AA in TRAPS

L Obici[1*], HJ Lachmann[2], I Touitou[3], G Palladini[1], S Donadei[4], M Pasotti[1], L Cantarini[5], K Gerhold[6], A Meini[7], N Toplak[8], P Woo[9], A Martini[10], G Merlini[1], M Gattorno[10]
[1]Amyloid Research and Treatment Centre, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [2]National Amyloidosis Centre, University College London Medical School, London, UK; [3]INSERM U844, CHRU Montpellier, Montpellier, France; [4]Laboratori Analisi Chimico Cliniche, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [5]Rheumatology Unit, Policlinico Le Scotte, Siena, Italy; [6]Department of Pediatric Rheumatology, Charité Hospital, Berlin, Germany; [7]Pediatric Immunology and Rheumatology, Spedali Civili, Brescia, Italy; [8]University Children's Hospital, University Medical Centre Ljubljana,

Ljubljana, Slovenia; [9]Centre of Pediatric and Adolescent Rheumatology, UCL, London, UK; [10]Giannina Gaslini Institute, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A161

Introduction: AA amyloidosis is the most severe complication of hereditary autoinflammatory diseases. In TRAPS it has been reported to occur in approximately 25% of patients in the absence of an effective treatment. However, susceptibility to AA is difficult to predict. Identification of key risk factors affecting the development of AA would improve the clinical management of TRAPS patients, by allowing a more tailored treatment approach.
Objectives: To evaluate the relative contribution of clinical and genetic factors to the risk of AA amyloidosis in TRAPS.
Methods: Clinical data were obtained from the EUROFEVER/EUROTRAPS web-based registry. Inclusion criteria for this study were: age ≥ 18 years at last follow-up, identification of a *TNFRSF1A* mutation and written informed consent. DNA was available for patients recruited into the EUROTRAPS research project and *SAA1* was genotyped by direct sequencing of exon 3.
Results: 104 patients (51 males, 49%) with TRAPS (39 different mutations) were included in the study. Median age was 41 years (range 18-88), median age at TRAPS onset was 6 years (range 0.5-63), and median age at diagnosis was 37 years (10-81). 21 patients had AA amyloidosis, with a median age at AA onset of 38 years (range 18-76). Family history for AA amyloidosis was observed in 33 patients (32%). *SAA1* genotype was established in 89/104 patients and 31 (35%) were homozygous for the *SAA1.1* allele. 77 patients (74%) had a clearly pathogenic *TNFRSF1A* variant. 27 had either P46L or R92Q.
At univariate analysis, family history for amyloidosis, *SAA1.1* homozygosity, disease course, age at TRAPS onset and the type of mutation were significantly associated with AA amyloidosis. At multivariate analysis homozygosity for *SAA1.1* and age at TRAPS onset independently predicted development of renal amyloidosis. *SAA1.1/1.1* genotype was the variable with the strongest influence on AA development, with a 5.3 fold increased risk whereas older age at TRAPS onset was associated with a reduced risk of AA amyloidosis. Survival according to SAA1 genotype (*SAA1.1/SAA1.1* versus all other genotypes) was estimated by Kaplan-Meier analysis. Median amyloid free survival from birth was 47 years vs. not reached (p=0.01).
Conclusion: Homozygosity for the *SAA1.1* allele is the strongest predictor of AA risk in TRAPS. This result is extremely relevant for the clinical management of TRAPS patients and supports *SAA1* genotyping on a routine basis to guide treatment approach.
Disclosure of interest: None declared.

A162

PW02-022 - Recurrent fever syndromes: multiple gene mutations

M Bohm[1*], P Dolezalova[1], H Lachmann[2], C Modesto[3], I Touitou[4], P Woo[5], M Finetti[6], V Hentgen[7], L Cantarini[8], F De Benedetti[9], R Gallizzi[10], L Obici[11], R Manna[12], E Gallo[13], N Ruperto[6], M Gattorno[6], EUROFEVER/PRINTO network
[1]Charles University in Prague, Prague, Czech Republic; [2]University College Medical School, London, UK; [3]Hospital Valle de Hebron, Barcelona, Spain; [4]Hôpital Arnaud de Villeneuve, Montpellier, France; [5]Great Ormond Street Hospital, London, UK; [6]Istituto Giannina Gaslini, Genova, Italy; [7]Centre de reference national des maladies auto-inflammatoires, Paris, France; [8]University of Siena, Siena, Italy; [9]Ospedale Pediatrico Bambin Gesù, Roma, Italy; [10]Università di Messina, Messina, Italy; [11]Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; [12]Policlinico Gemelli, Roma, Italy; [13]Clinica Pediatrica Università di Torino, Torino, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A162

Introduction: In patients with monogenic autoinflammatory diseases, the majority of detected mutations were found just in a single gene, either in homozygous, heterozygous or compound heterozygous state. However, in some patients mutations in several different genes have been detected. Clinical significance of this finding has yet to be established.
Objectives: To describe clinical characteristics of patients with rare combinations of genetic mutations.
Methods: Seventy-seven centres from 33 countries have been contributing to an international secured web-based registry for autoinflammatory diseases (EUROFEVER), hosted by the PRINTO website (Paediatric Rheumatology International Trial Organisation, http://www.printo.it).
The registry collects anonymised demographic, clinical, laboratory and molecular genetic data on patients with autoinflammatory diseases. Complete clinical information on 1868 consecutive children was available.
Results: In 31 patients (1.7%), the combination of mutations in two different genes, and in one patient in three genes was found with following distribution of clinical diagnoses: Cryopyrin-Associated Periodic Syndromes (CAPS)=9, Tumor necrosis factor (TNF)-Receptor Associated Periodic Syndrome (TRAPS)=7, Familial Mediterranean Fever (FMF)=5, mild Mevalonate Kinase Deficiency (MKD, also known as Hyper IgD Syndrome (HIDS))=2, undefined=9. Out of these patients 18 (56%) carried one high penetrance mutation each. The prevalence of high penetrance mutations among clinically defined groups was most prominent within the CAPS phenotype (8/9) followed by FMF (3/5), MKD (1/2) and TRAPS (3/7). In patients with low penetrance mutations in one of the 4 relevant genes the second mutation was found in one of the remaining 3 genes with the following characteristics: low penetrance (5/14), polymorphisms (3/14), unknown (4/14). In remaining 2 patients the combination of unknown penetrance mutation in one gene and polymorphism in the other was found (both with undefined phenotype).
Conclusion: As the availability of molecular genetic analysis for patients with recurrent fever syndromes increases, the amount of detected mutations in more than one gene will grow. The data currently available suggest that the high prevalence mutations overrule the clinical picture of the disease in majority of patients, though clinical significance of second mutations will have to be evaluated in larger patient series.
Disclosure of interest: None declared.

A163

PW02-023 - Qualitative aspects of autoinflammatory diseases

D Konukbay[1*], D Yildiz[1], C Acikel[2], D Karaman[3], B Eren-Fidanci[1], Y Bilginer[4], I Koné-Paut[5], J Frenkel[6], M Gattorno[7], S Ozen[4], E Demirkaya[8], FMF Arthritis Vasculitis and Orphan disease Research in Pediatric Rheumatology (FAVOR)
[1]Pediatric Rheumatology, Gulhane Military Medical Academy, School of Nursing, Ankara, Turkey; [2]FMF Arthritis Vasculitis and Orphan Disease Research Center, Ankara, Turkey; [3]Child and Adolescent Psychiatry, Gulhane Military Medical Faculty, Ankara, Turkey; [4]Pediatric Rheumatology, Hacettepe University, School of Medicine, Ankara, Turkey; [5]Pediatric Rheumatology, University of Paris SUD, Paris, France; [6]Pediatrics, University of Utrech , Utrech, The Netherlands; [7]Pediatric Rheumatology, Ospedale Gaslini, Genoa, Italy; [8]Pediatric Rheumatology, Gulhane Military Medical Faculty, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A163

Introduction: In pediatric rheumatology, the lack of scales showing activities of illness in the patients groups, the absence of biomarkers for the severity of damage led the scientific world to develop a scale where the patient can make an self-assessment with quantitative results. So, a necessity has been occurred to develop a multidimensional scale which is understandable, applicable and comprehensive in the evaluation of children with auto-inflammatory diseases.
Objectives: The aim of this study is to develop a multidimensional assessment instrument named "**J**uvenile **A**uto**i**nflammatory Disease **M**ultidimensional **A**ssessment **R**eport" (JAIMAR) to measure all the domains of the autoinflammatory diseases. In this study the data of "Qualitative Interviews", one of the steps of item generation in JAIMAR, will be presented.
Methods: 19 mothers who have children with autoinflammatory disease (8 FMF, 5 Behcet, 4 PFAPA, 1 HIDS, 1 TRAPS) and their children greater than 7 years old were enrolled in this study. Data were collected using both a demographic data form and a semi-structured interview form. The study was performed on individual patient face-to face interview. Data were collected by using both a demographic data form and a semi-structured interview form. Data analysis by grounded theory and N Vivo 10 software.
Results: Unknowing the time of attack, lifelong illness, difficulties in diagnosis and exposure to the other parts of the body were described as the worst parts of the illness. In addition to physical factors such as cold and fatigue, psychological factors such as overexcitement, worry and happiness were stated to be in the triggering factors of the attacks. Although decrease in attacks after treatments were stated, lifelong drug addiction and its side

effects were told to be the most worrying aspects. Problems at school (absenteeism, loss of performance, fear of having attack at school and bad peer relations) were explained as the biggest difficulties affecting the quality of life. Problems with friends, precocity, and extreme expressions such as depression/wanting to die due to back pain were to be the in the emotional difficulties.

Conclusion: These results provide an evidence based data for the assessment of children with autoinflammatory disease by several domains including physical, emotional and social aspects as well as treatment protocols. With this regard there is a need to develop a multidimensional instrument to measure important aspects of the illness gained from these results.

Disclosure of interest: None declared.

A164

PW02-024 - A case of candle syndrome treated with thalidomide

K Yamazaki*, T Miyamae, S Yokota
Pediatrics, Yokohama City University, Yokohama, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A164

Introduction: A new group of autoinflammatory diseases caused by immunoproteasome dysfunction has been recently reported. The mutation in the PSMB8 gene encoding immunoproteasome subunit β type 8 causes a number of clinical syndromes that described as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome and Nakajo-Nishimura syndrome (NNS).

Case Report: A Japanese girl presented with fever, annular erythematous plagues and elevation of hepatocellular enzyme at 2 months of age. She had deformed ears, a broad saddlelike nose and periorbital edema. At 16 years of age, she had lipodystrophy of the face and upper limbs, a protuberant abdomen, and severe fat deposition into the peritoneal and the pleural cavity. Painful nodular erythema, hepatosplenomegaly, muscle atrophy, mild joint contracture of ankle, and mild mental retardation were observed. She suffered from arthralgia without arthritis for her lifelong. Laboratory findings showed hypochromic anemia, and elevation of erythrocyte sedimentation rate and C-reactive protein. A brain computed tomographic image revealed basal ganglia calcification. After obtaining informed consent, the patient's DNA was analyzed for mutations in PSMB8, and heterozygous c145C>A mutation (Q49K) was found. She was unsuccessfully treated with NSAIDs, a variety of immunosuppressants: cyclosporine, tacrolimus and mycophenolate mofetil, and biologics: infliximab and tocilizumab. Pulsed intravenous methylprednisolone and high dose of oral prednisolone (PSL) were effective. Thalidomide had an efficacy for improvement in her symptoms and reduction of PSL dosage, but the treatment had to be terminated due to thrombocytopenia.

Discussion: We reported here the case of severe CANDLE syndrome. Although both her disease course and clinical presentations were typical, only heterozygous Q49K mutation in the PSMB8 gene was found. Recent studies showed that thalidomide combined with statin was involved in anti-myeloma action by p38 MAPK inhibition, as well as thalidomide inhibits lipopolysaccharide-induced tumor necrosis factor α production. The efficacy of thalidomide in our case indicates that thalidomide could regulate the inflammatory signaling induced by immunoproteasome dysfunction, and thalidomide might become key treatment other than PSL.

Disclosure of interest: None declared.

References

1. Liu Y, Ramot Y, Torrelo A, Paller AS, Si N, Babay S, *et al*: **Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity.** *Arthritis Rheum* **64(3)**:895-907.
2. Arima K, Kinoshita A, Mishima H, Kanazawa N, Kaneko T, Mizushima T, *et al*: **Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome.** *Proc Natl Acad Sci U S A* **108(36)**:14914-14919.
3. Slawinska-Brych A, Zdzisinska B, Mizerska-Dudka M, Kandefer-Szerszen M: **Induction of apoptosis in multiple myeloma cells by a statin-thalidomide combination can be enhanced by p38 MAPK inhibition.** *Leuk Res.*

A165

PW02-024-B - First report of AA amyloidosis in Blau syndrome

E Ruiz-Ortiz[1*], A Saurina[2], E González-Roca[1], M Solé[3], C Modesto[4], F Rius[1], S Plaza[1], J Yagüe[1], JI Aróstegui[1]
[1]Immunology, Hospital Clinic, Barcelona, Spain; [2]Nephrology, Consorci Sanitari de Terrassa, Terrassa, Spain; [3]Pathology, Hospital Clinic, Barcelona, Spain; [4]Pediatric Rheumatology, Hospital Vall Hebrón, Barcelona, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A165

Introduction: Systemic AA amyloidosis is a life-threatening complication of different chronic infectious and inflammatory diseases. The deposition of amyloid fibrils derived from the serum amyloid A (SAA) protein represents its pathological hallmark. A long lasting and increased serum level of SAA is a prerequisite to its development. The group of inherited autoinflammatory diseases includes different disorders consequence of a genetically-determined dysregulation of innate immune system. All these diseases are associated with a marked acute phase response. The incidence of AA amyloidosis varies widely among them, with the higher incidence in Muckle-Wells syndrome and in TNF receptor-associated periodic syndrome. Inversely, no cases of AA amyloidosis have been reported in some few inherited autoinflammatory diseases, including Blau syndrome, a dominantly-inherited disease caused by *NOD2* mutations.

Case report: The patient is a 30 years-old woman that referred since the 21 months of age persistent fever, chronic polyarthritis, skin rash, and ocular manifestations including recurrent bilateral uveitis. Granulomatous infiltration was detected in synovial biopsies, with negative results for microbiologic test. Her mother and sister were also affected by a similar disease, suggesting an autosomal dominant inheritance pattern. A clinical diagnosis of Blau syndrome was proposed. The *NOD2* analyses revealed a heterozygous c.1759C>T transition that provokes the novel p.Arg587Cys mutation. This mutation was subsequently detected in her affected relatives, and established the definitive diagnosis of Blau syndrome.

At 25 year-old she became pregnant, and during the pregnancy, proteinuria was detected in the first and second quarter, without other accompanying signs. Proteinuria disappeared in the third quarter. After delivery, several episodes of intense uveitis were detected, and treated with corticosteroids. Methotrexate (MTX) was also added to modify the inflammatory disease activity and to prevent the recurrence of uveitis. However, one year later, MTX was discontinued to avoid potential teratogenic effects in a future pregnancy.

During the follow-up, different routine tests revealed repeatedly glomerular-range proteinuria, with normal urine sediment and preserved renal function. A renal biopsy was performed and pathological studies revealed Congo-red positive deposits with glomerular and vascular involvement. Immunohistochemical studies revealed that these deposits were of AA-type. Antiproteinuric measures were started, and proteinuria decreased after 5 months of treatment.

Discussion: This case represents the first description of AA amyloidosis in Blau syndrome. It highlights the relevance of monitoring the renal function in the follow-up of patients affected by any inherited autoinflammatory disease, independently of the previously reported risk of AA amyloidosis development.

Disclosure of interest: None declared.

A166

PW02-025 - Programme necrosis by CAPS-associated NLRP3

T Satoh*, N Kambe, H Matsue
Dermatology, Chiba University School of Medicine, Chiba, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A166

Introduction: Cryopyrin-associated periodic syndrome (CAPS), clinically characterized by neutrophil-rich urticarial rash, is associated with missense mutations in *NLRP3*. NLRP3 is a pattern recognition receptor in the cytoplasm of cells and structurally related to plant resistance proteins, which detect pathogen- or danger-associated signals, leading to programmed cell death and hyper response at the local site in plants. In mammals, activated NLRP3 forms inflammasome, which orchestrates the early inflammatory process via IL-1β activation, and also cause programmed necrotic cell death termed "pyronecrosis". However, the mechanistic details are largely unknown.

Objectives: To investigate the mechanism of NLRP3-mediated pyronecrosis and its in vivo relevance.
Methods: We have established a system in which pyronecrosis was induced by the expression of CAPS-associated gain-of-function mutant of NLRP3, using a tetracycline-inducible expression (Tet-on) system. We also induced NLRP3-mediated cell death in mouse air-pouch and harvested the cells and fluid.
Results: Mutant NLRP3 expression without LPS pretreatment induced only necrotic cell death but not IL-1β secretion in this system. Silencing ASC gene by shRNA prevented pyronecrosis, while silencing caspase-1 did not. When the cell lines expressing NLRP3 mutants by Tet-on system were treated with cathesin B inhibitor, necrotic cell death and speckle patterns of ASC oligomerization were not observed. Interestingly, when these cells were treated with Z-VAD-fmk, the speckle patterns of ASC were seen while they were still alive.
Upon oral administration of doxycycline, injection of LPS-pretreated NLRP3-mutant cells into mouse air-pouch showed necrotic cell death in addition to IL-1β release, resulting in the significant increase in numbers of neutrophils in the pouch. Interestingly, non-pretreated mutant cells, which showed necrotic cell death without mature IL-1β release, also induced neutrophil infiltration, though smaller in number relative to neutrophil infiltration induced by LPS-pretreated mutant cells.
Conclusion: Pyronecrosis is provoked by downstream of NLRP3-induced ASC oligomerization, but does not require caspase-1 or IL-1β cleavage, and that cathesin B inhibitor and Z-VAD-fmk inhibit pyronecrosis before and after ASC oligomerization, respectively. This clarify the differences of pyronecrosis from pyroptosis which is mediated by ASC oligomerization but do not require NLRP3. In vivo study showed that necrotic cell death by pyronecrosis in itself can cause and exacerbate the neutrophilic inflammatory response.
Disclosure of interest: None declared.

A167

PW02-026 - Low frequency variants of NLRP3 in CAPS patients

M Lesche[1], A Dahl[1], A Kränkel[1], J Roesler[2], A Rösen-Wolff[2*]
[1]Deep Sequencing Group SFB655, BIOTEC, TU Dresden, Germany; [2]Department of Pediatrics, University Clinic Carl Gustav Carus, TU Dresden, Dresden, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A167

Introduction: Somatic mosaicism of *NLRP3* has been identified in a high percentage of "mutation-negative" patients suffering from chronic infantile neurologic, cutaneous, articular (CINCA) syndrome.
Objectives: The aim of the study was to detect and quantify low frequency variants of *NLRP3* in German patients suffering from cryopyrin associated periodic fever syndromes (CAPS) including CINCA, Muckle-Wells syndrome (MWS), and familial cold autoinflammatory syndrome (FCAS).
Methods: All exons of *NLRP3* were amplified by PCR (30 cycles) from genomic DNA isolated from PBMCs of healthy controls or CAPS patients. Thereafter, PCR products were concatenated, fragmented and subjected to NGS fragment library preparation followed by Illumina short read sequencing. For SNV calling a customized pipeline on basis of the GATK pipeline (1000 Genomes project) was utilized using a 40.000x coverage to assure sufficient sensitivity. In order to determine the accuracy of quantification, PCR products containing a known heterozygous mutation (T348M) were mixed with *NLRP3* wildtype PCR products to obtain dilutions of the mutated sequences of 25%, 12.5%, and 6.25%.
Results: We were able to exactly quantify the diluted low frequency mutation (T348M). In one CINCA patient a new variant (L359S) was detected in 30% of the DNA sequences that had not been identified by classical Sanger sequencing of an older sample. Up to now we could not detect low frequency *NLRP3* variants in MWS or in FCAS patients.
Conclusion: Massive parallel sequencing is a reliable method to quantify low frequency variants of *NLRP3*. A new *NLRP3* mutation could be detected in a patient suffering from typical CINCA syndrome. Somatic mosaicism may be less frequent in MWS and FCAS patients. Due to the fact that more "mutation-negative" CAPS patients need to be characterized, we will continue with this study.
Disclosure of interest: M. Lesche: None Declared, A. Dahl: None Declared, A. Kränkel: None Declared, J. Roesler: None Declared, A. Rösen-Wolff Grant / Research Support from: Novartis Pharma GmbH, Nürnberg, Germany

A168

PW02-027 - CAPS and cost-effectiveness analysis project

O Della Casa Alberighi[1*], L Accame[1], J Frenkel[2], M Gattorno[3], A Martini[3], B Neven[4], P Quartier[4], F Pierotti[5], G Turchetti[5]
[1]Clinical Pharmacology and Clinical Trial Unit, Giannina Gaslini Institute Pediatric Hospital, Genova, Italy; [2]Pediatric Rheumatology, University Medical Center Utrecht, Utrecht, The Netherlands; [3]2nd Division of Pediatrics, Giannina Gaslini Institute Pediatric Hospital, Genova, Italy; [4]Pediatric Immunology, Haematology and Rheumatology Unit , Assistance Publique-Hopitaux de Paris Hopital Necker, Paris, France; [5]Health Economy, Scuola Superiore S. Anna, Pisa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A168

Introduction: Ultra-orphan drugs are medicines used to treat exceptionally rare diseases that are chronically debilitating or life-threatening. Low patient numbers make it difficult for pharmaceutical companies to recoup research and development costs, and consequently these medicines are generally very expensive on a per patient basis. European Union (EU) regulations promote the development of orphan drugs; but to contain costs, EU healthcare systems will increasily need the cost-effectiveness analysis (CEA) of therapies when deciding if they should be funded. Conventional methods for CEA of drugs for common conditions do not apply to ultra-orphan drugs; therefore, additional factors need to be considered.
Objectives: Using the case of ultra-orphan cryopyrin associated periodic syndromes (CAPS) currently investigated by the EuroFever registry, the RaDiCEA (Rare Diseases & Cost-Effectiveness Analysis) project is aimed at collecting prospective efficacy, safety, tolerability, treatment adherence (effectiveness data), cost of illness (COI) information, and relative effectiveness of life-long treatment strategies, and at elaborating on CEA modeling in ultra rare diseases.
Methods: Design and setting: As a EuroFever registry spin-off, a three-year, international, multicentre, observational, cost-effectiveness study will be conducted in approx. 150 CAPS patients through the Paediatric Rheumatology INternational Trials Organisation (PRINTO) network.
Participants: The EuroFever registry project (http://www.printo.it/eurofever/) involves so far 170 centres of Paediatric Rheumatology and centres of reference for all autoinflammatory diseases in 45 Countries worldwide.
Results: Main outcome measures: They will be the retention on treatment and reasons of treatment withdrawal for effectiveness. For safety, the incidence rates of anti-IL-1 agents-emergent adverse events (AEs) and serious AEs will be evaluated in comparison with incidence rates observed in CAPS subjects not exposed to anti-IL-1 agents. The bases for a cost-effectiveness model in CAPS will be set by means of a COI evaluation, and of a comparative economic evaluation of different treatment strategies in the National Health Systems'(NHS) perspectives, using CEA of direct health costs (Incremental Cost Effectiveness Ratio - ICER), and by measuring quality adjusted life years (QALY), and organ/system damage prevention up to three years.
Expected results: The RaDiCEA project will assess the long-term effectiveness of different potentially life-long treatment strategies and COI, while exploring the feasibility of a new CEA model to be generated from a rare disease (CAPS) registry.
Conclusion: As expensive medications like "biologicals" show promising results in some patients with "ultra-orphan" diseases, it becomes more and more important to have detailed information on as many patients as possible. A promising new international collaboration aims to develop a model to evaluate both costs and (long-term) benefits in an ultra-orphan group of diseases known as CAPS. The same model may be used in other very rare disorders.
Disclosure of interest: None declared.

A169

PW02-028 - Association of novel NLRP3 mutations with CAPS phenotype in Turkish patients

A Berdeli[1*], S Nalbantoglu[2], D Tigli[2], I Demirel[2], M Atan[2], B Sozeri[3]
[1]Molecular Medicine and Pediatric Rheumatology, Izmir, Turkey; [2]Molecular Medicine Laboratory, Izmir, Turkey; [3]Pediatric Rheumatology, EGE UNIVERSITY, Izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A169

Introduction: Cryopyrin-Associated Periodic Syndromes (CAPS) are a group of rare, inherited, autoinflammatory diseases involved of Familial Cold Autoinflammatory Syndrome (FCAS),Muckle-Wells Syndrome (MWS) and Neonatal Onset Multisystem Inflammatory Disease (NOMID) (also called Chronic Infantile Neurologic Cutaneous Articular, or CINCA, Syndrome. The responsible gene NLRP3 (nucleotide-binding domain, leucine-rich family [NLR], pyrin domain containing, produces cryopyrin protein which participates in inflammasome complexes leading to production of interleukin-1β (IL-1β) and autoinflammation.
Objectives: We aimed to investigate possible variations of the genes linked to CAPS disorders.
Methods: Bidirectional DNA Sequencing analysis was performed in coding exons and exon-intron flanking regions of NLRP3 gene (NM_004895.4; NP_004886.3).
Results: Patients with manifestations of hereditary autoinflammatory disorder and MEFV gene mutation negative (n:194) had undergone mutation analysis of NLRP3 gene. Disease related mutations were obtained in 25 patients. p.Gln703Lys in 19 patients, p.Ser726Gly in 2 patients and p.Val198Met mutation in 1 patient were investigated. In 3 patients, novel pathogenic p.Ala154Gly, p. Ser726Gly missense, and p.K610fsX613 frameshift mutations were identified, and registered to ISSAID. Synonimous amino acid polymorphisms include: p.Thr219Thr (ACC/ACT; c.657C>T) %14, p.Ala242Ala (GCG/GCA; C.726G>A) %56.4, p.Arg260Arg (CGA/CGG; c.780A>G) %63.6, p.Ser434Ser (TCC/TCT; c.1302C>T) %23.4 and p.Leu411Leu (c.1231C-T) %0.4. Anti-IL1B treatment was started for those patients and good response was obtained.
Conclusion: In this study, DNA sequencing analysis revealed novel NLPR3 mutations which were found related to typical CAPS phenotype and registered to ISSAID. In particular, for patients with no identifiable MEFV gene mutation, genetic diagnosis helps early diagnosis, proper follow-up and treatment of patients in CAPS disorders. For the genetic and phenotypic heterogeneity of the patients and for patients with no identifiable mutations, possible presence of other genetic and non-genetic factors should be investigated.
Disclosure of interest: None declared.

A170

PW02-029 - Single cell fluorescent immunoassay of CINCA/NOMID

K Nakagawa[1*], N Shimura[2], Y Shirasaki[2], M Yamagishi[2], K Izawa[1], R Nishikomori[1], T Kawai[1], T Yasumi[1], T Heike[1], O Ohara[2,3]
[1]Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Research Center for Allergy and Immunology, RIKEN, Yokohama, Japan; [3]KAZUSA DNA research institute, Kisarazu, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A170

Introduction: CINCA syndrome, also known as NOMID, is a rare autoinflammatory disease caused by the *NLRP3* mutations. It has been known that conventional genetic analysis failed to detect disease-causing mutations in approximately 40% of patients. We have recently identified *NLRP3* somatic mosaicism on 70% of these "mutation-negative" patients in the international collaborative study (Tanaka N. and Izawa K. et al., Arthritis Rheum, 2011), and found no significant differences on systemic inflammation between heterozygous germline mutations and somatic mosaicism. This raises a question how a small number of *NLRP3*-mutated cells cause systemic inflammation as severely as 100% of germline mutations.
Objectives: To solve the question, we analyzed cytokine production from the single cells, especially IL-1β which is the key molecule of NLRP3 inflammasome. There are the 2 forms of IL-1β, namely preform and mature forms, and only the latter is said to be secreted. Although the IL-1β in the single cell can be measured by intracellular cytokine staining, the relationship of the amount of intracellular IL-1β in the single cells and secreted IL-1β from them is still unknown. From these issues, it would be a better approach to measure the secreted IL-1β at a single cell level.
Methods: In this work, we tried to establish a single cell fluorescent immunoassay system to measure IL-1β secretion from monocytes of CINCA/NOMID patients at a single cell level using a soft lithographic method called microengraving.
Results: In the healthy control, very small number of cells secreted low amount of IL-1β by the LPS stimulation. In contrast, significant number of cells secreted high amount of IL-1β by the LPS stimulation in CINCA/NOMID patients with heterozygous germline mutations. We were also able to observe a large number of IL-1β secreting cells from patients with somatic mosaic mutations by LPS stimulation alone.
To delineate whether only the cells with a mutation on *NLRP3* are responsible for IL-1b secretion in the mosaics, we pick up the single cells from the microwells positive on IL-1β secretion by micromanipulator. By performing genetic analysis on them, we are now trying to determine whether only the mutated cells secrete IL-1β or the small fraction of *NLRP3*-mutated cells causes *in vivo* bystander activation of the wild type monocytes in the somatic mosaic CINCA/NOMID patients.
In addition, this method could be offered to diagnose the somatic mosaicism of CINCA/NOMID easily on the basis of single cell functional analysis, which would complement the DNA sequencing based method (Izawa K. and Hijikata A. et al., DNA research, 2012) that might miss some rare CINCA/NOMID cases caused by other than *NLRP3* coding region mutations.
Disclosure of interest: None declared.

A171

PW02-030 - Clinical phenotype in individuals with Q703K

DM Rowczenio*, H Trojer, G Wang, PN Hawkins, HJ Lachmann, A Baginska, T Russell, R Al-Nackkash, A Bybee, NM Stewart, T Lane
Medicine, University College London, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A171

Introduction: Mutations in the *NLRP3* gene are associated with the dominantly inherited cryopyrin-associated periodic syndrome (CAPS) characterized by episodes of fever, urticarial rash, arthralgia, myalgia, eye inflammation, and, in its more severe forms, bony abnormalities and CNS inflammation. Of the 145 sequence variants in *NLRP3* reported to date, 30 are either nonpathogenic or of undetermined significance, the commonest of which, Q703K, has been reported in 5 to10% of general population.
Objectives: To characterize the clinical phenotype in individuals with Q703K in a single UK center.
Methods: 1017 subjects with hereditary periodic fever syndromes (HPFS) were screened for mutations in the *NLRP3* gene, individuals in whom genetic variants were not identified or those with low penetrance mutations underwent additional screening of *MEFV*; *TNFRSF1A*; *MVK* and *NOD2*.
Results: *NLRP3* Q703K was identified in 69 subjects (7% of screened), clinical information was available on 56. 4 cases had another mutation in *NLRP3*: 1 had A439V and 3 siblings had R260W. 18 subjects (32%) had aberration in another HPFS gene: 4 in *TNFRSF1A*: R92Q, C29F, H22Q and S57_E64del; 1 in *MVK*: V377I; 2 in *NOD2*: P268S and 12 in *MEFV*: 2 were compound heterozygotes, M680I/V726A and M694V/V726A, 2 were homozygous and 1 was heterozygous for M694V, 1 was heterozygous for V726A, 2 for S208C, 1 for S154P and 3 for E148Q.
The inflammatory syndromes were thought to be fully consistent with CAPS, TRAPS, MKD and FMF in the 4 cases with *NLRP3* variants other than Q703K; 4 subjects with *TNFRSF1A* mutations; a subject with *MVK* variant and 5 of the 12 cases with *MEFV* substitutions respectively. One subject with Q703K and E148Q was an asymptomatic carrier and in 4 cases a diagnosis of disease other than HPFS was made. 14 cases (25%) were diagnosed with AA amyloidosis (confirmed immunohistochemically and by SAP scintigraphy) the nature of the underlying inflammatory disease in 12 remains uncertain. In total we were unable to make a clinical diagnosis in 25 subjects (44%): in this group the median age at disease onset was 5 years (birth-59 years); fever, arthralgia and myalgia were the most prominent features - identified in over 50% of cases; 11 subjects (44%) had rash during febrile attacks (urticarial rash was reported in 4); 7 (28%) had symptoms triggered or worsened by cold exposure; 5 (20%) suffered from headache, GI symptoms or lymphadenopathy; 4 (16%) had hearing impairment; a delayed puberty was identified in 4 (16%) and one had growth deficit. Episodes occurred irregularly and lasted from 1 day to 2 weeks.
In 10 the inflammatory markers, serum amyloid A protein (SAA) and C-reactive protein (CRP), were measured during disease flare and were elevated to median values of 106.5 mg/L (range 40-438) and 68 mg/L (range 34 – 220) respectively.
Conclusion: We have identified Q703K in subjects displaying FCAS-like symptoms, in individuals with HPFS other than CAPS, in cases with uncharacterised autoinflammatory diseases, in AA amyloidosis, and in asymptomatic individuals. Given the high frequency of healthy carriers, the interpretation of Q703K presents a diagnostic challenge and the genetic

and/or environmental factors that may influence pathogenic consequences of this variant remain unknown.
Competing interests: None declared.

A172

PW02-031 - Genetic and clinical manifestations of CAPS

AK Bybee[1*], H Lachmann[1], E Omoyinmi[2], B Nedjai[3], P Woo[4], T Lane[5], S Savic[6], P Hawkins[1], M McDermott[7]
[1]National Amyloidosis Centre, UCL, London, UK; [2]Infectious Diseases, Institute of Child Health, London, UK; [3]Institute of Bio-Medical Engineering, Imperial College, London, UK; [4]Immunology and Molecular Pathology, London, UK; [5]Medicine, UCL, London, UK; [6]Clinical Immunology and Allergy, London, UK; [7]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A172

Introduction: Many variants in the NLRP3 gene are associated with a particular spectrum of autoinflammatory diseases or hereditary recurrent fevers (HRFs), including familial cold autoinflammatory syndrome (FCAS), Muckle Wells Syndrome (MWS), and chronic infantile neurologic, cutaneous and articular syndrome (CINCA), also known as neonatal onset multisystem inflammatory disease (NOMID). These are now subsumed under the umbrella term CAPS (cryopyrin-associated periodic syndromes). However, CAPS is rarely encountered in any population and is often difficult to identify. Extension of genetic screening to a genomic scale may soon become routine, but that development will require definition of the identities and ontology of the constellations of symptoms involved, in order for bioinformatics applications to keep pace. Patients with clinical features suggestive of these conditions were analysed for NLRP3 variants.
Objectives: To explore the prevalence of NLRP3 variants in patients with autoinflammatory disorders in order to establish a consistent pattern of associated symptoms.
Methods: Genomic DNA from 91 unrelated patients referred for genetic testing for any of the NLRP3-associated diseases was screened for variants in exon 3 of NLRP3 by PCR and automated DNA sequencing. Diverse symptoms were recorded when possible and compared with the outcome of genetic testing.
Results: Heterozygous missense substitutions in NLRP3 (NM_001243133.1) were found in 16 of the 91 patients (17.6%), including p.V198M, p.D303N, p.P315L, p.T348M, p.V351M, p.T436I, p.G569R, and p.Y570F variants. In addition to the well-known CAPS signs of recurrent fever, urticarial rash and sensorineural hearing loss, symptoms relatively frequently reported in NLRP3 variant-positive patients included chronic meningitis or headache (9/14 with NLPR3 variant vs. 6/38 without) uveitis or iritis (8/15 vs. 10/41), growth retardation (6/14 vs. 5/37) epiphyseal or patellar growth (5/14 vs. 1/35) and learning difficulties (4/14 vs. 3/35). In addition, several patients with multiple symptoms characteristic of CAPS were mutation-negative, and at least 9 patients with or without a genetic diagnosis developed AA amyloidosis.
Conclusion: In addition to the more typical symptoms, this study highlighted distinctive neurological characteristics of some CAPS cases. These results indicate a large degree of genetic and symptomatic heterogeneity in NLRP3-related syndromes and suggest that other, as yet unidentified, genes and other factors may be involved in producing a CAPS-like phenotype. Such factors accrue to produce symptomatic recurrent fever disease with an accompanying heightened risk of developing AA amyloidosis. Autoinflammatory diseases continue to drive investigation of the genes controlling fundamental aspects of the innate immune system.
Competing interests: None declared.

A173

PW02-032 - CNS manifestations and NLRP3/CIAS1 gene mutations

T Kümpfel[1*], E Schuh[1], R Hohlfeld[1], P Lohse[2]
[1]Institute of Clinical Neuroimmunology, LMU-Munich, München, Germany; [2]Section of molecular genetics, Institute for Laboratory Medicine Blessing, Singen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A173

Introduction: Central nervous system (CNS) involvement is common in cryopyrin-associated periodic syndromes (CAPS), especially in children. Neurological manifestations of the low-penetrance cryopyrin mutations V198M and Q703K encoded by exon 3 of the *NLRP3* gene have not been investigated so far.
Objectives: To determine the frequency of the V198M and Q703K substitutions in adult patients with possible inflammatory CNS disease and at least two symptoms compatible with CAPS and to describe the clinical phenotype of mutation-positive patients.
Methods: 94 unrelated, consecutive patients with possible inflammatory CNS disease and at least two symptoms compatible CAPS were prospectively screened for the V198M and Q703K mutations. In addition, the clinical, laboratory, and MRI features of mutation carriers were assessed.
Results: 15 patients (16%; 12 females) were identified to carry one of the two low-penetrance mutations in exon 3 of the *NLRP3* gene (V198M: n= 2; Q703K: n =13). CAPS-associated systemic symptoms consisted of recurrent inflammation of the eyes, arthralgias, myalgias, urticarial rash, abdominal pain, and severe fatigue. CNS manifestation included optic nerve inflammation and/or atrophy, cranial nerve palsy, migraine, recurrent meningitis, and sensoneurinal hypacusis. Eight patients (53%) fulfilled the diagnostic criteria for multiple sclerosis (MS) according to the McDonald criteria. Brain magnetic resonance imaging (MRI) showed abnormalities in all but one patient.
Conclusion: So far, the V198M and Q703K mutations have been only rarely described in association with MS or CNS inflammation. We observed a a surprisingly high frequency of these two low-penetrance mutations in the cohort studied, leading to a heterogeneous pattern of CNS manifestations in affected patients. Thus, molecular genetic testing should be considered in patients with an unusual CNS inflammation and/or MS, who report additional symptoms compatible with CAPS.
Competing interests: T. Kümpfel Grant / Research Support from: TK has received grant support by Novartis Pharma, E. Schuh: None declared, R. Hohlfeld: None declared, P. Lohse: None declared.

A174

PW02-033 - Cytokine profile in CSF in CAPS patients

E Schuh[1*], P Lohse[2], M Frankenberger[3], I Meinl[1], T Kuempfel[1]
[1]Neurology, Institut of Clinical Neuroimmunology, Munich, Germany; [2]Laborararoriumsmedizin, Singen; [3]Helmholtz Zentrum, Munich, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A174

Introduction: CAPS is a rare autoinflammatory syndrome caused by autosomal dominant mutations in the NLRP3/CIAS 1 gene on chromosome 1q44 encoding for the cryopyrin protein, an important component of the inflammasome, leading to excessive production of interleukin-1beta (IL-1ß). CAPS encompasses three different entities of variable clinical severity: familial cold auto-inflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous articular syndrome (CINCA)/ neonatal – onset multisystem inflammatory disease (NOMID). They are all characterised by recurrent episodes of systemic inflammation involving particularly skin, joints, central nervous system and eyes.
Objectives: To analyse and quantify various cytokines in sera and cerebrospinal fluid (CSF) in five patients with central nervous system (CNS) manifestations of cryopyrin-associated periodic syndromes (CAPS) carrying the Q703K mutation in heterozygosity.
Methods: Five Caucasian patients (mean age 37 ±10 years; one male) with CNS manifestations including optic nerve inflammation, recurrent cranial nerve palsy, migraine, fatigue, and recurrent meningitis were identified as heterozygous carriers of the cryopyrin Q703K substitution. CSF investigations were performed for diagnostic purposes in all patients and showed pleocytosis in 3 patients. In addition, concentrations of the proinflammatory cytokines interleukin beta (IL-1), interleukin-6 (IL-6), interleukin 17 (IL-17), tumor necrosis factor alpha (TNF-alpha) and FGF (Fibroblast growth factor) were determined in the CSF and sera using a multiplex assay.
Results: IL-6 concentrations in the CSF were clearly elevated in two patients during acute attacks of CAPS-associated CNS manifestations. The other three patients were investigated during remission and showed no IL-6 elevations in the CSF. The other serum cytokine levels were increased in one patient.
Conclusion: Our results show a correlation between CSF IL-6 concentrations and CAPS-associated disease activity in the CNS. IL-6 levels in the CSF

therefore may serve as a marker of disease activity in CAPS patients with CNS manifestations.
Competing interests: E. Schuh: None declared, P. Lohse: None declared, M. Frankenberger: None declared, I. Meinl: None declared, T. Kuempfel Grant / Research Support from: Novartis.

A175

PW02-034 - NLRP3 mosaicism detection in CAPS using NGS

M Stoffels[1,2,3*], M Elferink[4], P van Zon[4], J Frenkel[5], E Hoppenreijs[6], A Simon[1,2,3], M van Gijn[4]
[1]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Netherlands; [2]Nijmegen Centre for Infection, Inflammation and Immunity (N4i), Netherlands; [3]Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands; [4]Department of Medical Genetics, Netherlands; [5]Department of Pediatrics, UMC Utrecht, Utrecht, Netherlands; [6]Department of Pediatric Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A175

Introduction: Heterozygous germline mutations in *NLRP3* are a known cause of Cryopyrin associated periodic syndrome (CAPS). However, in a considerable number of these patients mutations cannot be detected by conventional genetic analyses. Somatic mosaicism has been detected in several mutation-negative patients, and is suggested to be a major cause of CAPS in these patients.
Objectives: Using next-generation sequencing (NGS), we aimed to investigate whether mosaicism for *NLRP3* mutations was present in mutation-negative CAPS patients.
Methods: Six well-defined mutation-negative CAPS patients were included. In addition two CAPS patients that were identified before as mosaics, by a subcloning and Sanger sequencing method, were included for validation purposes. In short, barcoded whole genome fragment libraries were generated for each patient, enriched for the coding regions of 300 inflammation related genes using a custom Agilent 1M microarray and subsequently sequenced on the SOLiD5500XL platform. Because almost all *NLRP3* mutations are located in exon 3 of *NLRP3*, this exon was analyzed using an in house bioinformatic pipeline, CARTAGENIA BENCH lab NGS and IGV2.2 software.
Results: In all patients all *NLRP3* exons were 100% covered, with an average coverage of 460x. The two patients that were identified before as being 6.3% mosaic for the E567K and G755R mutation demonstrated mosaicism for these mutations of subsequently approximately 8% and 10%. This indicated the method was valid to study mosaicism. We could not detect mosaicism for known pathogenic mutations in exon 3 in the six patients. However, in one patient 9% mosaicism for an E567G variant was detected. Although this variant has not been described as heterozygous mutation in CAPS patients, mosaicism for this variant has been described before in a mutation-negative CAPS patient. The clinical relevance of this variant, however, remains uncertain.
Conclusion: We demonstrated that our NGS method is a reliable, accurate method to identify mosaic percentages of at least 8-10%. In contrast to the earlier reported finding that in 70 percent of mutation-negative CAPS patients mosaicism for *NLRP3* mutations can be demonstrated, we could not find evidence for mosaicism in exon 3 of *NLRP3* in five of our six patients. Although very low mosaic percentages or mosaic mutations in other exons cannot be excluded, this suggests that next to germline and mosaic *NLRP3* mutations also other, not yet identified defects underlie CAPS.
Competing interests: None declared.

A176

PW02-035 - A role for thermo-TRP channels in innate immunity?

M Stoffels[1,2,3*], T Remijn[1], LM Elders[1], HD de Koning[1,2,3,4], JW van der Meer[1,2,3], A Simon[1,2,3]
[1]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Netherlands; [2]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Netherlands; [3]Nijmegen Centre for Molecular Life Sciences (NCMLS), Netherlands; [4]Department of Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A176

Introduction: Exposure to cold can induce an exaggerated (local and systemic) inflammatory response in a number of rare disorders, including cryopyrin-associated periodic syndrome (CAPS), and idiopathic cold urticaria (CU). Although it is widely recognized that temperature sensing in neurons is mediated by several transient receptor potential (TRP) channels, it is not known how immune cells sense cold temperatures.
Objectives: In the present study we aimed to explore how inflammatory cells sense cold.
Methods: qRT-PCR, western blot and immunohistochemistry were used to detect TRP mRNA and protein in several human-derived cell lines, primary cells and skin biopsies. Cytokine concentrations in culture supernatants of stimulation assays were detected by ELISA.
Results: mRNA of different thermo-TRPs was detected in PBMCs, macrophages and keratinocytes. The 'cool' menthol receptor TRPM8 is differentially expressed in glycosylated form in immune cells, human fibroblast and lymphoblast cell lines. TRPM8 expression was detected in skin biopsies and localized to the keratinocytes and epithelial cells lining blood vessels. No differences in expression were observed between biopsies from healthy controls and CAPS or CU patients. Preincubation of PBMCs with menthol, a TRPM8-agonist, resulted in enhanced interleukin-1 beta (IL-1β) secretion in response to TLR stimulation.
Conclusion: TRPM8 is differentially expressed in human immune cells in glycosylated form, indicating active regulation. *Ex vivo* stimulation of PBMCs with menthol results in an increased inflammatory response to TLR stimuli. We hypothesize that *in vivo* cold exposure results in a modulated inflammatory response, through activation of temperature sensitive ion channels. This activation is most likely regulated at the post-translational level.
Competing interests: None declared.

A177

PW02-036 - Thermosensitive CA2+ assay innate immune cells

T Remijn[1,2,3*], M Stoffels[1,2,3], A Simon[1,2,3], JW van der Meer[1,2,3]
[1]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Netherlands; [2]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Netherlands; [3]Nijmegen Centre for Molecular Life Sciences, Nijmegen, Nijmegen, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A177

Introduction: Temperature has immunomodulatory effects on innate immune cells: incubating monocytes at low temperatures for example increases pro-inflammatory responses after LPS-stimulation. The mechanisms mediating this effect are still unclear. The activity of several ion channels is sensitive to changes in temperature, leading to cytosolic calcium influx. Calcium is universally involved in cellular processes, its effects depending on the cellular context. Although not much is known about the role of calcium in immune cells, it has been shown that calcium is necessary for inflammasome assembly. Conventional methods of measuring calcium are not ideal to study the relation between temperature and intracellular calcium concentrations ($[Ca^{2+}]_i$).
Objectives: In this study, we aimed to optimize a fluorescence-based assay to measure $[Ca^{2+}]_i$ in innate immune cells using a qPCR-machine in a 96-well format.
Methods: $[Ca^{2+}]_i$ can be measured by loading cells with the indicator Fluo-4AM. However, its affinity (K_d) is highly dependent on temperature. This is not established in detail because the indicator is mostly used in temperature independent assays. Here, we determined $K_d^{Fluo\text{-}4}$ for temperatures in a range of 5-55°C, enabling calculation of exact intracellular calcium concentrations. We applied the assay in different cell lines and primary human innate immune cell populations.
Results: Exposing cells to decreasing temperature induces elevated intracellular calcium concentrations. Subsequently rising temperature restores $[Ca^{2+}]_i$ to basal levels. This $[Ca^{2+}]_i$ increase can be blocked by the aspecific ion channel antagonist Ruthenium Red.
Conclusion: We show that the intracellular calcium concentration in immune cells increases significantly upon decreasing temperature. Blockade by Ruthenium Red indicates that this effect is mediated by thermosensitive ion channels. We successfully optimized a 96-well format fluorescense-based assay using a qPCR machine, enabling $[Ca^{2+}]_i$ measurements in innate immune cell populations under tight temperature control.
Competing interests: None declared

A178

PW02-037 - The Eurofever cohort of 136 patients with CAPS

R Levy[1*], L Gerard[1], J Kuemmerle-Deschner[2], H Lachmann[3], I Koné-Paut[1], L Cantarini[4], P Woo[3], A Naselli[5], B Bader-Meunier[1], F De Benedetti[6], SM Al-Mayouf[7], S Ozen[8], M Hofer[9], J Frenkel[10], C Modesto[11], I Nikishina[12], A Martini[5], N Ruperto[5], B Neven[1], M Gattorno[5]

[1]for PRINTO and Eurofever Project, Paris, France; [2]for PRINTO and Eurofever Project, Tübingen, Germany; [3]for PRINTO and Eurofever Project, London, UK; [4]for PRINTO and Eurofever Project, Siena, Italy; [5]for PRINTO and Eurofever Project, Genova, Italy; [6]for PRINTO and Eurofever Project, Roma, Italy; [7]for PRINTO and Eurofever Project, Riyadh, Saudi Arabia; [8]for PRINTO and Eurofever Project, Ankara, Turkey; [9]for PRINTO and Eurofever Project, Lausanne, Switzerland; [10]for PRINTO and Eurofever Project, Utrecht, Netherlands; [11]for PRINTO and Eurofever Project, Barcelona, Spain; [12]for PRINTO and Eurofever Project, Moscow, Russian Federation

Pediatric Rheumatology 2013, **11(Suppl 1):**A178

Introduction: Cryopyrin associated periodic syndromes (CAPS) is a spectrum of autoinflammatory syndromes including familial cold autoinflammatory syndrome, Muckle Wells syndrome and CINCA (chronic infantile neurologic articular syndrome). All phenotypes are associated with gain of function mutation in *NLRP3* encoding cryopyrin, a protein involved in inflammasome and IL-1b processing.
Objectives: We describe clinical features in a large cohort of patients and test the hypothesis of a génotype/phénotype correlation.
Methods: Members of PRINTO were invited to report their patients to the Eurofever registry via a web-based questionnaire. Experts in CAPS validated diagnosis in 136 patients. Mean age of the cohort at inclusion was 26 years.
Results: Mean age at disease onset was 5 years. Skin rash, articular involvement and fever were the most prevalent features, respectively in 132, 117 and 108 patients. 6 patients suffered from severe articular involvement defined as flexion contractures, patella overgrowth or bone complications. Neurological involvement was noticed in 55 patients and characterized by morning headaches (n=39), aseptic meningitis (n=23), papilledema (n=29), Severe neurological involvement (hydrocephalus, mental retardation, seizures) was reported in 16 patients. Ophthalmological involvement was observed in 97 patients suffering from conjunctivitis (n=87), uveitis (n=7), or papilledema. 56 patients presented neurosensorial hearing loss. 78 had a chronic course, while 58 experienced only acute episodes. 76 cases were familial, 54 sporadic. NLRP3 sequencing had been performed in all patients; heterozygous germline mutation was reported in 133 patients. 7 mutations were recurrent and found in 106 patients: R260W (n= 36), T348M (n= 20), A439V (n= 14), V198M (n= 13), E311K (n= 9), Q703K (n= 9), D303N (n= 5), 27 patients carried a non recurrent mutation; Statistically significant associations between genotype and phenotype were found: patients with non recurrent mutations had the most severe phenotype (youngest age of onset, more frequent neurological involvement, including severe ones and severe arthropathies). Patients carrying R260W mutation were associated with acute pattern, cold triggering and onset > 6 months. T348M group was associated with onset < 6 months, a chronic course and frequent hearing loss. V198M, E311K and A439V were less frequently associated with neurological involvement.
Conclusion: This retrospective large survey of CAPS patients allowed to better define genotype-phenotype correlation. Patients with non-recurrent NLRP3 mutations were identified at risk of severe disease-related complications. Early effective treatment with anti-IL1b drugs and close monitoring should be recommended in these patients.
Competing interests: None declared.

A179

PW02-038 - Treatment-resistant NOMID with autoantibodies

L Broderick[1,2*], J Chang[2,3], I Szer[2,3], HM Hoffman[1,2,3,4]

[1]Medicine, University of California San Diego, La Jolla, USA; [2]Rady Children's Hospital San Diego, San Diego, USA; [3]Pediatrics, University of California San Diego, La Jolla, USA; [4]Ludwig Institute of Cancer Research, San Diego, USA

Pediatric Rheumatology 2013, **11(Suppl 1):**A179

Introduction: Neonatal Onset Multisystem Inflammatory Disorder (NOMID) is the most severe of the Cryopyrin-Associated Periodic Syndromes (CAPS) spectrum [1]. Given the persistent nature of CAPS, symptoms may be confused with autoimmune diseases. However, unlike autoimmune diseases, autoinflammatory disorders are not associated with high-titer autoantibodies or antigen-specific T cells. Here we describe a patient with severe and early onset features of NOMID confirmed by NLRP3 mutation, hepatitis, neonatal antibodies and a limited response to high dose anakinra.
Case report: N.D. is a 2.5 yo male with congenital hearing loss who initially presented at 2 months of age with direct hyperbilirubinemia and transaminitis. Liver biopsy showed giant cell hepatitis. He had a positive ANA (with strong cytoplasmic staining), anti-smooth muscle antibody, and mildly positive double-stranded DNA antibody although his mother had no autoimmune antibodies. At 3 months of age, he developed persistent fevers and an urticarial-like rash and at 1 year he developed hip arthritis followed by knee swelling. His physical exam was notable for poor growth, macrocephaly, and irritability. He had generalized lymphadenopathy, pronounced hepatosplenomegaly and arthralgia with effusion of the left knee. Laboratory evaluations demonstrated persistently elevated CRP up to 200 mg/L, ESR 76 mm/hr, and ferritin 114 ng/mL. Urine showed no proteinuria or mevalonic acid. MRI of the brain demonstrated severe volume loss and Xray of distal femur showed physeal irregularity. Skin biopsy showed neutrophilic urticaria particularly surrounding eccrine glands. Sequencing of NLRP3 revealed a c.926T>A mutation (Phe209Leu) which was described in a NOMID patient [1]. Despite treatment with anakinra, his clinical course has been complicated by uveitis, anemia, rib fractures with rachitic changes suggestive of vitamin D-deficiency rickets, chronic diarrhea and fat malabsorption, abnormal hearing, left knee reactive synovitis, seizures and significant developmental delay in speech, and both fine and gross motor skills. While he demonstrated an initial modest improvement on 4mg/kg anakinra [2,3] including decreased rash, improved mobility and development, and slow but steady weight gain, he continues to have intermittent rashes, irritability and enlarging head circumference despite dose escalation to 11mg/kg. In addition, he has a severe, persistent unilateral knee synovitis with minimal response to therapy.
Discussion: This complex patient with evidence for persistent inflammation emphasizes the variable clinical presentation of the CAPS spectrum. The severity of this case also illustrates that NLRP3-related inflammation may begin *in utero* and suggests that in some cases, autoinflammation and autoimmunity may not be mutually exclusive.
Competing interests: L. Broderick: None declared, J. Chang: None declared, I. Szer: None declared, H. Hoffman Consultant for: Sobi, Novartis and Regeneron.

References

1. Feldmann J, *et al*: *Am J Hum Genet* 2002, **71**:198.
2. Neven B, *et al*: *Arthritis Rheum* 2010, **62(1)**:258.
3. Sibley CH, *et al*: *Arthritis Rheum* 2012, **64**:2375.

A180

PW02-039 - Long-term anakinra treatment in CAPS: a metaanalys

M Leinonen, B Hallén, M Aldén-Raboisson, H Olivecrona[*]

SWEDISH ORPHAN BIOVITRUM, Stockholm, Sweden

Pediatric Rheumatology 2013, **11(Suppl 1):**A180

Introduction: The common denominator in CAPS (FCAS, Muckle-Wells syndrome, NOMID/CINCA) is an uncontrolled IL-1β release. An often complete response after treatment with the IL-1 blocker anakinra (Kineret®) has been demonstrated in all three entities of CAPS [1-3]. However, the overall documentation is limited due to the inherent difficulties in conducting randomized studies in the more severe forms of the disease, and the low prevalence of CAPS (1 in 1 000 000). The literature consists of uncontrolled, small clinical studies, and a large number of case reports.
Objectives: To estimate CAPS disease activity before and after long-term treatment with anakinra by a meta-analysis.
Methods: Five major data bases were searched to find published data on anakinra in the treatment of CAPS. To be included in the meta-analysis studies had to be prospectively designed and include longitudinal long-term data (at least 6 month follow-up) on the selected endpoints and published in a peer reviewed journal. Presence of primary disease symptoms (rash, headache, arthralgia, and fever) and on levels of inflammation markers (CRP, SAA) were selected as endpoints. Pooled estimates were calculated as

weighted average of the individual studies, using the inverse of variability as weights. The main meta-analysis was supported by a sensitivity analysis, conducted by including studies of retrospective nature and/or providing only short-term data. Data from 5 untreated patients were used as a control group [4].

Results: The search resulted in 14 clinical studies and a large number of published case reports (n=79) on anakinra treatment in all CAPS subtypes. Three studies fulfilled the inclusion studies for the main analysis and three additional studies for the sensitivity analysis. Selected studies comprised both pediatric and adult patients in all three CAPS entities. All four disease symptoms were present in a large proportion of patients at baseline, rash being most frequently (95% CI from 83.5% to 97.5%) and fever least frequently reported (95% CI from 46.6% to 71.8%). At the last follow-up visit (11-60 months) the estimated proportion of affected patients was <20% for each symptom. Almost all patients had abnormal SAA (95% CI from 85.0 to 98.9% of patients) and CRP (95% CI from 91.5% to 100.0%) at baseline. Mean SAA decreased from a baseline value of 41.0 mg/L to 6.9 mg/L at the last visit and CRP from 28.8 to 6.4 mg/L. Among the 5 untreated control patients (NOMID/CINCA), all symptoms except self-reported fever were still present at the last visit at follow-up (median 52 months). The sensitivity analyses showed comparable findings.

Conclusion: The results of the present meta-analysis of long-term efficacy measured as presence of primary disease symptoms (rash, headache, arthralgia, and fever) and levels of inflammation markers (CRP, SAA), confirm the efficacy of anakinra in the treatment of CAPS, including NOMID, MWS, and FCAS.

Competing interests: M. Leinonen Consultant for: Swedish Orphan Biovitrum AB, B. Hallén Employee of: Swedish Orphan Biovitrum AB, M. Aldén-Raboisson Employee of: Swedish Orphan Biovitrum AB, H. Olivecrona Employee of: Swedish Orphan Biovitrum AB.

References

1. Hawkins PN: *N Engl J Med* 2003, **348**:2583-2584.
2. Hoffman HM: *Lancet* 2004, **364**:1779-1785.
3. Goldbach-Mansky : *N Engl J Med* 2006, **355**:581-592.
4. Lepore: *J Pediatr* **157(2)**:310-315.

A181

PW02-040 - Low-penetrance NLRP3 variants

T Endres[1], F Hofer[1], R Goldbach-Mansky[2], HM Hoffman[3], N Blank[4], K Krause[5], C Rietschel[6], G Horneff[7], P Lohse[8], J Kuemmerle-Deschner[1*]
[1]Department of Pediatrics, Division of Pediatric Rheumatology, University Hospital Tuebingen, Tuebingen, Germany; [2]Translational Autoinflammatory Disease Section, NIAMS/NIH, Bethesda, MD, USA; [3]Division of Rheumatology and Allergy/Immunology, University of California at San Diego, San Diego, CA, USA; [4]Hämatologie, Onkologie u. Rheumatologie, Universitätsklinikum Heidelberg, Heidelberg, Germany; [5]„Allergie-Centrum Charité", Klinik für Dermatologie, Charité Campus Mitte, Berlin, Germany; [6]Kinder- und Jugendrheumatologie, Clementine-Kinderhospital, Frankfurt, Germany; [7]Abteilung für Allgemeine Kinder- und Jugendmedizin, Asklepios-Klinik Sankt Augustin, Sankt Augustin, Germany; [8]Institut für Laboratoriumsmedizin und Humangenetik, Singen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A181

Introduction: Cryopyrin-associated periodic syndrome (CAPS) presents as rare, autosomal dominant disease spectrum, due to mutations in the *NLRP3* gene which result in an excessive interleukin-1 (IL-1) release.
In patients with low-penetrance NLRP3 variants, the clinical presentation varies widely. So far, a correlation with a specific phenotype could not be demonstrated.

Objectives: The aim of this study was to analyze the association of the V198M, R488K, and Q703K substitutions with a specific phenotype, laboratory markers, and the response to IL-1 inhibitors anakinra and canakinumab.

Methods: This multi-center observational study included 44 patients (25 children and 19 adults). All patients were symptomatic with some symptoms suggesting possible CAPS at the time of baseline examination. Genetic analysis detected one of the following NLRP3 variants: Q703K (n=18), R488K (n=6), and V198M (n=20).
Clinical phenotypes were described and laboratory markers were analyzed. In order to review the response to IL-1 inhibitors, data from follow-up visits were also evaluated.

Results: At baseline examination, patients reported signs of systemic inflammation such as fever (75%), headache (73%), musculoskeletal symptoms (84%), and fatigue (77%). Other CAPS-specific features were rash (82%), conjunctivitis (43%), and sensorineural hearing loss (25%).
More than half of the patients (57%) reported abdominal pain and other gastrointestinal symptoms. A history of gastro-esophageal reflux was described by 23% of the patients, and 39% of the patients had oral ulcers.
Inflammation markers were only slightly increased: ESR was elevated in 26% (n=34) and C-reactive protein (CRP) in 38% (n=40).
Serum amyloid A (SAA) was raised in 36% (8/22) of the patients. Eight out of nine patients (89%) had elevated TNF-α-levels at baseline examination.
At baseline evaluation, 25 patients were treated with IL-1 inhibitors (anakinra or canakinumab). Data from follow-up visits during the first year of treatment were available from 21 patients: clinical disease activity was reduced in all cases; five patients (24%) achieved full remission, 13 (62%) still had mild symptoms, and three patients (14%) showed only a partial response.

Conclusion: Heterozygous carriers of NLRP3 variants V198M, R488K, and Q703K display distinct clinical characteristics compared to CAPS patients with definite disease causing mutations, including a high incidence of gastrointestinal symptoms, only slightly elevated inflammatory parameters, and a potentially inferior response to IL-1 inhibition.

Competing interests: None declared.

A182

PW02-041 - Canakinumab treatment regimens in CAPS-patients

F Hofer[1], T Endres[1], B Kortus-Götze[2], N Blank[3], E Weißbarth-Riedel[4], C Schuetz[5], T Kallinich[6], K Krause[7], C Rietschel[8], G Horneff[9], J Kuemmerle-Deschner[1*]
[1]Department of Pediatrics, Division of Pediatric Rheumatology, University Hospital Tuebingen, Tuebingen, Germany; [2]Klinik für Innere Medizin, Schwerpunkt Nephrologie, Universitätsklinikum Marburg, Marburg, Germany; [3]Hämatologie, Onkologie u. Rheumatologie, Universitätsklinikum Heidelberg, Heidelberg, Germany; [4]Kinderrheumatologische Ambulanz, Universitätsklinikum Eppendorf, Hamburg, Germany; [5]Klinik für Kinder und Jugendmedizin, Universitätsklinikum Ulm, Ulm, Germany; [6]Kinderklinik Sektion Rheumatologie, Charité Campus Virchow, Germany; [7]„Allergie-Centrum Charité", Klinik für Dermatologie, Charité Campus Mitte, Berlin, Germany; [8]Rheumatologische Ambulanz , Clementine Kinderhospital, Frankfurt, Germany; [9]Abteilung für. Allgemeine Kinder- und Jugendmedizin, Asklepios Klinik Sankt Augustin, St. Augustin, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A182

Introduction: Canakinumab is a recombinant monoclonal fully human antibody against Interleukin-1β and currently the only drug approved for the treatment of CAPS in Europe. Current dose recommendations are 150mg (body weight >40kg) respectively 2mg/kg bodyweight (15 to 40kg) every 8 weeks but yield insufficient response in some individuals, especially in children and patients with severe phenotypes [1].

Objectives: In this study we analyzed the response to daily practice (in contrast to trial condition) canakinumab treatment regimens in CAPS-patients with focus on age, mutation and clinical presentation and the necessity and effect of dose adjustment.

Methods: An observational national multicenter study was conducted. CAPS-Patients were included if they received at least two doses of canakinumab. Data included information regarding demographics, treatment, clinical disease activity and inflammatory markers (including SAA, CRP, S100, ESR, IL-6). Response to treatment was assessed using CAPS-disease activity scores, CRP and/or SAA levels.

Results: A cohort of 68 patients with CAPS was analyzed. At the beginning of treatment 27 patients had been younger than 18 years with a median age of 25.4 years (range 22 months to 73 years). The most frequent mutations were R260W, A439V, E311K, V198M, Q703K and most patients showed MWS or FCAS/MWS phenotype (3 patients with NOMID, 4 with MWS/NOMID). The median treatment duration was 855 days (range: 28-1973 days). In 57% (39) of patients full response was sustained until next scheduled drug application (34% (23) partial remission). With standard treatment 31% (21) of patients achieved full response. In 44% (30) of all patients canakinumab dose and/or application interval was increased above the standard regimen (2/3 NOMID, 3/4 MWS/NOMID). Two serious adverse events were reported (severe infection, osteonecrosis), mild and moderate adverse events were mostly upper respiratory tract infections but almost no injection site reactions.

Conclusion: Most CAPS-Patients achieve full remission with canakinumab. However, almost 50% of patients, particularly children, require dose adjustment. Dose increase was well tolerated and full remission was achieved without an increased rate of adverse events.
Competing interests: F. Hofer: None declared, T. Endres: None declared, B. Kortus-Götze: None declared, N. Blank: None declared, E. Weißbarth-Riedel: None declared, C. Schuetz: None declared, T. Kallinich: None declared, K. Krause: None declared, C. Rietschel : None declared, G. Horneff: None declared, J. Kuemmerle-Deschner Grant / Research Support from: NOVARTIS, Consultant for: NOVARTIS

Reference

1. Kuemmerle-Deschner JB: Efficacy of antibiotic therapy for SAPHO syndrome is lost after its discontinuation: an interventional study. *Ann Rheum Dis* 2011, **70**:2095-2102.

A183

PW02-042 - Induction of MDSC in Muckle-Wells syndrome

N Rieber*, A Brand, D Neri, T Hall, I Schäfer, S Hansmann, J Kümmerle-Deschner, D Hartl
Children's Hospital, University of Tübingen, Tübingen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A183

Introduction: Muckle-Wells syndrome (MWS) is caused by mutations in the *NLRP3*-gene encoding cryopyrin, leading to overproduction of IL-1β and other NLRP3 inflammasome products. Myeloid-derived suppressor cells (MDSCs) represent a novel innate immune cell subset, are generated in tumor, infective, and proinflammatory microenvironments and are capable of suppressing T cell responses. Consequently, MDSCs are considered a key intermediary in balancing innate and adaptive immune responses, particularly under chronic disease conditions.
Objectives: We hypothesized that NLRP3 inflammasome-dependent factors induce the generation of MDSCs in MWS.
Methods: We studied granulocytic MDSC numbers in 25 MWS patients under anti-IL-1 therapy with canakinumab and 20 healthy controls. After Ficoll density gradient sedimentation, granulocytic MDSCs were characterized as $CD33^{high}CD66b^{high}IL\text{-}4Ra^{inter}HLA\text{-}DR^{low}$ neutrophilic cells in the PBMC fraction, according to previously established human MDSC analysis methods. The functionality of MACS-isolated MDSCs was assessed using polyclonal T cell proliferation and cytokine / chemokine secretion tests. Physician's global assessment of disease activity, CRP, ESR, and T helper cell subsets were determined at the same time points and correlated with MDSC levels. Serum samples of 22 MWS patients and 5 healthy controls were examined by multiplex technique for possible MDSC inducing factors.
Results: MWS patients under anti-IL-1 therapy displayed significantly elevated MDSC numbers (mean 1.65 ± 0.33 %; range 0.16 – 5.17 %) compared to healthy controls (mean 0.45 ± 0.05 %; range 0.12 – 1.04%; p = 0.0025), although clinical MWS-disease activity was generally low at time of examination. MDSCs were functionally competent, as they suppressed polyclonal T cell proliferation, Th1, Th2, and Th17 responses. MDSCs correlated directly with Treg/Th17 and Treg/Th1 ratios indicating an influence on T helper cell subsets. Multiplex assays revealed the established MDSC-inducing growth factors GM-CSF and VEGF elevated in MWS sera even under anti-IL-1 therapy with canakinumab.
Conclusion: MWS patients under anti-IL-1 therapy display significantly elevated numbers of granulocytic MDSCs. Increased MDSCs in MWS might represent a novel autologous anti-inflammatory mechanism in autoinflammatory conditions and may serve as a future therapeutic target.
Competing interests: N. Rieber Grant / Research Support from: I obtained research grant from Novartis GmbH in 2012, Paid Instructor at: I held a paid talk for Novartis GmbH in 2012, A. Brand: None declared, D. Neri: None declared, T. Hall: None declared, I. Schäfer: None declared, S. Hansmann: None declared, J. Kümmerle-Deschner Grant / Research Support from: Obtained research grants from Novartis GmbH, D. Hartl Grant / Research Support from: Obtained research grants from Novartis GmbH.

A184

OR9-001 - Exome sequencing in monogenic Behçet-like disease

Q Zhou[1*], R Laxer[2], M Pelletier[3], M Ramaswamy[3], H-Y Wang[4], D Chin[1], A Gül[5], C Sibley[6], M Barat-Houari[7], R Siegel[3], DL Kastner[1], I Aksentijevich[1]
[1]Inflammatory Disease Section, National Human Genome Research Institute (NHGRI/NIH), Bethesda, USA; [2]Hospital for Sick Children, Toronto, Canada; [3]Immunoregulation Section, National Institute of Arthritis and Musculoskeletal and Skin (NIAMS/NIH), USA; [4]Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases (NIAID/NIH), Bethesda, USA,; [5]Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [6]Office of Clinical Director, National Institute of Arthritis and Musculoskeletal and Skin (NIAMS/NIH), Bethesda, USA; [7]Département de Génétique, LBM -CHU de Montpellier, Montpellier, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A184

Introduction: A Caucasian family with an affected mother and 2 affected daughters presented with early onset Behçet–like disease, manifesting with arthralgia/arthritis, mouth and genital ulcers, and uveitis. They are negative for *HLA**B51. Their symptoms are significantly ameliorated with TNF-inhibitors.
Objectives: To identify the causative mutation(s) in this family we sequenced the exomes of the 3 affected patients.
Methods: Exome data were filtered for novel variants not present in dbSNP, the 1000 Genomes Pilot Project, NHLBI Exome Sequencing Project (ESP5400), and 124 exomes from in-house data.
Results: We identified 21 putative candidate variants that are both novel and consistent with dominant inheritance. Sanger sequencing validated all 21 variants. Three variants identified in *TNFRSF9*, *MGEA5*, and *TNFAIP3* genes were confirmed to have arisen *de novo* in the affected mother based on the genotyping of the healthy maternal grandmother, the maternal unaffected brother, and the unaffected paternal aunt. The candidate variant in *MGEA5* was predicted as benign by PolyPhen-2. The 2 candidate variants that remained for consideration are p.C78W (*TNFRSF9;* CD137; 4-1BB) and p.L227X (*TNFAIP3;*A20). Haplotype analysis showed that p.C78W occurred *de novo* on the haplotype inherited from the grandmother. The p.L227X mutation-associated haplotype was found on the grandfather's haplotype; his sample is not available for analysis. Because we reached the limit for further analysis of candidate variants in the family, we studied 6 Turkish familial Behçet cases and 56 sporadic Caucasian cases. All of these individuals were negative for mutations in either candidate gene. Both 4-1BB and A20 are strong candidates and potentially in the same signaling pathway. 4-1BB is a TNF-family receptor that costimulates T cell responses and promotes survival of lymphocytes and dendritic cells; A20 is a negative regulator of NF-kB activation by TNF and TLR family receptors. Mutant 4-1BB expressed in Jurkat cells was associated with reduced expression on the plasma membrane, and activated T cells from all 3 patients had a marked decrease in surface expression, especially in $CD8^+$ T cells. Despite reduced surface expression, the C78W TNFRSF9 mutation could have a gain-of-function phenotype like TNFR1 mutations in TRAPS, leading to intracellular retention of mutant protein and spontaneous signaling, which in this case could amplify immune responses by T cells and other cell types expressing 4-1BB. Patient peripheral blood cells also had lower total A20 protein levels relative to controls and, consistent with A20 lack of function, increased I-κB degradation after cellular activation.
Conclusion: Behçet-like disease in this family is associated with mutations in 2 genes that affect immune cell survival and production of inflammatory cytokines. Additional functional studies will determine whether 1 or both mutations may contribute to this dominantly-inherited phenotype.
Competing interests: None declared.

A185

OR10-001 - Altered mitochondrial ROS and metabolism in TRAPS

R Siegel[1*], L Billingham[1], M pelletier[1], C Cudrici[1], A Ombrello[2], M Murphy[3], D Kastner[2]
[1]NIAMS, Bethesda, USA; [2]NHGRI, NIH, Bethesda, USA; [3]Cambridge University, Cambridge, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A185

Introduction: Mutations in TNFR1 cause the familial autosomal-dominant autoinflammatory disorder TNF receptor-associated periodic syndrome (TRAPS). Most TRAPS-associated mutations in TNFR1 disrupt normal receptor function and cause retention of the mutant protein in the ER. Cells expressing mutant TNFR1 have enhanced MAP Kinase activation at baseline and hyper-responsiveness to innate immune stimuli, which is aided by the wild-type receptor. We have previously found that reactive oxygen species (ROS) generated by mitochondrial respiration is critical for this phenotype [1]. TRAPS patient cells, and cells from TNFR1 mutant

mice display enhanced basal and maximal oxygen consumption, suggesting that enhanced mitochondrial respiration can in some circumstances contribute to acute inflammatory responses through increased generation of ROS.
Objectives: To identify the metabolic and mitochondrial abnormalities underlying increased ROS production and hyper-inflammatory responses of TRAPS cells and more generally, the role of mitochondrial ROS in acute inflammatory responses and production of pro-inflammatory cytokines.
Methods: Oxygen consumption, reactive oxygen species generation, and cytokine production was measured in mouse embryonic fibroblasts and macrophages from mice harboring TRAPS-associated TNFR1 mutations and peripheral blood monocytes from TRAPS patients. Agents that disrupt specific complexes in the electron transport chain (ETC) or import of key substrates into mitochondria were used to dissect the sources of increased ROS production, mitochondrial oxygen consumption and transcription of pro-inflammatory cytokines and chemokines after treatment with innate immune activating stimuli such as LPS.
Results: Experiments with permeablized cells fed with ETC substrates showed no intrinsic abnormalities in complexes I-IV of the ETC. Fatty acid supplementation and etomoxir, which blocks fatty acid uptake by mitochondria, showed that fatty acid oxidation and delivery to mitochondria is a key driver of increased spare respiratory capacity in TNFR1 mutant cells. However inflammatory cytokine production in response to LPS is not reduced in the presence of etomoxir in normal cells or those harboring TNFR1 mutations. Rather, inhibition of glycolysis was more effective in reducing macrophage production of IL-6 and IL-1.
Conclusion: Enhanced inflammatory cytokine production in TRAPS is a result of altered cellular metabolism rather than intrinsic abnormalities in the mitochondrial ETC. Increased mitochondrial fatty acid metabolism accounts for the increased mitochondrial respiratory reserve, but not inflammatory cytokine production in TNFR1 mutant or normal macrophages. These results emphasize the pathogenic role of mitochondrial ROS in inflammatory responses, which is is being investigated using mitochondria-targeted antioxidants in mouse models and a planned human trial of the mitochondrial antioxidant Mito-Q.
Competing interests: None declared.
Reference
1. Bulua, *et al*: *J. Exp Med* 2011.

A186

OR10-002 - A novel TNFR1 transcript of TRAPS gene

C Rittore[1], E Sanchez[1], S Soler[1], M Albers[2], L Obici[3], MF McDermott[4], I Touitou[1], S Grandemange[1*]
[1]INSERM / CHU A.DE VILLENEUVE, Montpellier, France; [2]University Medish Centrum, Utrecht, Netherlands; [3]IRCCS Fundazion Policlinico San Matteo, Pavia, Italy; [4]6.NIHR-Leeds Musculoskeletal Biomedical Research Unit, Leeds, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A186

Introduction: Mutations in the *TNFRSF1A* gene encoding the TNF cell surface receptor, TNFR1, cause TNFR-associated periodic syndrome (TRAPS) and polymorphisms in *TNFRSF1A*, including rs4149570, rs767455 and rs1800692, are associated with inflammatory diseases.
Objectives: We describe a novel exon 2-spliced transcript, named TNFR1-d2, and the impact of these 3 SNPs on exon 2 splicing, transcriptional activity of *TNFRSF1A* and TRAPS phenotype.
Methods: Expression of *TNFRSF1A* transcripts was performed by RT-PCR in a range of human cells and tissues. Exon 2 splicing and transcriptional activity were analysed in HEK293T and SW480 cells by *in vitro* alternative splicing and luciferase assays, respectively. We constructed haplotypes containing rs4149570, rs767455 and rs1800692 in controls (n=70), TRAPS (n=111) and TRAPS-like patients (n=450) to compare their distribution and association with clinical features of TRAPS.
Results: TNFR1-d2 was expressed in a tissue-specific manner, whereas TNFR1 expression was ubiquitous. Alternative splicing assays revealed that the T-A-T haplotype at rs4149570-rs767455-rs1800692 showed the highest expression of exon 2-skipping product (p=0.02). Transcriptional activity from the T-T haplotype at rs4149570-rs1800692 was increased compared to the G-C haplotype (p=0.03). In TRAPS patients, rs1800692 T/T homozygotes were excessively rare ($p<10^{-4}$) and TRAPS-like patients with this genotype experienced less fever.
Conclusion: Our study provides a novel mechanism of *TNFRSF1A* regulation whereby three polymorphisms in the promoter, exon 1 and intron 4 have a functional and combined effect on exon 2 splicing, via a coupling mechanism between transcription and splicing. These polymorphisms may impact the phenotype of TRAPS and TRAPS-like patients.
Competing interests: None declared.

A187

OR10-004 - Circulating micrornas in TRAPS

OM Lucherini[1*], L Obici[2], M Ferracin[3], V Fulci[4], MF McDermott[5], G Merlini[6], I Muscari[7], F Magnotti[1], LJ Dickie[5], M Galeazzi[7], M Negrini[3], CT Baldari[8], R Cimaz[9], L Cantarini[10]
[1]Department of Medical Sciences, Surgical and Neuroscience. Rheumatology Unit, Università di Siena, Siena, Italy; [2]Amyloid Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, and Department of Molecular Medicine., University of Pavia, Pavia, Italy; [3]Laboratory for Technologies of Advanced Therapies (LTTA) and Department of Morphology, Surgery and Experimental Medicine., University of Ferrara, Ferrara, Italy; [4]Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Genetica Molecolare, Sapienza., Università di Roma, Roma, Italy; [5]NIHR-Leeds Musculoskeletal Biomedical Research Unit (NIHR-LMBRU), Leeds Institute of Rheumatic and Musculoskeletal Medicine, Leeds, UK; [6]University of Pavia, Pavia, Italy; [7]Università di Siena, Siena, Italy; [8]Department of Life Sciences, Università di Siena, Siena, Italy; [9]Department of Pediatrics, Rheumatology Unit, Anna Meyer Children's Hospital and University of Florence, Florence, Italy; [10]Department of Medical Sciences, Surgical and Neuroscience. Rheumatology Unit., University of Siena, Siena, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A187

Introduction: To the best of our knowledge circulating miRNAs in TRAPS, as well as in other monogenic autoinflammatory disorders have never been investigated.
Objectives: To evaluate circulating microRNAs (miRNAs) levels in patients with tumor necrosis factor-receptor associated periodic syndrome (TRAPS), in comparison to healthy controls, and to correlate their levels to parameters of disease activity and/or disease severity.
Methods: Expression levels of circulating miRNAs were measured by Agilent microarrays in 29 serum samples from 15 TRAPS patients carrying mutations known to be associated with high disease penetrance and 8 healthy controls. Differentially expressed and clinically relevant miRNAs were detected using GeneSpring GX software.
Results: We identified a 6 miRNAs signature able to discriminate TRAPS from healthy controls. Moreover, 4 miRNAs were differentially expressed between patients treated with the interleukin (IL)-1 receptor antagonist anakinra and untreated patients. Of these, miR-92a-3p expression was found to be reduced in untreated patients, while its expression levels were similar to healthy controls in samples obtained during anakinra treatment. MiR-92b levels were inversely correlated with the number of fever attacks/year during the 1st year from the index attack of TRAPS, while miR-377-5p levels were positively correlated with serum amyloid A (SAA) circulating levels.
Conclusion: Serum miRNAs levels show a baseline pattern in TRAPS, and may serve as potential markers of response to therapeutic intervention.
Competing interests: None declared.

A188

OR10-005 - Treatment responses in TRAPS: Eurofever/ Eurotraps

HJ Lachmann*, K Minden, L Obici, A Naselli, A Insalaco, V Hentgen, R Merino, C Modesto, N Toplak, R Berendes, R Cimaz, A Jansson, A Martini, P Woo, I Touitou, M Gattorno
for PRINTO, Eurotraps/Eurofever Projects, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A188

Introduction: TRAPS is a rare lifelong disease. Optimal treatment is not established but tends to rely either on corticosteroids with risks of known short and long term side effects or anti cytokine agents which are expensive, and both types of agents lead to higher risk of infection.
Objectives: To analyze treatments used and their responses in patients with clinical TRAPS associated with a pathogenic sequence variants (PSV) in TNFRSF1A enrolled in the Eurofever/Eurotraps registry.
Methods: The Eurofever Project (agreement n 2007332, EAHC) built a common web-based registry for all Autoinflammatory diseases in collaboration with the Eurotraps Project (FP7, HEALTH-F2-2008-200923).
Results: In total there was treatment data on 113 patients with 45 different PSV of *TNFRSF1A*. Patients came from 14 countries and 94.5% were of

European Caucasian ancestry. 16 patients had only received symptomatic treatment.
Of 48 patients given steroids only with attacks 20 (42%) reported complete success (CR) in terminating acute attacks but 38 (79%) were either converted to biologic therapy or had them added to improve disease control. Of 22 patients on maintenance steroids 6 (27%) reported complete attack prevention but 14 (64%) were converted to biologic therapy. 37 patients received etanercept (in the 19 where data was available for a median of 51 months). 9 patients had a CR and 26 a partial response (PR). 10 remain on etanercept. Of the 27 who discontinued etanercept inadequate disease response was sole or contributory reason for discontinuing etanercept in 21 and side effects in 9. 20 patients converted to anti IL-1 therapy. 38 patients received anakinra. 34 (89%) reported a CR and 4 a PR. 92% remain on anakinra with a median treatment duration date of 23 months (range 1- 89 months).
Conclusion: This is the largest survey of treatment of TRAPS to date. The marked predominance of patients from Western Europe may be reflected in the high use of biologic agents which are not necessarily widely available. The most significant findings are that corticosteroids are effective in more than 40% of patients initially but almost 80% of patients have been converted to anti cytokine agents. Anakinra is completely effective in 89% of cases and continued as long-term treatment in 92%. Its use is associated with a 90% reduction in the requirement for corticosteroids to treat acute attacks. Etanercept is significantly less effective and is discontinued in almost 75% of cases. Although these data strongly support use of anti IL-1 agents to treat TRAPS follow up remains short and re-evaluation will be required.
Competing interests: None declared.

A189

OR10-006 - Canakinumab in patients with TRAPS

HJ Lachmann[1*], L Obici[2], A Meini[3], V Tormey[4], K Abrams[5], N Davis[5], C Andrews[6], SG Bhansali[5], M Gattorno[7]
[1]University College London Medical School, London, UK; [2]IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; [3]Pediatric Immunology and Rheumatology, Brescia, Italy; [4]Galway University Hospitals, Galway, Ireland; [5]Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA; [6]Novartis Pharmaceuticals UK Limited, Surrey, UK; [7]G Gaslini Institute, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A189

Introduction: TNF-receptor associated periodic syndrome (TRAPS) is a rare, dominantly inherited periodic fever syndrome due to mutations of the *TNFRSF1A* gene. The IL-1 receptor antagonist anakinra has been reported to be an efficacious daily treatment. Canakinumab (CAN) is a fully human monoclonal selective anti-IL-1β antibody with a $T_{1/2}$ of ~4 wks. Interim clinical and PK data of CAN treatment in patients with active TRAPS are presented.
Objectives: To assess the efficacy, PK, and safety of canakinumab in patients with active TRAPS.
Methods: 14 adults and 6 children (7-78 yrs) with active TRAPS entered a 3-part trial: 4 months open-label 150 mg (or 300 mg) CAN every 4 wks followed by up to 5 months treatment withdrawal, then 24 months open-label CAN. Primary endpoint was complete or almost complete response at Day 15 based on physician assessed absent or minimal TRAPS signs/symptoms and normal or ≥70% reduced CRP and/or SAA. Those without response by Day 8 were eligible for another 150 mg dose and then 300 mg thereafter. Patients were observed after last dose until relapse (5 month max) before restarting CAN. Population PK analysis was performed using NONMEM based on CAN concentrations determined by ELISA from blood samples collected at pre-specified times points during the first month, at each pre-dose of CAN, and at flares thereafter.
Results: At Day 15, 19 (95%) patients achieved complete/almost complete response, including all 4 patients without it at Day 8. Two patients were dose up titrated. Clinical remission was maintained by all from Day 15 onwards except 1 who relapsed at Day 85 (during 4 month treatment period), responding to that visit's CAN dose. Upon CAN withdrawal, all patients relapsed after a median of 92 days (range 72-122 days). 18 regained response 8-27 days after restarting CAN and 2 relapsed at final visit following last dose administered during treatment period without follow-up at time of this analysis. Population PK analysis showed that serum clearance and volume of distribution of CAN were dependent on bodyweight. The estimated apparent serum clearance (CL/F) was 0.238 ±0.0139 L/day and the corresponding volume of distribution (V_{ss}/F) was 8.06 L. Following the first dose, mean±SD observed C_{max} was 16.4±4.62 μg/mL and the median T_{max} was 7.4 days. Apparent weight normalized PK parameters were comparable to the PK observed in other indications. All patients reported at least one adverse event (AE); infections, mostly of the upper respiratory tract, (n=15, 75%), followed by headache (n=9) and abdominal pain (n=7). Two serious AEs, an upper respiratory tract infection and a TRAPS relapse, were reported. All patients are ongoing in the trial.
Conclusion: Canakinumab produced a rapid clinical and serological benefit which was maintained with continued monthly dosing. Relapse occurred at a median of 92 days after last dose and remission achieved upon re-dosing. Weight normalized PK parameters were comparable to PK observed in other indications. Further studies are needed to better define CAN therapy in TRAPS.
Competing interests: H. Lachmann Consultant for: Novartis, L. Obici Consultant for: Novartis, A. Meini Consultant for: Novartis, V. Tormey: None declared, K. Abrams Shareholder of: Novartis, Employee of: Novartis, N. Davis Employee of: Novartis, C. Andrews Shareholder of: Novartis, Employee of: Novartis, S. Bhansali Shareholder of: Novartis, Employee of: Novartis, M. Gattorno Grant / Research Support from: Novartis, Consultant for: Novartis, Speaker Bureau of: SoBI

A190

OR11-001 - Protein misfolding in mevalonate kinase deficiency

S Stojanov[1,2*], SW Gersting[1], L Reitzle[1], D Reiß[1], BH Belohradsky[2], M Vogeser[3], B Maier[3], D Teupser[3], AC Muntau[1]
[1]Department of Molecular Pediatrics, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University, Munich, Germany; [2]Department of Infectious Diseases and Immunology, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University, Germany; [3]Institute of Laboratory Medicine, Ludwig-Maximilians-University, Munich, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A190

Introduction: Mevalonate kinase deficiency (MKD) is an early-onset autosomal recessive autoinflammatory fever syndrome lacking specific treatment options. Current data point to protein misfolding as underlying molecular mechanism.
Objectives: To characterize the molecular pathophysiology of eight MK variants associated with a hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) (W188X, V203A, V377I, H380R) and/or mevalonic aciduria (MA) (H20P, L264F, I268T, A334T) phenotype.
Methods: Recombinant wild-type (WT) and variant MK proteins N-terminally fused to a maltose binding protein (MBP) were expressed in *E. coli*. Following affinity purification and size exclusion chromatography, the MBP-MK fusion (fMK) or cleaved MK (cMK) proteins, respectively, were characterized with regard to oligomerization, thermal unfolding monitored by differential scanning fluorimetry, thermal aggregation analyzed by right angle light scattering, and enzyme activity. These data were correlated with the disease phenotype and MK activities measured in blood cells and/or fibroblasts of 101 homozygous or compound heterozygous MKD patients, respectively, that were collected retrospectively from our center and the literature.
Results: MK dimer assembly was impaired in all analyzed MK variants. Oligomerization profiles of V377I and V203A were most similar to that of WT, A334T showed reduced amounts of fMK and cMK dimers, while H20P and I268T revealed only small peaks of fMK dimers, and W188X, H380R, and L264F lacked any dimers, thus pointing to altered protein conformation of various degree. This was confirmed by partial unfolding in the native state, and variably accelerated thermal unfolding of all MK variants. Furthermore, thermal aggregation kinetics investigated in cMK V203A, A334T, and V377I, respectively, were altered. Catalytic function varied from high residual activity in cMK V377I (100% of WT), to moderately decreased activity in cMK V203A (70%) and fMK I268T (80%), markedly decreased activity in cMK A334T (3.1%), and almost no activity in fMK H20P (0.4%), as well as W188X (0.7%), L264F (0.9%), and H380R (1.5%). Consistently, MKD patients carrying at least one V377I mutation presented the HIDS phenotype, while those being compound heterozygous or homozygous for H20P, L264F, I268T, and/or A334T mutations were associated with MA. While patient MK activities were highest among V377I homozygous or V377I/I268T and V377I/H20P genotypes, there was a considerable variability of MK activities for other genotypes analyzed being associated with HIDS or MA.

Conclusion: These results support the hypothesis of protein misfolding with loss of function being the molecular basis in MKD, and thus may assist the development of novel targeted therapeutic strategies.
Competing interests: None declared.

A191

OR11-002 - Mutations in MVK cause non-syndromic RP

AM Siemiatkowska[1], M Stoffels[2*], K Neveling[1], A Simon[2], PM van Hagen[3], AI den Hollander[4], FP Cremers[1], LI van den Born[5], RW Collin[1]
[1]Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [4]Department of Ophthalmology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [5]The Rotterdam Eye Hospital, Rotterdam, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A191

Introduction: Retinitis pigmentosa (RP) is a genetically heterogeneous retinal disease. Typically beginning with night blindness, RP is characterized by rod cell degeneration followed by cone cell death, which may ultimately lead to complete blindness. Despite extensive knowledge about genes involved in RP pathogenesis, in several cases the genetic cause remains elusive.
Objectives: We aimed to identify novel genes that are involved in the etiology of RP.
Methods: After detailed clinical characterization including funduscopy and optic coherence tomography, exome sequencing analysis was performed in a proband of Dutch origin with non-syndromic autosomal recessive RP. Identified mutations were tested for segregation within the family and in a large cohort of genetically unsolved RP patients. Upon identification of mutations in *MVK*, encoding mevalonate kinase (MK), patients with mutations in this gene underwent extensive clinical re-examination. MK enzyme activity was analyzed in cultured lymphoblastoid cells and mevalonic acid levels were measured in urine samples.
Results: Exome variant filtering and prioritization led to the identification of compound heterozygous mutations in *MVK* (p.I268T and p.A334T) in the proband and her affected brother. Screening of 269 non-syndromic RP patients revealed an additional individual who was homozygous for the p.A334T alteration. Clinical re-evaluation of all three patients revealed a relatively classic form of RP with variable extra-ocular symptoms, such as history of recurrent childhood febrile crises in two, and mild ataxia in one patient. All three affected individuals showed a significantly decreased mevalonate kinase activity and strongly elevated levels of urinary mevalonic acid.
Conclusion: Although the MK activity in cells and mevalonic acid concentrations in urine are strongly aberrant as in patients with systemic mevalonate kinase deficiencies (MKD), only mild clinical symptoms related to these phenotypes are observed in our patients, who were initially classified to have non-syndromic RP. Herewith, we add another phenotype to the spectrum of diverging disorders associated with mutations in *MVK*.
Competing interests: None declared.

A192

OR11-003 - The NLRP3 inflammasome is regulated by CaSR

JJ Chae[1*], G-S Lee[1], N Subramanian[2], DL Kastner[1]
[1]Medical Genetics Branch, National Human Genome Research Institute, Bethesda, USA; [2]Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, Bethesda, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A192

Introduction: Mutations in the gene encoding NLRP3 cause a spectrum of autoinflammatory diseases known as the cryopyrin-associated periodic syndromes (CAPS). NLRP3 is a key component of one of several distinct cytoplasmic multiprotein complexes (inflammasomes) that mediate the maturation of the proinflammatory cytokine interleukin-1β (IL-1β) by activating caspase-1. Although several models for inflammasome activation, such as K^+ efflux, generation of reactive oxygen species, and lysosomal destabilization have been proposed, the precise molecular mechanism of NLRP3 inflammasome activation, as well as the mechanism by which CAPS-associated mutations activate NLRP3, remains to be elucidated.
Objectives: To investigate how extracellular DAMP signals activate the NLRP3 inflammasome and the molecular pathogenesis of CAPS.
Methods: Using a combination of genetic, pharmacological, and biochemical approaches, we provide evidence that the CaSR is essential for NLRP3 inflammasome activation, which is directly controlled by intracellular Ca^{2+} and cAMP.
Results: Ca^{2+} or other CaSR agonists activate the NLRP3 inflammasome in the absence of exogenous ATP, whereas knockdown of *CaSR* reduces inflammasome activation in response to known NLRP3 activators. The CaSR activates the NLRP3 inflammasome through phospholipase C (PLC), which catalyzes inositol trisphosphate (IP_3) production and thereby induces release of Ca^{2+} from endoplasmic reticulum (ER) stores. The increased cytoplasmic Ca^{2+} promotes the assembly of inflammasome components, and intracellular Ca^{2+} is required for spontaneous inflammasome activity in cells from CAPS patients. CaSR stimulation also results in reduced intracellular cAMP, which independently activates the NLRP3 inflammasome. cAMP binds to NLRP3 directly to inhibit inflammasome assembly, and downregulation of cAMP relieves this inhibition. The binding affinity of cAMP for CAPS-associated mutant NLRP3 is substantially lower than for wild-type NLRP3, and the uncontrolled mature IL-1β production from CAPS patients' peripheral blood mononuclear cells is attenuated by increasing cAMP.
Conclusion: Taken together, these findings suggest that Ca^{2+} and cAMP are two key molecular regulators of the NLRP3 inflammasome that have critical roles in the molecular pathogenesis of CAPS. In addition, our data suggest a broader spectrum of potential targets for therapy of CAPS as well as other inflammatory conditions involving the NLRP3 inflammasome, including gout, type 2 diabetes mellitus, atherosclerosis, and Alzheimer's disease.
Competing interests: None declared.

A193

OR11-004 - IL-1, IL-18 and cell death in NLRP3 driven disease

L Broderick[1*], SD Brydges[1], MD McGeough[1], CA Pena[1], JL Mueller[1], HM Hoffman[1,2,3]
[1]University of California San Diego, La Jolla, USA; [2]Rady Children's Hospital, San Diego, USA; [3]Ludwig Institute of Cancer Research, San Diego, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A193

Introduction: Missense mutations in cryopyrin (NLRP3) result in a hyperactive inflammasome that drives overproduction of the pro-inflammatory cytokines interleukin-1b (IL-1b) and IL-18. Mice expressing mutations associated with familial cold autoinflammatory syndrome (FCAS) or Muckle-Wells syndrome (MWS) exhibit severe, spontaneous inflammation, early death, and hyperresponsiveness to stimuli *in vitro*. Abrogating IL-1 signaling either genetically or pharmacologically results in modest improvement of life expectancy in murine CAPS, but clearly indicates a role for players in addition to IL-1b.
Objectives: To examine the role of other caspase-1 dependent mediators, namely IL-18, in the context of inflammasome-mediated disease.
Methods: Mice heterozygous for the A350V MWS mutation or the L351P NOMID mutation were bred to IL-1R-/- or IL-18R-/- knockout mice, and weighed and assessed daily. Bone marrow macrophages and peritoneal cells were evaluated *in vitro* with inflammatory stimuli. Peripheral blood was drawn for complete blood counts and serum cytokine analyses. Pathology was examined in both young and old mice. Bone marrow transplant experiments were used to elucidate the role of cellular signaling compartments.
Results: Similar to IL-1b, hematopoietic cells derived from our mutant mice and monocytes from FCAS patients hyper-secrete IL-18, in response to low amounts of inflammatory stimuli or cold temperature. Breeding *Nlrp3* mutations onto an IL-18R null background resulted in partial phenotypic rescue that abolished skin and visceral disease in young mice, and normalized serum cytokines to a greater extent than breeding to IL-1R null mice. However, significant systemic inflammation developed in aging *Nlrp3* mutant IL-18R null mice, implicating a role for pyroptosis, a caspase-1 mediated form of cell death. Bone marrow transplant studies demonstrate that hematopoietic cells are driving disease in murine CAPS but signaling requirements differ between IL-1 and IL-18.
Conclusion: These studies demonstrate a previously underappreciated role for IL-18 signaling in murine CAPS pathogenesis. We also confirm our previous findings that CAPS is inflammasome-dependent by demonstrating that intact caspase-1 is required for disease, yet other downstream

mechanisms besides IL-18 and IL-1b mediated inflammation are involved in this autoinflammatory syndrome constellation. Our results may have important implications for patients with CAPS and residual disease and emphasize the need to explore other NLRP3 mediated pathways and the potential for inflammasome targeted therapy.
Competing interests: L. Broderick: None declared, S. Brydges: None declared, M. McGeough: None declared, C. Pena: None declared, J. Mueller: None declared, H. Hoffman Consultant for: Regeneron, Novartis, Sobi Pharmaceuticals.

A194

OR11-005 - Mast cells respond to pathogen signals with IL-1ß

L Kraas[1*], O Schmetzer[1], K Krause[1], E Latz[2], M Maurer[1]
[1]Dermatology and Allergy, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Institute of Innate Immunity, University Hospitals Bonn, Bonn, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A194

Introduction: Mast cells, key effector cells of allergic and innate immune responses, have recently been reported to be an important source of IL-1ß in patients with autoinflammatory conditions such as cryopyrin-associated-periodic-fever syndromes (CAPS). CAPS patients show IL-1beta-driven systemic inflammation together with non-histamine dependent urticarial rash, which are caused by activating mutations of the inflammasome, a multiprotein oligomer responsible for the initiation of inflammatory responses to pathogens.
Objectives: To determine if mast cells can produce and release IL-1ß in response to pathogenic signals that target the inflammasomes NLRP3, NLRC4, or AIM2.
Methods: Peritoneal mast cells (PMCs) were obtained through lavage from adult (>8 weeks) C57BL/6 mice and WBB6F1 Kit+/+ mice, purified via CD117+ bead selection (>96 % purity) and cultured for 7-14 days. 10^5 cells/well were primed with LPS (100ng/ml) for 15 hrs. Then the PMCs were stimulated with 10 μM Nigericin (NLRP3), 5mM ATP (NLRP3), 100μM R837 (NLRP3) for 45 min or for 4 hours with 600 ng Flagellin (NLRC4) transfected with DOTAP or 200 ng polydAdT (AIM2) transfected with Lipofectamine. IL-1 beta production was measured in the supernatants by Elisa.
Results: PMCs produced significant amounts (mean ± SEM) of IL-1ß upon stimulation with Nigericin (467 ± 41pg/ml), ATP (152 ± 88pg/ml), R837 (21 ± 2 pg/ml), Flagellin (245 ± 44pg/ml) and polydAdT (571 ± 194pg/ml) as compared to no stimuli (7,1 ± 0,8 pg/ml) only.
Conclusion: We show that mouse mast cells incubated with inflammasome activators produce significant amounts of IL-1ß ex vivo. Our data suggest that inflammasome-driven mast cell activation and subsequent IL-1ß production and release may importantly contribute to innate immune responses to pathogens.
Competing interests: None declared.

A195

OR11-006 - A mutation in NLRP1A causes autoinflammation

SL Masters[*], M Gerlic, BT Kile, BA Croker
THE WALTER AND ELIZA HALL INSTITUTE, Parkville, Australia
Pediatric Rheumatology 2013, **11(Suppl 1):**A195

Introduction: The NLRs (Nucleotide-binding domain and Leucine-rich repeat containing Receptors) are a family of intracellular innate immune receptors involved in host defense. Upon activation, NLRs form large protein complexes called "inflammasomes" that bind and activate Caspase-1, resulting in proteolytic activation of the pro-inflammatory cytokines pro-IL-1β and pro-IL-18 and also induce a Caspase-1-dependent form of cell death known as pyroptosis.
Objectives: Activating mutations in NLRP3 trigger the inflammasome and cause a spectrum of auto-inflammatory disease. Therefore our objective was to establish if activating mutations in NLRP1 also cause autoinflammatory disease.
Methods: We performed an *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen for dominant mutations that cause neutrophilia in G_1 mice and isolated a pedigree with a mutation in NLRP1a.
Results: Mice with the mutation $Nlrp1a^{+/Q593P}$ were fertile and remained healthy to at least 8 months of age, despite histological evidence of a multi-organ neutrophilic inflammatory disease characterised by meningitis, hepatitis, pneumonitis, pancreatitis, pulmonary peri-arteritis, myocarditis and inflammatory bowel disease. In $Nlrp1a^{Q593P/Q593P}$ homozygotes, a similar but lethal condition developed by 3-5 months of age. Neutrophil counts in these animals were 15-fold higher than wild-type, and they exhibited lymphopenia and splenomegaly. By breeding with genetically deficient mice we showed that the lethal systemic inflammatory disease was ameliorated by removing Caspase-1 and IL-1R but was independent of ASC. On the other hand, deletion of IL-18 increased the number of neutrophils in the blood, and greatly accelerated the onset of disease.
Conclusion: In summary we show for the first time *in vivo* the effect of an activating mutation in NLRP1, which causes autoinflammatory disease. We demonstrate that this disease is caused by IL-1β and Caspase-1, but not ASC. Surprisingly, IL-18 is beneficial for this condition, suggesting that caution should be employed when blocking IL-18 in human autoinflammatory diseases. Our results strongly suggest that mutations in human NLRP1 would cause autoinflammatory disease.
Competing interests: None declared.

A196

P03-001 - PFAPA and MEFV genes

F Salehzadeh[1,2*], M Vahedi[2], S Jahangiri[1], S Hosseiniasl[3]
[1]Pediatric Rheumatology, Bouali Hospital, Iran, Islamic Republic Of; [2]Pediatric Rheumatology, ARUMS, Iran, Islamic Republic Of; [3]Genetic, Emam Khomeini Hospital, Ardabil, Iran, Islamic Republic Of
Pediatric Rheumatology 2013, **11(Suppl 1):**A196

Introduction: Marshall Syndrome or PFAPA is an inflammatory periodic disease characterized by periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis. Restless, headache, abdominal pain, vomiting, hepatosplenomegaly and arthralgia are less common symptoms seeing in this disease. The diagnosis is established on the basis of clinical criteria that require the presence of a recurrent fever of early onset (<5 years) and ≥1 of the 3 associated symptoms (aphthosis, cervical adenitis, and pharyngitis), in the absence of upper respiratory tract infections and cyclic neutropenia.
Objectives: Although PFAPA is an auto inflammatory disease, it doesn't have genetic basis such as other periodic fevers. This study evaluates the 12 common MEFV gene mutations in patients with PFAPA syndrome. This study evaluates the 12 common MEFV gene mutations in patients with PFAPA syndrome.
Methods: 21 patients with PFAPA syndrome who had diagnostic criteria were enrolled in this study and 12 common MEFV gene mutations were evaluated in them. The 12 most common MEFV gene mutations (P369S, F479L, M680I (G / C), M680I (G / A), I692del, M694V, M680I, K695R, V726A, A744S, R761H, E148Q) were analyzed by using amplification refractory mutation system for 11 of the first and the PCR was performed for E148Q.
Results: The age of patients was between 6 months to 14 years old, and 15 were male. Seven patients had heterozygote and one had compound heterozygote (K695R, V725A) mutation. There were 4 alleles M694V, 3 alleles V726A, 1 allele E148Q and 1 allele K694R. No significant difference between mutated patients with non-mutated in symptoms like aphthous and stomatitis, duration of attacks, episodes of fever and response to treatment. Gaslini score test was not helpful to predict the probability of gene mutations.
Conclusion: About 30 percent of patients had MEFV gene mutations but these mutations don't play a main role in presentation of PFAPA symptoms.
Competing interests: None declared.

A197

P03-002 - Different phenotypes associated with Q703K variant

A von Scheven-Gête[1*], I Moix[2], G Simon[1], N Busso[1], M Morris[2], M Hofer[1,2]
[1]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [2]Hopitaux Universitaires Genevois, Geneve, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A197

Introduction: PFAPA syndrome is characterized by recurrent fever with aphtosis, pharyngitis and cervical adenitis. It is suspected to be an auto-inflammatory disease (AID). For some AID, a monogenic origin has been

established in the past years. In PFAPA no clear aetiology has been found yet. However in some patients variants in the NLRP3 gene can be found. The variant Q703K was described so far in patients with CAPS as well as healthy carriers.
Objectives: To describe the phenotype of our patients with recurrent fever presenting the NLRP3 variant Q703K.
Methods: In patients presenting to our consultation with periodic fever suspected to be auto-inflammatory, we screened genomic DNA by PCR and sequenced for genetic variants of NLRP3 genes. The symptoms, treatment, response to treatment and family history of the patients with the variant Q703K have been retrospectively extracted and described.
Results: We found the NLRP3 variant Q703K in 11 patients. Ten were PFAPA patients among 97 patients from our cohort and one had a CAPS phenotype: 8 boys and 3 girls with a median age of 12 months at disease onset and a median age of 52 months at diagnosis. In the PFAPA patients, family history was positive for febrile episodes or for tonsillectomy in 6 patients. The median duration of fever was 4 days and the median interval was 4 weeks. Pharyngitis was always present in 6 patients and in most episodes in 3 patients. Cervical adenitis presented in every episode in 6 patients, in most episodes in 2 patients and rarely in 1 patient. Aphtosis was found only in 1 patient in every episode, in 6 patients sometimes and in one patient in most episodes. 5 patients expressed abdominal pain that accompanied most fever episodes. 1 patient showed sometimes arthralgia, 2 patients had headaches in most episodes and one patient had once a cutaneous rash. All patients were well and without symptoms in between febrile episodes. 5 out of 7 patients treated by corticosteroids responded promptly. In the other two patients two doses were often necessary. 3 patients underwent tonsillectomy: one with no effect, in 2 the fever episodes resolved but one patient had persistent episodes of aphtosis. In 4 patients genomic sequencing of the parents was done; one parent positive for Q703K had a history of recurrent febrile episodes, but the 3 other parents did not present a history of recurrent fever episodes nor recurrent pharyngitis nor tonsillectomy. The patient with CAPS phenotype presented with urticarial rash, partial deafness, arthralgias and elevated inflammatory parameters.
Conclusion: 11 of our patients presented with the variant Q703K but 10 had clearly a phenotype of PFAPA and only one the phenotype of CAPS. This could suggest that variants in fever genes can be associated with different phenotypes and that probably more than one gene could be implicated in the pathogenesis.
Competing interests: None declared.

A198

P03-003 - Sacroilitis with propionibacterium acnes

C Galeotti*, I Koné-Paut
Pediatric Rheumatology, CHU BICÊTRE, Le Kremlin Bicêtre, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A198

Introduction: The syndrome of synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) is a rare autoinflammatory syndrome, affecting essentially the adults. Osteitis with Propionibacterium Acnes reported in the literature is generally connected to the presence of a foreign body or with the SAPHO syndrome. Chronic recurrent multifocal osteomyelitis (CRMO) is a disease related to the SAPHO syndrome, affecting the children. There is essentially an isolated bone inflammation. We report the case of a 16 year-old girl, affected with a sacroiliitis for 5 years.
Case report: This girl presented chronic pain at left sacroiliac for 5 years, with regional amyotrophy and limping. The scanner showed a left condensed sacral fine, with multiple geodes. The right sacroiliac was normal. In the hypothesis of an infection of atypical germs, a left sacroiliac biopsy was realized. There was no inflammatory infiltrate, in particular no epithelioid granuloma. But Propionibacterium acnes has been recovered from bone biopsy samples. The whole body MRI showed an aspect of sclerosis of the left sacral wing iliac joint, and the absence of lesion of the other side and the whole body. The patient was treated with NSAID then clindamycin in the hypothesis of a SAPHO syndrome. We observed a decrease of pain but the stability of the lesions after 4-months treatment with antibiotic.
Discussion: The association sacroiliitis and P.Acnes thus directs us to a SAPHO syndrome, exceptionally reported in children; furthermore, our patient has an isolated osteitis lesion, without cutaneous lesion. Assman et al treated in 2009 30 patients having a SAPHO syndrome by azithromycin, doxycycline or clindamycin [1]. He has found P.Acnes by 14 patients who had a bone biopsy. They received a 16-week treatment with antibiotics. For the period of application, the antibiotic therapy seems to have controlled the disease. After antibiotic discontinuation, however, disease relapse was observed. Our patient could have a CRMO, which is related to the SAPHO. There is no case of CRMO associated with P.Acnes reported in the literature. On the other hand, Schilling et al treated 13 teenagers having a CRMO by azithromycin, without infectious documentation [2]. Seven children had a very fast decrease of the pain and an improvement of mobility. Other diagnostic hypothesis at these patient would be a spondylarthropathy, but the fact that she has an unilateral sacroiliitis with P.Acnes and that she has no HLA B27 antigen are not in favour. The bone biopsy finding P.Acnes guided us to a cause of ostiitis and allowed us to propose a treatment which seems to be suspensive.
Competing interests: None declared.
References

1. Assman G, Kueck O, Kirchhoff T, Rosenthal H, Voswinkel J, Pfreundschuh M, Zeidler H, Wagner AD: **Efficacy of antibiotic therapy for SAPHO syndrome is lost after its discontinuation: an interventional study.** *Arthritis Res Ther* 2009, **11(5)**:R140.
2. Schilling F, Wagner AD: **Azithromycin: an anti-inflammatory effect in chronic recurrent multifocal osteomyelitis? A preliminary report.** *Z Rheumatol* 2000, **59(5)**:352-3.

A199

P03-004 - Production of proinflammatory cytokines in PFAPA

A Kozlova[1*], O Barabanova[1], N Davydova[2], A Shcherbina[1]
[1]Immunology, Federal Research and Clinical Center of Pediatric Hematology, Moscow, Russian Federation; [2]Immunology, Children's Clinical Hospital N9, Moscow, Russian Federation
Pediatric Rheumatology 2013, **11(Suppl 1):**A199

Introduction: The PFAPA syndrome, associated with aphthous stomatitis, pharyngitis and cervical adenitis which is characterized by monthly flare-ups of fever is quite common in early childhood. Affected children experience their first attack before the age of 5, with fever episodes usually abating in adolescence or young adulthood. Usually a single administration of oral corticosteroids aborts attacks. Tonsillectomy is successful in the prevention of recurrence of further episodes. To date, the cause of this syndrome is unknown. Several studies demonstrated increased serum levels of proinflammatory cytokines(interleukin 6, interleukin 1b) in these patients during the attacks.
Objectives: Determining the level of intracellular proinflammatory cytokines in patients with PFAPA syndrome during and between the attacks compared to the level of these cytokines in the control group
Methods: Four patients with PFAPA syndrome were studied during and outside febrile episodes. We determined intracellular cytokines (IL-1β, IL6, IL8, TNFa) in resting and LPS-stimulated monocytes
Results: Not surprisingly, and concurrent with previous publications during the PFAPA attack we saw increased basal levels of IL1, which rose somewhat upon LPS stimulation. Basal level of IL6 was also increased during the attack and did not respond to LPS stimulation. TNF level in PFAPA patients with or without attacks was not significantly different from the control group. Interestingly, intracellular levels of the proinflammatory chemokine IL8 was drastically decreased in PFAPA patients during the attack.
Conclusion: During the attack of PFAPA there is a significant increase in IL1 production, which is concurrent with systemic symptoms during the flare-ups and makes use of IL1 inhibitor drugs potentially effective in the treatment of the flares. Decreased IL8 levels might represent abnormal regulation of inflammation in PFAPA and require further investigation.
Competing interests: None declared.

A200

P03-005 - MEFV heterozygous mutations in PFAPA patients

A Kozlova[1*], O Barabanova[1], N Kuzmenko[1], N Zinovieva[2], O Molochnikova[2], A Shcherbina[1]
[1]Immunology, Federal Research and Clinical Center of Pediatric Hematology, Oncology, and Immunology, Moscow, Russian Federation; [2]Immunology, Children's Clinical Hospital N9, Moscow, Russian Federation
Pediatric Rheumatology 2013, **11(Suppl 1):**A200

Introduction: PFAPA syndrome (acronym for periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) is the most common cause of periodic fever in childhood. It is considered part of the wide family of the autoinflammatory diseases, but a genetic or molecular marker hasn't been identified yet, therefore, its etiology is still unknown. Diagnosis is essentially based on clinical criteria but, especially in younger children, it is sometimes difficult to differentiate it from other hereditary periodic fever syndromes. Familial (Mediterranean) fever (FMF) is caused by MEFV gene mutations, mostly inherited in autosomal recessive fashion. Yet, there are reports of heterozygous MEFV mutation carriers with clinical features of FMF.
Objectives: To assess the effects of heterozygous MEFV mutations on the clinical features of PFAPA syndrome.
Methods: We studied 27 patients with typical clinical manifestations of PFAPA syndrome, ages 2 -12 years (mean age 9.56 years). Direct sequencing of MEFV gene was performed, 21 patient had no mutations, and 6 patients had various heterozygous mutations of MEFV. We analyzed the spectrum of clinical features, response to prednisone treatment and tonsillectomy in patients who carried MEFV mutations in the heterozygous state and patients without mutations.
Results: In comparison with PFAPA group without MEFV mutation patients with heterozygous mutations in MEFV gene had the following features: the symptoms begin at a somewhat younger age (15.7 months versus 19 months), the duration of attacks was shorter (3.92 days vs 5,1 days) and the interval between the attacks of the disease was found to be shorter, these patients rarely has had arthralgias, hepatosplenomegaly and aphthous stomatitis was present less frequently, during the attack CRP levels were higher, yet (unlike HPF) they always normalized between the attacks. In the group of carriers of heterozygous MEFV mutations all patients responded to steroid treatment and had full effect after tonsillectomy.
Conclusion: In opposite to some other reports in our PFAPA group, patients with heterozygous MEFV mutation had typical PFAPA symptoms and no features suspicious of FMF. Thus, the pathologic meaning of the MEFV mutation in these group is not clear and does not seem to influence the course of the disease.
Competing interests: None declared.

A201

P03-006 - Pamidronate rapidly decreases CRP and TNFA in CNO

MM Kostik[1*], LI Vasyakina[2], VM Ponomarenko[2], IA Chikova[1], AV Minkin[3], OV Kalashnikova[1], IV Shvedovchenko[3], VG Chasnyk[1]
[1]Hospital Pediatry, Saint-Petersburg State Pediatric Medical University, Saint-Petersburg, Russian Federation; [2]Department of Immunology, Federal Institute of Public Health, The Nikiforov's Russian Center of Emergency and Radiation Medicine, Saint-Petersburg, Russian Federation; [3]St. Petersburg Scientific and Practical Center of Medical and Social Expertise, Prosthetics and Rehabilitation n.a. G.A. Albrecht , Saint-Petersburg, Russian Federation
Pediatric Rheumatology 2013, **11(Suppl 1):**A201

Introduction: Pediatric chronic nonbacterial osteomyelitis (CNO) is a sterile inflammatory bone disorder of presumed autoimmune or autoinflammatory etiology. Sometimes CNO associated with other rheumatologic conditions, such spondyloarthritis, sacroileitis, IBD, pyoderma gangrenosum and psoriasis as well as a part of distinct autoinflammatory disease (AID), such as DIRA syndrome.
Case report: 14 year old girl was admitted to our department with pain, low and moderate grade fever, delay to thrive and history of osteomyelitis. Her family history was unremarkable. The onset of disease was in the age of 10 months with small bone lesion in distal epiphysis of femur and intensive irritability. After 1 month of immobilization she had an intensive bone overgrowth with periostal reaction and deformity in distal epiphysis. The bone biopsy confirmed non-specific osteomyelitis, malignancy and infection was excluded. Antibiotic treatment was ineffective. Her disease had wide-spread course involving the whole femur from distal to proximal part. The main features included bone lesions, bone overgrowth, intensive periostal reaction and sclerosis. The disease had persistant course without remission episodes, accompanied with pain, irritability, fever and lead to elongation of femur (+8 cm). She had failure to thrive since the age of 6 years. Currently she looks like as 8 year old girl in her 14 years. Intellectual development is normal. No signs of any other diseases, particularly recurrent infections and involvement of other bones. A Tc99m bone scan revealed increased uptake only in the whole femur (+400%). Laboratorial features were specific for AID: persisted microcytic anemia (Hb-8.0 gr/dl), ESR>110 mm\h (n.v.<15), CRP >150 mg/l (n.v.<5), sideropenia. Immunological assessment was detected increased Ig A (5.2 gr\l), Ig G (26.2 gr\l) and decreased zymozan-induced chemoluminescence (11 Units, lower limit -160). Chronic granulomatose disease (CGD) was confirmed without any known foci of serious infection during her life. Also high levels of IL1β, IL-6 and TNFα were detected. NSAIDs appeared short temporary effect.
In our clinic we started to treat her with pamidronate 1,5 mg/kg on 1 cycle, with monthly repeated courses. After first cycle the CRP was decreased from 150 to 19 mg/l, ESR from 58 to 12 mm\h, TNFα from 169 to 19 pg\ml. Hb increased up to 10.2 gr/dl. No pain, irritability and fever after initiating pamidronate therapy.
Discussion: We describe a case of early presented chronic nonbacterial osteomyelytis affected of only one bone. Early onset (first year of life), permanent progredient course, systemic features (fever, failure to thrive), typical laboratorial changes (microcytic anemia, very high ESR and CRP), increased levels of IL1β, IL-6 and TNFα are characteristic for AID. The described case can be new form of an AID with clinical features resembling CRMO or DIRA diseases. Biologics can be considered to be a promising way of further treatment as it has been reported to be successful already for small number of cases.
Competing interests: None Declared.

A202

P03-007 - Mevalonate kinase gene in Behçet's disease

D Arslan Tas[1*], E Erken[2], F Yıldız[1], S Dinkçi[1], H Sakallı[3]
[1]Rheumatology-Immunology Department, ADANA, Turkey; [2]Cukurova University, Faculty Of Medicine, ADANA, Turkey; [3]Internal Medicicne, Medline Hospital, ADANA, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A202

Introduction: Genetics is suggested to play role in the development of Behçet's disease (BD). Shared phenotipic features requires an approach to differential diagnosis from periodic febrile syndromes.
Objectives: We planned to study for mevalonate kinase (MVK) as a candidate for a susceptibility gene for Behçet's disease.
Methods: Consecutive Behçet patients and apperently healthy subjects were included. Severity score of Behçet disease was calculated. Genotyping of mevalonate kinase gene was done by polymerase chain reaction /sequence-based typing technique.
Results: 50 BD patients (median age: 38.30±11.06 years) and 51 controls (median age: 33.88±12.47 years) were recruited. Three types of mutations have found. First: A single nucleotide polymorphism (SNP) c.769-38C>T (rs35191208) in 21 of 50 BD patients and in 15 of 51 controls. Both groups were comparable for the frequency of c.769-38C>T (p>0,05). In all of the cases with c.769-38C>T, a second SNP: c885+24G>A(rs2270374) was also present (previously reported to be in linkage disequilibrium with the first SNP). Third SNP: c.769-7T>G(rs104895331) was found in 3 of 50 BD patients and in 1 of the control group. We found this SNP together with c769-38C>T and c.885+24G>A. The neurological involvement was found to be more frequent in the BD patients with c.769-38 C>T when compared to the BD patients without this polymorphism (p:0,012).
Conclusion: Our results suggested that the effects of MVK mutations in Behçet's disease could be an additional genetic susceptibility factor for the patients with neurological involvement. However these results need confirmation in larger study populations and in different ethnic groups.
Competing interests: None Declared.

A203

P03-008 - Gastrointestinal involvement in Behçet's syndrome

I Hatemi[1], G Hatemi[2*], Y Erzin[1], AF Celik[1], H Yazici[2]
[1]Gastroenterology, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey; [2]Rheumatology, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A203

Introduction: Gastrointestinal involvement in Behçet's syndrome (BS) can be a severe complication resulting in perforation and massive bleeding. Controlled data regarding treatment is lacking and long term prognosis is not well known.
Objectives: To report the demographic and disease characteristics, type of involvement, treatment modalities and outcome of BS patients with gastrointestinal involvement (GIBS).

Methods: We retrospectively reviewed the charts of all BS patients evaluated with a suspicion of gastrointestinal involvement. We identified those with GIBS and surveyed their demographic features, other BS manifestations, clinical, endoscopic and histologic gastrointestinal findings, and treatment modalities. Patients were evaluated either in the outpatient clinic or if not possible by phone calls to assess their outcome.
Results: Among the 8058 recorded BS patients in our multidisciplinary outpatient clinic, 69 had symptoms suggesting gastrointestinal involvement and lesions on endoscopy. Among these, 18 patients had other reasons for their gastrointestinal symptoms and endoscopic lesions. The remaining 51 patients had GIBS (Table). The presenting symptoms were acute abdomen caused by perforations in 4/51 patients, massive bleeding in 8/51 patients and abdominal pain and/or diarrhea in 39/51 patients. Surgery had to be performed in 20/51 patients, and 4 of them had to be re-operated for development of stricture, progressive disease, relapse, and corrective surgery, 1 patient each. The most commonly used drugs for initial management were azathioprine 2-2.5 mg/kg/day (n=33) and 5 ASA compounds 3-4 g/day (n=13). Remission was observed and there were no relapses during a mean follow-up of 44.3±46.9 months in 22/33 (67%) patients who had initially been prescribed azathioprine (2.5 mg/kg) and during 45.0±50.1 months in 9/13 (68%) patients who had been prescribed 5 ASA compounds. Other than the 33 patients who used azathioprine as their initial treatment, remission was also obtained with azathioprine in 3/4 patients who were resistant to 5 ASA compounds. Among the 10 patients who had relatively severe symptoms and persistent large ulcers despite at least 6 months of azathioprine treatment, endoscopic and symptomatic remission could be obtained with thalidomide in 4 patients, infliximab in 4 patients and adalimumab in 2 patients. After a mean follow-up of 7.1± 4.8 years (range 0.25 – 17 years), 42 (84%) patients were in remission and 14 (28%) of these were off treatment. Four (8%) patients were still active, 3 (6%) patients had died due to non-GI releated reasons and 2 (4%) were lost to follow-up. The reasons for death were pulmonary artery thrombosis, infection and acute renal failure due to amyloidosis in 1 patient each.
Conclusion: 84% of patients with GIBS were in remission after a mean of 7 years of follow-up. Surgery was required in 40% of patients with GIBS. 5 ASA compounds or azathioprine provided remission and prevented relapses in two thirds of the patients .The latter was also beneficial in some patients resistant to 5 ASA compounds. Resistant and relapsing cases could be managed with thalidomide or TNF-alpha antagonists.
Competing interests: None Declared.

A204

P03-009 - Experiences of in pediatric Behçet uveitis

B Sozeri[1*], S Yilmaz[2], S Mir[3], H Ates[2]
[1]Pediatric Rheumatology, Ege University, Izmir, Turkey; [2]Ophtalmology, Ege University, Izmir, Turkey; [3]Peadiatric Nephrology, Ege University, Izmir, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A204

Introduction: Behcet disease (BD) is a chronic systemic inflammatory disease of unknown origin. Although,the clinical feature of juvenil BD is similar to adults, neurologic and gastrointestinal involvement have concluded more in juvenile BD.
Objectives: To evaluate the efficacy and safety of immunosuppressive therapy including conventional therapy and anti-tumor necrosis factor-alpha (anti-TNF-α) agents in pediatric patients in Behcet uveitis.
Methods: A retrospective study was made of 6 consecutive pediatric patients with BD. Inclusion criteria were fulfillment of the classification criteria of the International Study Group for Behçet Disease and onset of uveitis at 16 years of age or younger. The main outcome measures were sex, age at onset of uveitis, the initial symptom of Behcet disease, clinical ocular features, ocular complications and systemic treatment.
Results: Four patients were female, 2 male were. Mean age at onset of uveitis was 11.8±3.2(7 to16) years. The most common extra-ocular clinical manifestations were recurrent oral ulcer in all patients and arthritis in 4 patients(50%) and pseudo folliculitis in 3 patients(66.7%). Pan uveitis was bilateral in83.3%, retinal vasculitis and retinitis were seen in83.3% and100% of the involved eyes, respectively.Cataract, maculopathy, glaucoma and optic atrophy were seen in 36.4%,18.1%,18.1 and0.9 %of the involved eyes, respectively.
Treatment modalities applied to treat either uveitis or its complications were classified as topical, and systemic. Corticosteroid drops (dexamethasone 0.1%, prednisolone 1%) with frequent instillation and cycloplegic drops (cyclopentholate 1%) 3 times daily were used in eyes with panuveitis. Systemic corticosteroid treatment was performed to suppress acute inflammatory episodes.
The mean duration of oral corticosteroid therapy for the treatment of acute inflammatory conditions was 3.4± 0.5 months (range, 3-4). All patients had used conventional immunosuppressive (IS) agents including azathioprine and cyclosporine, and 4 (66.7%) patients had additionally used anti-TNF treatment to control panuveitis attacks. Before starting to anti-TNF agents, screening for latent tuberculosis was performed using the local guideline. The majority of the patients (n:3) received only ADA subcutaneous injections once in every two weeks, while the one patient switched from IFX to ADA due to loss of clinical response. Ocular manifestations (panuveitis and retinal vasculitis) responded rapidly and reduction in the number and dose of standard immunosuppressive agents in patients with adalimumab. Overall, mean treatment period for anti-TNF agents was 9.5 ± 4.1 (range 6 to 14) months. Considering the 8 eyes of 4 patients with these anti-TNF agents, basal uveitis relapse rate of 4.0±0.8 decreased to 0.5±1.0 ($p<0.05$) during fallow-up. In 2 patients who completed the first year of anti-TNF treatment without any relapses, anti-TNF treatment could be stopped only in a single case using ADA, while anti-TNF treatment had to be continued in other. No adverse effect requiring cessation of anti-TNF agents was observed.
Conclusion: In line with the previous data, our findings also suggest that anti TNF alpha agents may be tried in the treatment of pediatric Behçet uveitis resistant to other therapeutic approaches.
Competing interests: None Declared.

A205

P03-010 - IL10 SNPs associated with BD in Western Algeria

O Khaib Dit Naib[1*], M Aribi[1], A Idder[2], A Chiali[3], H Sairi[3], I Touitou[4,5,6], G Lefranc[7], M Barat-Houari[8]
[1]Immunology, Tlemcen University, Tlemcen, Alger; [2]Ophtalmology, Hamou Boutlelis Clinic, Alger; [3]Dermatology, Chu, Oran, Algeria; [4]INM U844, Inserm, France; [5]Montpellier University, France; [6]Genetic, Chru de Montpellier, France; [7]Immunogenetic, CNRS UPR1142 IGH, France; [8]Genetic, Chru, Montpellier, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A205

Introduction: Behcet's disease (BD) is a multisystem inflammatory disease, characterized by recurrent, oral and genital ulceration, skin lesions and uveitis. Several publications in the last decades showed the complex role of genetic factors; recent studies have revealed that SNPs of the *IL10* gene promoter are associated with BD in various populations.
Objectives: We aimed to test the hypothesis that two SNPs of the *IL10* gene promoter (c.-819C>T, rs1800871 and c.-592C>A, rs1800872) may act as predisposing factors for BD in Algerian patients.
Methods: Fifty one BD patients and 96 unrelated controls from Western Algeria were genotyped for the two SNPs by direct sequencing. Allele and genotype distributions were compared between cases and controls, using Chi2 or Fisher's exact tests.
Results: The minor alleles c.-819T and c.-592A, were significantly more frequent (i) in BD patients than in controls (44% versus 27%, p= 0.003, OR= 2.18; 95% CI 1.33, 3.90) and (ii) in patients with genital ulcers or skin lesions than those without (OR=2.28, p= 0.002, 95% CI 1.10, 1.60 and OR = 2.18, p= 0.0035, 95% CI 1.27, 3.72, respectively).
Conclusion: Our results showed that two investigated SNPs play a role in BD and in most of its related phenotypes in the population of Western Algeria. These observations are consistent with those reported for other ethnic groups, but need to be confirmed in a larger sample.
Competing interests: None Declared.

A206

P03-010-B - A novel mutation in MEFV gene is not enough

D Martorana[1], M Carrabba[2,3*], G Fabio[2,3]
[1]Unit of Molecular Genetics, University Hospital of Parma, Parma, Italy; [2]Internal Medicine Department and Medical Science and Community Health, Fondazione IRCCS Ca' Granda, Italy; [3]Universita' degli Studi, Milan, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A206

Introduction: Genotype-phenotype correlation is still challenging in FMF patients especially when the disease is part of a complex autoinflammatory disease.

Case report: A 23-year-old female was diagnosed for FMF in 2002 after a 3-year course of recurrent atypical attacks. Administration of colchicine 1mg was started and symptoms improved.
In 2005 she developed fever and epilepsy. CT-scans demonstrated splenic and cerebral lesions of uncertain etiology. Autoantibodies tests were negative. Infectious diseases were ruled out and therapy with steroids and carbamazepine was started under suspicious of vasculitis. Patient improved and in 2006 brain MRI showed resolution of cerebral lesions. She tapered steroids, gave up carbamazepine and was well under colchicine 1mg with rare mild FMF attacks but no signs of amyloidosis. She got pregnant in 2012. During pregnancy she experienced several mild FMF attacks with slight improvement despite colchicine increasing to 1.5mg. She developed gestosis. Her delivery was complicated by big vaginal hematoma that was surgically drained in two subsequent times. Further investigations on haemophilic acquired diseases were negative as well as platelets functional tests.
DNA sequence analysis did not find classic MEFV mutations and revealed heterozygosity for a novel c.460T>C in exon 2 , leading to the replacement of a polar Serine by an hydrophobic Proline at amino acid position 154 (S154P), not reported in Infevers database. This nucleotide substitution was not present in the genomic DNA of 734 control chromosomes. No mutations of the exons 2 and 11 of the Mevalonate Kinase gene and the exons 2,3,4 and 6 of the TNFRSF1A gene were found The woman was found to be heterozygous for the c.362G>A in the exon 4 (R92Q). Her father is heterozygous for the R92Q low penetrance variant in the TNFRSF1A gene, while her mother is heterozygous for the S154P mutation in the MEFV gene; both the parents do not report any inflammatory attack and are asymptomatic for autoinflammatory syndromes.
Discussion: About 200 MEFV gene mutations have been described so far in patients with FMF. Recently it has been shown that in up to 25% of the patients, one MEFV mutation is probably enough to manifest the disease. This behavior could be explained assuming an interaction between the single MEFV mutation with another mutated inflammatory gene. R92Q is considered a low penetrance TNFRSF1A mutation that has been found to have a population frequency between 2-5%. The role of the R92Q has not been wholly understood. Given the fact that the carrier rates for R92Q are very similar and do not significantly differ from the carrier rate in controls, it might be concluded that an interaction between TNFRSF1A and MEFV is minimal or does not exist. A number of patients with heterozygous mutations in two autoinflammatory genes have been described so far in MEFV-TNFRSF1A, MVK-TNFRSF1A, and CIAS1-MEFV. The scarcity of such patients provides support to the conclusion that these autoinflammatory genes do not interact.
Based on the notion that some individuals who harbour only one mutation may have the second hit in currently unrecognized autoinflammatory genes, a whole-exome sequencing in selected cases may be performed.
Competing interests: None Declared.
References

1. Sarrauste de Menthiere C: 2003.
2. Ben-Chetrit E: 2009.
3. Booty MG: 2009.
4. Hoffmann F: 2005.
5. Singh-Grewal D: 2007.
6. Aksentijevich I: 2009.
7. Ng SB: 2010.

A207

P03-011 - Differential for granulomatosis with polyangiitis

SM Rawn[1*], P Miettunen[2], H Schmeling[2]
[1]Department of Medicine, Calgary, Canada; [2]Department of Pediatric Rheumatology, University of Calgary, Calgary, Canada
Pediatric Rheumatology 2013, **11(Suppl 1):**A207

Introduction: Granulomatosis with Polyangiitis (GPA) is a rare and potentially lethal vasculitis, which predominantly affects small vessels. GPA can present with palpable purpura, hemorrhagic macules and bullae similar to skin lesions found in the most common small vessel vasculitis in childhood, Henoch-Schoenlein Purpura (HSP), leading to diagnostic confusion.
Case report: We report the case of a previously healthy 14-year-old girl who presented with fever, congestion and sinus pain in May 2012. Initially diagnosed with bacterial sinusitis, she received antibiotics, but experienced ongoing fatigue and malaise. Within weeks, she developed abdominal pain, hematuria, migratory arthritis, palpable purpura on her lower and upper extremities and was diagnosed with HSP. Two weeks later, she was admitted with hemoptysis, abnormal urinalysis, ischemic digits and progression of the rash into large bullae and ulcerations. Her renal biopsy revealed pauci-immune necrotizing glomerulonephritis, and her antineutrophil cytoplasmic antibodies and anti-proteinase 3 antibodies were high positive leading to a diagnosis of GPA. She received six months of treatment with oral cyclophosphamide and high dose intravenous and oral steroids. The skin lesions required debridement. Due to ongoing inflammation, with progression to nasal septal perforation, Rituximab, an anti-B-cell antibody, was initiated at month seven. This allowed her clinical picture and laboratory abnormalities to improve, with return to normal renal function.
Discussion: In this case, a pediatric patient with GPA initially presented with the classic tetrad of HSP: palpable purpura, arthritis/arthralgias, abdominal pain and hematuria. This child subsequently developed hemoptysis and progression of her rash and ischemic digits prior to a diagnosis of GPA being made. In conclusion, if a patient with a severe case of HSP has extensive skin involvement, it is vital to consider a diagnosis of GPA to avoid serious organ or life threatening consequences.
Competing interests: None Declared.
References

1. Twilt M, Benseler S, Cabral D: **Granulomatosis with Polyangiitis in Childhood.** *Curr Rheumatol Rep* 2012, **14**:107-115.
2. Ruperto N, Ozen S, Pistorio A, Dolezalova P, Brogan P, *et al*: **EULAR/PRINTO/PRES criteria for Henoch-Schonlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part I: Overall methodology and clinical characterisation.** *Ann Rheum Dis* 2012, **69**:790-797.
3. Marzano AV, Vezzoli P, Berti E: **Skin involvement in cutaneous and systematic vasculitis.** *Autoimmun Rev* 2012, [Epub ahead of print].
4. Deng F, Lu L, Zhang Q, Hu B, Wang SJ, Huang N: **Henoch-Schonlein purpura in childhood: treatment and prognosis. Analysis of 425 cases over a 5-year period.** *Clin Rheumatol* 2010, **29**:369-74.

A208

P03-012 - A P268S NOD2 mutation in one Blau patient

M Pardeo[1], E Cortis[2], C Bracaglia[1], R Nicolai[1], F De Benedetti[1], A Insalaco[1*]
[1]Division of Rheumatology, Department of Pediatric Medicine, Ospedale Pediatrico Bambino Gesù, Rome, Italy; [2]Pediatric department, Santa Maria della Stella Hospital, Orvieto, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A208

Introduction: Blau syndrome (BS) is a rare autosomal dominant, autoinflammatory syndrome characterized by the clinical triad of granulomatous, recurrent uveitis, dermatitis and symmetric arthritis. The arthritis is usually a polyarticular exuberant synovitis and tenosynovitis and represents the characteristic phenotypic feature. Uveitis occurs in most patients and commonly evolves to a panuveitis. In the majority of patients, the disease is characterized by early onset, usually before 3-4 years of age. The gene responsible for BS has been identified in the caspase recruitment domain gene NOD2/CARD15, and the most common mutations were found in codon 334 (R334Q and R334W) [1]. NOD2 is a member of a family of intracellular proteins with N-terminal caspase recruitment domains (CARDs). Since the first report of an association of the NOD2 variants with Crohn disease by Hugot et al. [2], extensive studies have been focused on an association of NOD2 with inflammatory bowel disease (IBD), pediatric Blau syndrome, NOD2-associated autoinflammatory disease (NAID) and rheumatic disease [3].
Case report: We describe a 4-year-old male, the first child of healthy unrelated parents, who presented at 13 months of age, with arthritis of the ankle and of the second proximal inter-phalangeal of the right hand, without the typical puffy appearance. Laboratory test revealed mild increase in inflammatory parametrs (erythrocyte sedimentation rate 35 mm/h, C-reactive protein 0.77 mg/dl) and the presence of antinuclear antibody (titer 1:640, homogeneous pattern). A diagnosis of ANA positive oligoarticular

juvenile idiopathic arthritis was made and an infiltration of the ankle joint with TXA was performed, with insufficient response. Persistence of the ankle arthritis led to initiate treatment with methotrexate that was not associated with clear benefit. Four months later the patient developed recurrent episodes of fever and skin rash on limbs and trunk, with spontaneous resolution, and subsequently recurrent episodes of bilateral anterior uveitis. Based on the presence of persistent arthritis, recurrent uveitis, fever and rash, Blau Syndrome was suspected and molecular analysis of NOD2/CARD15 gene was performed. Sequencing analysis demonstrated a heterozygous c.802C>T mutation (P268S /SNP5) in exon 4.

Discussion: Until now the c.802C>T mutation (P268S /SNP5) in exon 4 of NOD2 had only been reported in association with Crohn's disease, rheumatoid arthritis, spondylarthropathy and ulcerative colitis. This is, to the best of our knowledge, the first case of c.802C>T mutation (P268S /SNP5) that appears to be associated with typical clinical features of Blau syndrome.

Competing interests: None Declared.

References

1. Sfriso P, *et al*: Blau syndrome, clinical and genetic aspects. *Autoimmun Rev* 2012, **12(1)**:44-51.
2. Yao Q: Nucleotide-binding oligomerization domain containing 2: Structure, function, and diseases. *Semin Arthritis Rheum* 2013.
3. Hugot JP, *et al*: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001, **411(6837)**:599-603.

A209

P03-012-B - Lupus erythematous chronicus: a new etiology of macrocheilitis

M Mael-Ainin[1*], H Boudhir[1], A Saidi[2], B Hassam[1], K Senouci[1]
[1]Dermatologie - Vénérologie, Centre Hospitalier Universitaire ibn Sina, Rabat, Morocco; [2]Centre of Anatomical Pathology, United Nations, Rabat, Morocco
Pediatric Rheumatology 2013, **11(Suppl 1):**A209

Introduction: Macrocheilitis etiologies are diverse, particularly granulomatous diseases. We report the first case occurring in the context of lupus erythematous chronicus.

Objectives: To show the importance of investigating lupus in etiological diagnosis of macrocheilitis.

Methods: Mrs. E.F a 30 years old woman, was followed up, since 2003, for a lupus erythematous chronicus. In 2010, she presented a painful cheilitis of the upper lip that gradually increases in volume. Clinical examination showed a scaly macrocheilitis, the rest of the clinical examination was within normal limits. The labial biopsy showed a dyskeratotic hyperkeratosis, keratotic plugs, a pseudo-epitheliomatous hyperplasia, an inflammatory infiltrate of the chorion without granuloma, a direct immunofluorescence that was negative, antinuclear antibodies were positive at 1/80, anti-native DNA antibodies were negative. The systematization work up was unremarkable. The diagnosis of lupus erythematous chronicus was retained. The patient was put under Chloroquine at 4mg/kg/day dose causing total regression of symptoms within 6 months without recurrence for one year.

Results: Macrocheilitis etiologies are diverse, mainly of granulomatous nature especially Melkersson Rosenthal syndrome, Crohn's disease or sarcoidosis. Macrocheilitis in the lupus disease has never been reported in the literature. Indeed, lupus erythematous chronicus manifest in keratotic whitish lesions of the vermilion, systemic lupus in erosive and crusting cheilitis. These lesions can be the initial symptom or occur during the evolution of the disease.

The etiologic diagnosis of macrocheilitis can rely on several clinical and paraclinical data. In our case, the antecedent of the lupus disease of the patient, the clinical exam, the histology of lip biopsy and the antinuclear antibodies positivity have retain lupus erythematosus as an etiology of this cheilitis.

The treatment of macrocheilitis is difficult; various therapies have been used with varying results, including oral or intralesional corticosteroids, antimalarials, immunosuppressives, antibiotics and biotherapies, or in some cases cheiloplasty reduction. In our case, improvement was obtained under Chloroquine at a dose of 4mg/kg/day.

Conclusion: To our knowledge, we present the first case of cheilitis occurring in the context of erythematosus lupus, this exceptional etiology is worth being known as it allows for an appropriate treatment.

Competing interests: None Declared.

A210

P03-013 - Symptomatic neuromuscular sarcoidosis

M Mulazzani[1*], C Haberler[2], F Zimprich[1], EV Hartl[3], H Cetin[1]
[1]Department of Neurology, Medical University of Vienna, Vienna, Austria; [2]Institute of Neurology, Medical University of Vienna, Vienna, Austria; [3]Landesklinikum Horn, Horn, Austria
Pediatric Rheumatology 2013, **11(Suppl 1):**A210

Introduction: Sarcoidosis is a granulomatous disease possibly affecting all organ systems. Less than 10% of sarcoidosis patients develop symptomatic neurological involvement. Although muscular non-caseating granulomas (NCGs) can be found in up to 75% of sarcoidosis patients, muscular symptoms develop only in less than 0.5% of sarcoidosis patients. Corticosteroids (CS) are the mainstay of medical therapy, but their effect on chronic myopathy is not consistent. Additive immunosuppressive therapy with azathioprine, cyclophosphamide, methotrexate, or chloroquine may be necessary in patients inadequately responding to CSs.

Case report: A pathological routine x-ray of an asymptomatic 43-year-old farmer led to a pulmonary CT, which showed mediastinal and hilar lymphadenopathy. Sporadic epitheloid cells were found in lymph node biopsy. Bronchoalveolar lavage contained predominantly lymphocytes with a moderately elevated CD4/CD8 ratio (3.6). Tuberculosis-PCR and -culture were negative. Spirometry demonstrated only marginal obstruction. Because of iridocyclitis, prednisolone therapy (100 mg/d for two weeks, 50 mg/d for two months, 25 mg/d for six more months, then 30 mg/d) was initiated. After five months of CS therapy, the patient first experienced stabbing pain (up to 3 out of 10 according to the visual analog scale, VAS) in the left thigh without paresis. Six more months later he presented with pain aggravation (VAS: up to 5) and additional moderate paresis of left hip flexion and -extension. MRI demonstrated a slight T2 (TIRM) hyperintensity in the left vastus lateralis muscle. Electromyography showed spontaneous fibrillation and positive sharp waves with normal motor unit potentials. A muscle biopsy illustrated two processes: an inflammatory myopathy with prominent endomysial and perimysial infiltrations (mainly CD4-lymphocytes, some multinucleated giant cells and epitheloid cells) and pronounced neurogenic changes (grouplike atrophy of muscle fibers and florid disintegration of muscle fibers). Laboratory analysis showed elevated creatine kinase (893 U/l with an upper limit (UL) of 200 U/l), and lysozyme (22.1 mg/l with UL of 17.6). Four weeks after the muscle biopsy, he still suffered from stinging pain in the left vastus lateralis muscle sensitive to activity and pressure (VAS: up to 8) despite medical therapy with diclofenac (150 mg/d) and hydromorphone (4-5 mg/d). He had no signs of arthritis or of cardiac, pulmonary, and visual involvement; lupus pernio was not visible. Oral methylprednisolone was increased to 60 mg per day. Five weeks later, he additionally presented stabbing pain in the left semitendinosus muscle with new pain-related moderate paresis of foot dorsal extension, foot plantar flexion and adduction of the left hip. Another inpatient stay for MRI, EMG, NCS, nerve biopsy, semitendinosus muscle biopsy, laboratory analysis and additive immunosuppressive therapy is planned (results will be presented on the congress).

Discussion: Chronic symptomatic neuromuscular sarcoidosis is often refractory to medical therapy and potentially leading to severe and permanent disability. Only little data are available regarding optimal therapy of this rare manifestation of systemic sarcoidosis.

Competing interests: None Declared.

Reference

1. Zisman, *et al*: Sarcoidosis involving the musculoskeletal system. *Sem Resp Crit Care Med* 2002, **23**:555-70.

A211

P03-013-B - Inaugural palate nodule of a systemic sarcoidosis

M Mael-Ainin[1*], M Bouaddi[1], K El Morabite[1], S Abil[1], A Abdou[1], K Senouci[2], B Hassam[1], I El Meknassi[1]
[1]Dermatologie - Vénérologie, Centre Hospitalier Universitaire ibn Sina, Morocco; [2]Centre of Anatomic Pathology, United Nations, Rabat, Morocco
Pediatric Rheumatology 2013, **11(Suppl 1):**A211

Introduction: Oral manifestations of the sarcoidosis are rare. We report a case of a systematic sarcoidosis revealed by a nodule of the palate.

Objectives: To emphasize on mucosa localization of sarcoidosis when addressing nodular lesion of the palate in order to allow an appropriate treatment.
Methods: Mrs M. L a 29-year-old woman, presented an isolated nodule of the soft palate. The nasofibroscopy was normal, the histology showed a non specific inflammatory infiltrate. Two years later, the patient presented papulonodular lesions of the face associated with anosmia and a second stage dyspnea of NYHA.
Results: Oral sarcoidosis is rare. Labial and lingual effects are at the forefront; effects of the palate remain exceptional. The lesions may take the form of a nodule, ulceration or perforation. Treatment based on systemic corticosteroids, sometimes with the addition of immunosuppressives or synthetic antimalarials.
Conclusion: Sarcoidosis of the soft palate is rare and probably under-diagnosed. The risk of perforation or occurrence of systemic localization requires a careful examination of the oral mucosa in all patients with sarcoidosis.
Competing interests: None Declared.

A212

P03-014 - Biological therapy for autoinflammatory disorders

S Bujan Rivas[1*], JI Arostegui Gorospe[2], F Martinez Valle[1], R Solans laque[1], JF Andres Cordon[1], J Ordi Ros[1], M Vilardell Tarres[1]
[1]Internal Medicine, Hospital vall Hebron, Spain; [2]Immunology, Hospital Clinic i Provincial, Barcelona, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A212

Introduction: Autoinflammatory diseases (AD) are innate immune system disorders, most of them with genetically identified basis. Usual therapeutic approach until last decade included non-steroidal antiinflamatories (NSAID), corticosteroids (CS) and colchicine. More recently, biological therapies (BT) have proved to be useful for refractory patients, especially those involving IL-1β blockade.
Objectives: To describe and analyze the experience with BT of a single center cohort of adult patients diagnosed with autoinflammatory disorders, including familial Mediterranean fever (FMF), TNF-receptor antagonist periodic syndrome (TRAPS), Muckle-Wells syndrome (MWS), undefined periodic fever (UPF), Blau syndrome (BS) and periodic fever, aphtous stomatitis, pharyngitis and adenitis (PFAPA).
Methods: Clinical files of 30 adult patients diagnosed with AD followed by the same clinical team of the Internal Medicine Service of Vall Hebron hospital were reviewed. Demographic, clinical, laboratory, and therapeutic data prior and after starting BT were collected. Data from BT treated patients included: reason for prescription, drug, dosage, treatment duration, response to treatment, tolerance and adverse effects related to the drug.
Results: 13 out of 30 patients (5 FMF, 1 TRAPS, 4 MWS, 1 UPF, 1 BS, 1 PFAPA) received BT: 7 patients (5 FMF, 1 BS, 1 TRAPS) received anti-TNF (6 etanercept 50 mg/weekly SC, 1 patient adalimumab 40 mg/15 d SC) and 10 patients (3 FMF, 4 MWS, 1 UPF, 1 BS, 1 PFAPA) received rIL-1RA anakinra 100 mg/d SC. 4 patients (2 FMF, 1 BS, 1 UPF) received first etanercept and were switched to anakinra due to incomplete / lack of response. Prescription reasons for BT were: first option 5/13 patients (etanercept for 1 TRAPS and anakinra for 4 MWS) and refractory disease in 8/13 patients (anti-TNF: 4 FMF, 1 UPF, 1 BS; anakinra: 3 FMF, 1 BS, 1 PFAPA, 1 UPF). Clinical improvement defined as reduction in number and/or intensity of attacks was achieved in 4/7 patients treated with anti-TNF and in 9/10 patients receiving anakinra. 6 patients, all treated with anakinra, presented adverse effects: 6 patients local erythema at punction site -2/6 were moderate reactions-, and 1 patient transient alopecia. No opportunistic infection was detected.
Conclusion: Although BT are off-label indications in AD, its use has to be considered a helpful and safe therapeutic alternative for refractory cases of FMF, UPF, BS or PFAPA. In this serie, IL-1β blocking approach showed better response than anti-TNF with limited side effects.
Competing interests: None Declared.

A213

P03-015 - Dapson treats chronic Pupura Schoenlein (PSH)

G Dueckers[1*], M Frosch[2], C Assaf[3], H Wiggermann[4], D Schneider[5], T Niehues[1]
[1]Childrens Hospital, Helios Kliniken, Krefeld, Germany; [2]Pediatrics, Vestische Kinderklinik, Datteln, Germany; [3]Dept. Dermatology, Helios Klinken, Krefeld, Germany; [4]Pediatrics, Private Practice, Schwerte, Germany; [5]Childrens Hospital, Kliniken Dortmund GmbH, Dortmund, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A213

Introduction: PSH is common and, with few exceptions, resolves spontaneously. In complicated cases treatment can be difficult.
Case Report: Here we present an 11 year old Iranian girl, of healthy parents suffered from chronic PSH with chronic exanthema, severe ulcerative dermatitis and arthralgia. Clinical observation: no fever, no arthritis, no signs of polyserositis or hepatosplenomegaly; Laboratory: normal values (full blood count, CRP, complement (C3, C4), no autoantibodies (ANA, ANCA, anti-ds-DNA), no cryoglobulins, zinc, IgG, IgM). Abnormal findings: highly elevated IgA in serum (maximum 2030 mg/dl), elevation of ESR and MRP8/ 14. Skin biopsy: leukocytoclastic infiltrates in subcutaneous fatty tissue. Immunohistochemistry: IgA deposits in tip of papillae and upper corium. No mutation in Marenostrin was found. Multimodal therapeutic approaches (Cortisone, Methotrexate, Azathioprine, Colchicine, i.v. Immunoglobulins) remained without success for 8 years. With the administration of Dapsone symptoms resolved within days and remain under control for > 8 months now. Met-Hb level is tolerable.
Discussion: Anti-inflammatory potency of dapsone is illustrated. Therapeutic efficacy of Dapsone has been reported in chronic PSH[1,2], but the mechanism remains to be fully elucidated. Hypothesis: Endothelial cells and hyperreactive B-cells (as illustrated by IgA elevation) secrete IL-8. IL-8 is elevated in patients with PSH [3]. IL-8 stimulates perivascular invasion by neutrophils. Dapsone can inhibit secretion of IL-8 [4], thereby impairing neutrophil function [5-7].
In conclusion Dapsone might be benefical for complicated cases of PSH. The mechanism of its anti-inflammatory potency remains to be elucidated.
Competing interests: None Declared.
References

1. Evans, *et al*: *Arch Child Dis* 2004.
2. Papandreou T, *et al*: *Eur J Dermatology* 2010.
3. Yang, *et al*: *Ann Rheuma Dis* 2004.
4. Schmidt, *et al*: *Clin Exp Immunology* 2011.
5. Thuong-Nguyen V, *et al*: *J Invest Dermatol* 1993.
6. Debol, *et al*: *J Leuko Biol* 1997.
7. Suda, *et al*: *Br J Dermatology* 2005.

A214

P03-016 - ANTI IL1 refractory CINCA respondes to ANTI IL6

G Dueckers[1*], K Sinha[2], V Soditt[3], G Ganser[4], T Niehues[1]
[1]Helios Kliniken Krefeld, Krefeld, Germany; [2]Helios Kliniken Wuppertal, Wuppertal, Germany; [3]Klinikum Solingen, Solingen, Germany; [4]St. Josef Stift, Kinderrheumatologie, Sendenhorst, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A214

Introduction: CINCA is thought to be mainly due to a dysregulation of IL1 synthesis and many patients respond to IL1 inhibition. The role of other cytokines in CINCA disease pathogenesis is less clear.
Case Report: A 13 year old boy from a healthy non-consanguineous family suffers from severe autoinflammatory symptoms (persistent fever, polyserositis, urticaria, arthritis, developmental delay, deafness and hepatosplenomegaly) since his first year of life. Based on clinical presentation and genotype (heterozygous mutation (CAG)>Lysin (AAG)-p. Gln703Lys/Q705K substitution in Exon 3 of NLPR3) he was diagnosed for CINCA, an IL-1 driven cryopyrinopathy. Despite intensive pretreatment (NSAI, GC pulses, MTX, Anakinra and Canakinumab), no durable control of autoinflammation was achievable. With the initiation of Tozilizumab, an IL-6 receptor antagonist, all signs of autoinflammation disappeared, ESR and Serum Amyloid normalized within days. Finally the boy was even able to build a snowman without any joint stiffness or arthralgias for the first time of his life. The patient is now in stable clinical remission for > 6 months.
Discussion: This is the first report of a patient with CINCA, who achieved clinical remission with the inhibition of IL6 pathway for more than 6 months. If Canakinumab fails to control autoinflammation in CINCA inhibition of IL-6 pathway might be an alternative. The mechanism of action of Tozilizumab in cryopyrinopathies remains to be elucidated.
Competing interests: None Declared.

A215

P03-017 - Health related quality of life in adult with HRFS

HJ Lachmann[1*], N Stewart[2], T Lane[2], DM Rowczenio[2], PN Hawkins[2]
[1]University College London Medical School, London, UK; [2]National Amyloidosis Centre, University College London Medical School, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A215

Introduction: Hereditary recurrent fever syndromes (HRFS) can have a profound impact on health related quality of life (HRQoL) but there have been relative few prospective studies.
Objectives: To assess HRQoL in patients aged over 17 years with confirmed CAPS, TRAPS and MKD and to assess the effect of treatment with biologic therapies.
Methods: Patients completed the 36-item Short Form Health Survey (SF-36). Data were collected on untreated patients and those on long term treatment with biologics. SF-36 measures the impact of disease on overall quality of life. Results are expressed as physical-component summary score (PCS) and a mental-component summary score (MCS). Scores range from 0 at worst to 100 at best, with values greater than 50 indicating a better HRQoL than that of a normal adult in the United States.
Results: In CAPS pre treatment HRQoL scores were available on 18 patients, 11 female, median age 43.6 years (IQ 27.9-47.1). The most affected domains were bodily pain, mean score 44, general health 32.5 and vitality 40.3. After treatment with anti IL-1 agents HRQoL was available on 31 patients, 13 female, median age 40.1 years (IQ 27.7:47.8). There was an improvement of more than 20 across all domains except role emotional which improved by 11 and scored above the US average on all domains. There was no significance difference in HRQoL in the 26 patients on long term canakinumab and the 8 on anakinra (one patient provided data on both agents). Data were available on 7 cases of TRAPS who have never received biologics (5 females, median age 443yrs (IQ: 24:44)) and 13 prior to biologics (10 females, median age 39 yrs (IQ 18.5:37)). HRQoL in patients managed with episodic steroids was near normal across all domains, mean PCS 49.7, MCS 52.6. In patients whose disease was severe enough to require biologics pretreatment scores were significantly lower, mean PCS 38.7 and MCS 43.0. In 19 TRAPS patients on biologics (13 female, median age 42.6 yrs (IQ 28:50), 3 with AA amyloidosis) HRQoL was significantly better across all domains, mean PCS 49.1 and MCS 51 than prebiologics but no different to those adequately managed with steroids. Of 6 patients with MKD (1 female, median age 28.1 yrs (IQ 24.4:31.8)) all had severe disease (5 on biologics, 4 refractory to etanercept, 3 refractory to anakinra, 1 refractory to tocilizumab, 1 with AA amyloidosis). Quality of life on no treatment in 2 cases and ineffective biologics in the others was poor, particularly general health (mean 22.8) and social function (mean 22.5), mean PCS 36.1, MCS 28.0. With effective treatment HRQoL (anakinra 1, canakinumab 1, tocilizumab 2) improved significantly across all domains in 4 assessed patients, mean PCS 48.5, MCS 46.8.
Conclusion: HRQoL improves with effective long term treatment. Mean scores were worst in untreated MKD. Self reported improvement was most marked in CAPS c who reported sustained high scores on anti IL-1 agents. There was also improvement in patients with TRAPS and MKD and in treated patients with both diseases HRQoL was almost the US norm. HRQoL data aids holistic assessment of disease activity; the improvements reported here mirror changes in clinical disease activity and inflammatory markers on treatment and help justify the cost and potential long-term risks of biologic therapies.
Competing interests: None Declared.

A216

P03-018 - Diversity in presenting manifestations of AUTOINFL

S Boiu[1*], B Neven[1,2], S Compeyrot-Lacassagne[1], R Mouy[1], C Wouters[1,2], B Bader-Meunier[1,2], M Gattorno[3], Eurofever[1], P Quartier[1,2]
[1]Pediatric Immunology, Hematology and Rheumatology Unit, Necker-Enfants Malades Hospital, Assistance Publique Hôpitaux de Paris, Paris, France; [2]Paris-Descartes University and IMAGINE Institute, Paris, France; [3]Departement of Pediatric Rheumatology, Gaslini Institute, Genoa, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A216

Introduction: The autoinflammatory diseases (AID) include monogenic and polygenic disorders characterized by primary dysfunction of the innate immune system.
Objectives: To describe the clinical spectrum, genetic background and therapy in a cohort of AID patients followed in a reference Pediatric Rheumatology center.
Methods: Medical records of AID patients followed between May 2007 and November 2010 and entered in the Eurofever Registry were studied.
Results: Fifty six patients were included: 17 Cryopyrin-Associated Periodic Syndromes (CAPS), 4 TNF-Receptor-Associated Periodic fever Syndrome (TRAPS), 5 Hyperimmunoglobulinaemia D with periodic fever Syndrome (HIDS), 18 Familial Mediterranean Fever (FMF), 6 Chronic Recurrent Multifocal Osteomyelitis (CRMO), 2 Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis (SAPHO) syndrome and 4 Behçet's Disease (BD). The median follow-up was 2 years (0-14). The male/female ratio was 20/36. The median age was 2.5 years at disease onset and 4 at diagnosis. Family history was positive in 34% of patients. Clinical manifestations included fever (79%), musculoskeletal (77%), gastrointestinal (63%), mucocutaneous (61%), neurological (41%), ocular (34%), cardiorespiratory (13%), and genitourinary (2%) findings, lymphadenopathy with/or hepatosplenomegaly (16%) and growth impairment (25%). Complications/sequelae developed in 45% of patients. Six patients presented with unusual manifestations: neonatal peritonitis (1 CAPS), pancreatitis (1 TRAPS), acute glomerulonephritis (1 FMF), complicated Henoch -Schönlein purpura (1 FMF), peritoneal adhesions with intestinal occlusion (1 FMF), periorbital pain (1 CRMO) and cerebral thrombosis (1 BD). AID was associated with other diseases in 2 patients (FMF/Henoch-Schönlein purpura and CRMO/enthesitis-related arthritis). One mutant allele was found in 16/17 CAPS, 4/4 TRAPS and 4/18 FMF patients. Two mutant alleles were present in 5/5 HIDS and 11/18 FMF patients. The most used therapeutic agents were biologics (54%) (Anakinra, Canakinumab, Etanercept, Adalimumab), NSAIDs (48%), colchicine (45%) and corticosteroids (29%). Anti-interleukin-1 therapy and colchicine proved efficacy in CAPS and FMF patients, respectively. In addition, favorable responses demonstrated anti-interleukin-1 therapy in TRAPS, HIDS and colchicine-resistant FMF patients, as well as Etanercept in TRAPS, HIDS and CRMO patients non-responsive to NSAIDs. 57% and 41% of patients were in complete and partial remission, respectively, at last visit.
Conclusion: AID in children are associated with a broad spectrum of manifestations. Early diagnosis and referral are essential as efficient therapy can be proposed in most cases.
Competing interests: None Declared.

A217

P03-019 - Anakinra for sweet syndrome treatment

G Passaro, L Cerrito, M Giovinale, A Marinaro, A Soriano, D Rigante, R Manna*
Periodic Fever Research Center, Catholic University of Sacred Heart, Rome, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A217

Introduction: Sweet syndrome (SS) is a rare autoinflamamtory neutrophilic dermatosis, sometimes associated with hematologic malignancies. We describe two cases of SS related to hematologic diseases successfully treated with an interleukin-1 (IL-1) receptor antagonist (Anakinra).
Case report: 1- A 72-year-old man with diagnosis of Relapsing Polychondritis and Myelodysplastic syndrome (MDS) was referred for a 4-yr history of recurrent fever (37.5 to 39°C) accompanied by sudden onset of cutaneous purple papules on the extremities and hypodermic painful nodules on the forehead and scalp. The patient suffered of autoimmune atrophic gastritis and severe metasteroidal osteoporosis, but also complained of recurrent auricular chondritis, recurrent conjunctival inflammation, hearing loss, aphonia, and itching. Blood tests revealed high C-reactive protein (43.2 mg/l), pancytopenia, 1/160 titer of ANA, and elevated beta2microglobulin (10.10 mg/ml). A host of blood tests and broncho-alveolar lavage fluid were negative. Bone marrow (BM) biopsy confirmed MDS. Histopatological evaluation of papules demonstrated neutrophilic nodular dermatitis with karyorrhexis without vasculitis, consistent with SS.
2- A 43-year-old man, with a 3-yr history of fever, periodic bone pain, and presence of maculo-papular erythematous skin lesions came to our observation. Histological examination of skin lesions demonstrated a diffuse infiltrate, consisting predominantly of mature neutrophils located in the upper dermis, framed as SS. MRI of pelvis and spine showed multiple sclerotic vertebral bone lesions. BM biopsy did not detect tumor cells, but instead identified inflammatory cells with intense sclerosis and calcification.

Diagnosis of Chronic Recurrent Multifocal Osteomyellitis (CRMO) was established. Treatment with etoricoxib, colchicine, corticosteroid was only partially successful, whereas bisphosphonate and anti-TNF therapy were completely uneffective.
In both these patients Anakinra was administered (100 mg/day by daily subcutaneous injection), obtaining the suppression of neutrophylic-mediated dermatologic manifestations. In the first case we also observed fever spike reduction, whereas in the second one there was the complete remission of fever and bone pain.
Discussion: Pathogenesis of SS involves cytokines and chemokines, as granulocyte-macrophage colony-stimulating factor and interleukins (e.g, IL-1, IL-3, IL-6, and IL-8). The optimal effect of Anakinra in these cases supports the major contributing role of IL-1beta in the physio-pathogenetic process of SS. The choice of alternative strategies, as anti-IL1beta therapy, is feasible in the absence of detectable infections to reduce adverse effects of long-term steroid therapy or in cases of insufficient response to conventional treatment.
Competing interests: None Declared.
References

1. Diamantino Fda E, Raimundo PM, Fidalgo AI: "Sweet's Syndrome and relapsing polychondritis signal myelodysplastic syndrome.". *Anais Brasileiros de Dermatologia* 2011, **86(4)**:173-177.
2. Calistru AM, Lisboa C, Azevedo F: "Paraneoplastic relapsing polychondritis and Sweet syndrome coexisting in a patient with myelodysplasia.". *Indian Journal of Dermatology, Venereology, and 3.Leprology* 2011, **77(6)**:730.
3. Schilling F, Kessler S: Chronic recurrent multifocal osteomyelitis. *Klin Padiatr* 2001, **213(5)**:271-6.

A218

P03-020 - A novel 15-HPGD mutation in pachydermoperiostosis

E Erken[1*], Ç Köroğlu[2], F Yıldız[1], HT Özer[1], B Gülek[3], A Tolun[2]
[1]Rheumatology-Immunology, Cukurova University Faculty of Medicine, Adana, Turkey; [2]Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey; [3]Radiology, Adana Numune Hospital, Adana, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A218

Introduction: Autosomal recessive primary hypertrophic osteoathropathy (PHO), also known as pachydermoperiostosis (PDP), is a rare genetic disease characterized by clubbing of the fingers, arthritis, periostosis and pachydermia and results from mutations in 15-hydroxyprostaglandin dehydrogenase (HPGD). Recessive mutations in 15-hydroxyprostaglandin dehydrogenase in PHO subjects. has been identified since 2008. Both homozygous and compound heterozygous mutations in HPGD have been reported. Homozygous patients had increased sustained prostaglandin E2 levels and prominent clinical and biochemical PHO.
Objectives: To perform clinical investigations, to attempt medical treatment, and to find the HPGD mutation, the gene responsible for the disease, in a 22-year old Turkish male and his 23-year old sister afflicted with primary hypertrophic osteoarthropathy as well 14 members of their family.
Methods: In combination with NSAIDs and colchicine, sulfasalazine was commenced to both of them, and methotrexate was added to the treatment regimen of the female patient at the end of the first year. All seven exons of gene *HPGD* including 5′ and 3′ UTRs were analyzed by direct sequencing. After the identification of the mutation, a primer pair was designed for the PCR amplification of a 152 bp-region harbouring the mutation site. Mutational analysis was repeated via high-resolution melting curve analysis performed on LightCycler 480 system, along with samples from 136 control individuals.
Results: A homozygous 2-bp deletion (c.310_311delCT or p.L104AfsX3) was identified. Eight relatives carrying the mutation in the heterozygous state were examined and none was found affected. The patients were found typical PHO. Ultrasonographic examination of the joints revealed synovitis and inflammation by B mode and power doppler ultrasonography. One of the patients had emphysema in addition to other findings reported as associated with PHO. Joint symptoms responded to Sulfasalazine treatment in both patients. However, after addition of methotrexate, the female patient had better remission.
Conclusion: Novel p.L104AfsX3 in *HPGD* underlies PHO in the family. Emphysema is an additional clinical finding associated with PHO. Sulfasalazin as well as methotrexate can be used for the treatment of joint symptoms.
Competing interests: None Declared.

A219

P03-021 - Characterization of BM-MSC from osteopetrotic mice

F Schena[1*], E Caci[2], N Lo Iacono[3,4], V Marrella[3,4], M Gattorno[1], A Martini[1], C Sobacchi[3,4], A Villa[3,4], E Traggiai[5]
[1]Second Pediatric Division, Gianna Gaslini Institute, Genova, Italy; [2]Molecular Genetics Laboratory, Gianna Gaslini Institute, Genova, Italy; [3]UOS/IRGB, Milan Unit, CNR, Milano, Italy; [4]Humanitas Clinical and Research Center, Milano, Italy; [5]Novartis Institute for Biomedical Research, Basel, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A219

Introduction: Autosomal Recessive Osteopetrosis (ARO) is a severe bone disease characterized by increased bone density due to impairment in osteoclast bone resorptive function (osteoclast-rich forms) or differentiation (osteoclast-poor forms). The latter form carries mutations in Tnfsf11 gene, which codifies for the receptor activator of NF-κB ligand (RANKL), an essential cytokine expressed in stromal cells. It contributes (with M-CSF) to the differentiation and activation of specialized osteoclasts from monocyte precursors in bone marrow niche. These patients, differently from other forms, do not benefit from HSC transplantation, demonstrating a pathogenetic role of stromal cells in unbalanced bone remodeling.
Objectives: Aim of the project is to characterize the stromal compartment in a murine model of osteopetrosis, RANKL deficient. In order to verify whether a defect in RANKL protein might cause an impairment in this compartment we generated bone marrow mesenchymal stromal cells (BM-MSC) from knockout mice. We investigated whether nonfunctional RANKL alters physiological features such as morphology and phenotype of MSCs. In order to evaluate if a defect in RANKL affects the physiological functions of MSCs, the clonogenic, proliferative and differentiation potential towards the osteogenic and adipogenic lineages was assayed.
Methods: MSCs were isolated from bones of wt and ko mice and were cultured in vitro with selective medium. Cell surface markers and proliferation were analyzed through flow citometry and CFSE labeling. Differentiation in vitro was induced with a specific medium for osteoblastic or adipogenic lineage. Alizarin Red and Oil Red staining was performed to confirm the differentiation respectively for OBs and adipocytes.
Results: 7 BM MSC lines from wt and 6 MSCs from ko mice have been generated. We analyzed the immune-phenotype using the following markers: CD45, CD34, CD9, SCA1, CD62L, CD117, CD44 and we observed a comparable phenotype despite a reduction in CD9 expression in *Rankl-/-* MSCs.We tested the clonogenic potential of KO MSCs and we observed a reduced capacity to form CFU, whereas the proliferation of ko MSCs was not different from the one of wt MSCs. Finally we evaluated the differentiation potential in vitro of Rankl -/- MSCs and we found that they are able to differentiate into osteoblasts (OBs) and adipocytes, but the osteoblastogenesis is reduced with respect to wt cells, even if not in significant manner. Indeed we analyzed the expression of osteoblastic markers (RUNX2, OSP, ALP, COL1) and we observed that during the differentiation ko MSCs express OSP and ALP at lower level than wt MSCs.
Conclusion: These results show that nonfunctional RANKL affects the expression in CD9, influences the intrinsic clonogenic potential of MSCs and the capacity to differentiate to OB suggesting a possible role of RANKL cytokine in stromal cell physiology and a consequent pathogenic role of bone marrow stroma in osteopetrosis.
Competing interests: None Declared.

A220

P03-022 - Calprotec in chronic nonbacterial osteomyelitis

J Brunner
Pediatrics, Medical University Innsbruck, Innsbruck, Austria
Pediatric Rheumatology 2013, **11(Suppl 1):**A220

Introduction: The cytoplasmic S100 proteins derived from cells of myeloid origin. Calprotectin (MRP8/14 protein complex) might be a biomarker either for autoinflammation and autoimmunopathy. Since autoinflammatory diseases might be a diagnostic challenge calprotectin may be helpful in the diagnosis of autoinflammatory diseases. Chronic nonbacterial osteomyelitis (CNO) is an autoinflammatory, noninfectious disease. CNO describes a wide

spectrum from a monofocal bone lesion to the chronic recurring multifocal osteomyelitis (CRMO). Laboratory and histopathological findings are nonspecific. In some patients systemic inflammatory signs such as elevated acute phase proteins cannot be found.
Case Report: To test the ability of Calprotectin (MRP8/14 protein complex) serum concentrations to monitor disease activity in patients with CNO.
Methods: Serum concentrations of Calprotectin (MRP8/14 protein complex) in a patient with CNO were determined by a sandwich ELISA.
Results: Calprotectin (MRP8/14) level were raised heralding active disease when acute phase proteins (CrP, erythrocyte sedimentation rate). The calprotectin level was 7872,7 ng/ml (normal range 0-3000 ng/ml).
Discussion: Calprotectin (MRP8/14) serum concentrations correlate closely with disease activity and may herald a flare before clinical manifestation. Therefore MRP8/14 serum concentrations are a biomarker indicating disease activity in CNO patients.
Competing interests: None Declared.
References

1. Gerss: *Ann Rheum Dis* 2012, **71**:1991-1997.
2. Hofmann: *International J rheumatology* 2012, **2012**:310206.

A221

P03-023 – Autoinflammatory diseases database in Japan

T Kawai[1*], R Nishikomori[1], M Awaya[1], K Nakagawa[1], K Izawa[1], T Yasumi[1], O Ohara[2], T Heike[1]
[1]Department of Pediatrics, Kyoto University Hospital, Kyoto, Japan; [2]Research Center for Allergy and Immunology, RIKEN Institute Yokohama Research Promotion Division, Yokohama, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A221

Introduction: In recent years, responsible genes for autoinflammatory diseases have been increasingly known and clinical phenotype-genotype correlations of these diseases have been explored through international clinical databases such as EUROFEVER project. However, clinical features of genetic disorders could be affected by countries and races of the patients. Actually, patients with Familial Mediterranean fever in Japan show the different distribution of both clinical features and *MEFV* mutations. Up to now, however, no nationwide research of autoinflammatory diseases with standardized contents which can integrate with data of other international studies has been performed in Japan.
Objectives: We have established autoinflammatory diseases database in Japan. In this research, we designed this database for integration with that of EUROFEVER project in cooperation with Pediatric Rheumatology international trials organization. This integration will enable us to evaluate clinical features and genotypes of autoinflammatory diseases patients in Japan in comparison with the European patients, which will provide further evidences of these diseases, leading to appropriate diagnosis and treatment for the affected patients.
Methods: Most of diagnoses of the patients with autoinflammatory diseases in Japan have been performed in the central specialized hospitals. In this research, we have collaborated all these hospitals and will collect the patients diagnosed as autoinflammatory diseases through these hospitals. In addition to the diseases devoted to EUROFEVER project, other candidate diseases such as Nakajo-Nishimura like syndromes are included. Inclusion criteria and database contents of the patients are designed by reference to that of EUROFEVER project. Patients' information will be obtained from collaborated hospitals and the hospitals which actually will treat the patients. Before participation of this study, each collaborated hospital conformed to institutional review board of each hospital and the Declaration of Helsinki.
Results: Pilot study using paper-based registrations and questionnaires has been closed, and this analysis is ongoing. Now this research is in the transition to web-based system. We hope to show these results in Autoinflammation 2013.
Conclusion: We have established autoinflammatory diseases database in Japan. This research is ongoing now.
Disclosure of interest: T. Kawai Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, R. Nishikomori Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, M. Awaya: None declared, K. Nakagawa Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, K. Izawa Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, T. Yasumi Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, O. Ohara Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare, T. Heike Grant / Research Support from: the Japanese Ministry of Health, Labor and Welfare.

A222

P03-024 – Early onset IBD treated by tocilizumab

R Helbling[1], A Nyddeger[1], F Angelini[2*], A Von Scheven Gete[2], M Hofer[2]
[1]Gastroenterology Unit, Department of Pediatrics, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland; [2]Immunology, Allergology and Rheumatology Unit, Department of Pediatrics, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A222

Introduction: Inflammatory bowel diseases (IBD) normally manifest in adolescent and young adults; nevertheless, in about 1% of the cases, disease onset occurs before the 1st year of age. The clinical picture is often characterized by an indeterminate colitis whose clinical remission is difficult to achieve. Current treatment for IBD consists of either immunosuppressant or biological (Infliximab, Adalimumab) agents.
Case report: A girl born from non-consanguineous parents of Swiss, Spanish and Korean origin, presented, at the age of 5 months, with bloody diarrhea, anal tags, vomiting, anorexia, mimicking a severe proctocolitis due to cow's milk protein allergy, non-responsive to elimination diet. Infections, celiac disease, cystic fibrosis were also excluded. Immunogical investigations, NBT, ANA, ASCA and ANCA were negative, and immunoglobulins were within the normal range.Due to persistent symptoms and failure to thrive, upper and lower endoscopiy was performed showing an ulcerative ileo-pancolitis. The histological analysis confirmed a focally erosive, non-granulomatous ileo-colitis Leading to a differential diagnosis of Crohn's disease versus indeterminate colitis. The patient clinically improvend with steroids and azathioprine. The follow-up was marked by arthritis of the ankle and knee, and erythema nodosum. She did not present severe or recurrent infections, besides those commonly associated with immunosuppresive therapy. Digestive symptoms were first cortico-dependent and eventually cortico-resistent, therefore, in alternative to azathioprine, infliximab, adalimumab and methotrexate were sequencially introduced. Despite these multiple therapies, a sustained remission could not be achieved. On the basis of the important systemic inflammation associated to joint involvement, the patient was started on tocilizumab, an anti-IL-6 receptor known for its efficacy in sytemic-onset juvenile idiopathic arthritis. The new treatment was well tolerated and induced a substantial improvement of the digestive and articular symptoms, together with the laboratory parameters, over 6 months follow up.
Discussion: We report the efficacy of anti-IL-6R therapy in a child affected by a severe early onset IBD, not responding to traditional therapy. Recent works indicate a role of IL-10 pathway in the pathogenesis of early onset colitis. In our patient further immunological and genetical investigations are ongoing.
Disclosure of interest: None declared.
References

1. Kotlarz D, *et al*: Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy. *Gastroenterology* 2012, **143(2)**:347-55.
2. Smolen JS, *et al*: Consensus statement on blocking the effects of interleukin-6 and in particular by interleukin-6 receptor inhibition in rheumatoid arthritis and other inflammatory conditions. *Ann Rheum Dis* 2013, **72(4)**:482-92.

A223

P03-025 – Differential diagnosis of autoimmune disorders

A Kozlova[1*], N Zinovieva[2], J Bustamante[3], S Boisson-Dupuis[4], J-L Casanova[4], A Shcherbina[1]
[1]Immunology, Federal Research and Clinical Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation; [2]Immunology, Children's Clinical Hospital #9, Moscow, Russian Federation; [3]Laboratory of Human Genetics of Infectious Diseases, Institut National de la Santé et de la Recherche Médicale, Paris, France; [4]St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, New York, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A223

Introduction: Mendelian susceptibility to mycobacterial disease (MSMD) is a rare form of primary immunodeficiencies characterized by predisposition for poorly virulent infection agents, primarily non-tuberculous mycobacteria and Salmonella. It has been shown that molecular basis of these diseases is mutations in at least 7 genes in the IL12-dependent IFN-g axis including *IFNGR1*, *IFNGR2*, *IL-12/IL23RB1*, *IL-12B*, *IRF8*, *NEMO*, *CYBB* and *STAT1*. There are about 140 patients with IL-12Rb1/IL-23Rb1 deficiency reported up to date, who manifest with various nonspecific inflammatory features due to chronic BCG infection and salmonellosis.
Case report: Here we report two patients ages 4 and 8 who were referred to us with preliminary diagnosis of autoimmune disorder and very similar symptoms of enlarged lymph nodes of several groups, recurrent fever and vasculitic rash on extremities. In the second patient the skin rash was considered a manifestation of vasculitis and, as a result, she was treated with glucocorticoids and azathioprine for more than 5 years with short interruptions and minimal effect. Laboratory tests in both patients showed: anemia 75-100 g/l, increased inflammatory activity (CRP, ESR), hypergammaglobylinemia (IgG 27-39.4 g/l; IgA 0.65 – 9.17; IgM 1.5 - 3.11 g/l). Both patients had very high levels of rheumatoid factor (with no arthritis), low levels of C4 complement. ANF levels were normal.
Discussion: It was known that both patients had regional BCG infection following vaccination in early infancy, that required massive anti-mycobacterial therapy. Salmonellosis complicated by pneumonia was diagnosed at 2 and 6 years of age in the 1st and 2nd patient, respectively.
Upon admission to our Center both patients' blood cultures were positive for *Salmonella D. enteritidis*. The extensive anti-bacterial therapy and IFN-g s.c. without any antiinflamatory treatment provided good control of the disease in both girls.
The diagnosis of IL12Rb1 deficiency in these patients was based on *in vitro* findings of nulle expression of CD212 (IL-12Rb1) on PHA-stimulates T cell blasts, decreased *in vitro* production of INFg by PHA activated PBMC and was genetically confirmed.
Conclusion: In accessing patients with periodic fever and autoimmune-like features it is important to keep in mind a group of rare primary immunodeficiencies, affecting IL12\IFN gamma axis.
Disclosure of interest: None declared.

A224

P03-026 – Sweet's syndrome: report of a new case

M Mael-Ainin[1*], H Boudhir[1], A Saidi[2], K Senouci[1], B Hassam[1]
[1]Dermatologie - Vénérologie, Centre Hospitalier Universitaire IBN Sina, Morocco; [2]Centre of Anatomical Pathology, United Nations, Rabat, Morocco
Pediatric Rheumatology 2013, **11(Suppl 1):**A224

Introduction: Sweet syndrome is a neutrophilic dermatosis usually idiopathic; it may be associated in one third of cases with inflammatory or neoplastic diseases, or subsequently to drug intake. Its association with pregnancy has been rarely described. Through this observation, we report the case of a pregnancy discovered by the etiological diagnosis of Sweet's syndrome.
Objectives: To emphasize on the importance of seeking pregnancy during etiological Sweet's syndrome in a young woman of childbearing age.
Methods: Mrs. S.H 31 years old, whose antecedents show irregular menstrual cycles, mechanical contraception used in anarchic way, was hospitalized for painful nodular erythematous papulopustular lesions located in the face and limbs, associated with fever (38.5°C) and arthralgia of the ankles. Clinical exam showed limited papulo-nodular erythematous lesions of variable size, in the face, the upper limbs and thighs with the dermal-hypodermic nodule in legs.
Tests showed neutrophilic leukocytosis at 13000 elements/mm3, a sedimentation rate accelerated to 100mm in the first hour and a C-reactive protein increased to 79mg/l. Liver function was normal and 24h proteinuria was negative.
Skin biopsy was in favor of Sweet's syndrome. An etiological test was conducted. No abnormality was observed for blood smear, electrophoresis of proteins, lactate dehydrogenase dosage and carcinoembryonic antigen. Given the gynecological history of the patient, a dosage of Beta-HCG was performed and showed an increase to 119358mUI/ml. Endovaginal ultrasonography showed mono fetal pregnancy with a gestational age estimated to six weeks. The diagnosis of pregnancy Sweet Sydrome was retained. Therapeutic abstention was advocated with clinical monitoring and the evolution was spontaneously favorable after 6 weeks.
Results: Sweet's syndrome in pregnancy represents only 2% of all etiologies of this neutrophilic dermatosis. Its pathogenesis, likely due to a hormonal mechanism, remains unclear. Fetal-maternal prognosis is not affected by the occurrence of Sweet's syndrome, the only risk is the recurrence in subsequent pregnancies. Treatment consists mainly on corticosteroids at a dose of 0.5 to 1mg/kg/jour allowing apyrexia and rapid regression of symptoms. In our case, no therapy has been advocated giving the conservation of the general condition of the patient, which resulted in a good evolution.
Conclusion: This observation shows that Sweet's syndrome pregnancy may occur at a very early gestational age. Seeking pregnancy is thus necessary in any patient of childbearing age with Sweet's syndrome to avoid exhaustive etiological and allow appropriate treatment.
Disclosure of interest: None declared.

A225

P03-027 - A brief Case report on specific undiagnosed condition, whose provisional diagnosis on the basis of provisional lab investigation proved as one of the component of autoinflammatory disorder

S Sathyanandan*, LK Md
Pediatrics, SAT Hospital, Medical College Trivandrum, Kerala, India
Pediatric Rheumatology 2013, **11(Suppl 1):**A225

Introduction: The autoinflammatory syndromes are a newly recognized group of immune disorders that lack the high titers of self-reactive antibodies and T cells characteristic of classic autoimmune disease. , patients with these illnesses experience unprovoked inflammatory disease in the absence of underlying infection.(Curr Top Microbiol Immunol. 2006;305:127-60).
In the category of autoinflamation this disease is a a lesion particularly known as CRMO or chronic recurrent mutifocal osteomylitis a rare condition in which bones have an lesion, inflammation and pain but without a focus of infection .the result of its inheritance pattern is still being debated and the distinguishing feature from the autoimmune pattern is still not clear.
It is classified as an auto inflammatory disease due to its recurrent out breaks and lack of any known pathogen.this disease tends to range from 4 to 14 years of age and 10 years as median. As by current statistics it occur 1:1000000 and primarly in girls with a 5:1 ratio.
CRMO may similar to a painful bone lesion such as arthritis , RF , bacterial osteomylitis ,ewings sarcoma, lymphoma, eosinophilic granuloma or LAH. When all the prevoious illness are ruled out and a bone biopsy turns negative for any known cancer CRMO is usually diagnosed.
As such the common medication given for reducing the inflammation are such as NSAIDS and steroids, antibiotics are not prescribed as there is no evident focus of infection. Congenital dyserthropoitic anaemia and CRMO , uncommon childhood diseases an association with sweet syndrome may be interelated .(journal of paediatrics 115 (5, part 1):730-4. Dol10.1016/S0022-3476(89)80650.
A retrospective review of autoinflamatory diseases in Saudi children at rheumatology clinic showed that of the 34 children with a mean age 118 months , consanguinity was present in 40 %. And in which FMF 50%, CRMO 23.5%, EOS 8.8%, MWS 6 %.(Ann Saudi med . 2012 ;32(1);43-8 (ISSN;0975-4466).
CRMO is sometimes associated with palmoplantar pustulosis and frequent relapses may be present with remission lasting for 4-16 weeks. The most distal case if the affected series . classical Xray diagnosis reveled skeletal changes consistent with appearance of acute or chronic heamoatogenic osteomylits.morphologically CRMO begins as an acute inflammatory process either predominance of PMN leucocytes, with occasionally form absesses and osteoclastic bone resorption. At a later stage it may present as lymphocytes and inflamatoty infiltrate and occasional granulomatous foci and signs of new bone formation.
Case report: The pathological basis of the inflammation in the various inflammatory foci of bone lesions without the exact non infectious cause is much debated part in the pathogenesis of chronic recurrent multifocal osteomylitis and the recurrent attacks that are caused may be due to an inherent autoimmune part of the disease in the body.
To identify the pathophysiological cause of the recurrent inflammatory attacks on the bone with due consideration to the time period of each attack.

The specific treatment stratergy is presently soley based on steroid therapy and the judicious use of NSAIDs for each specific attack that is manifested therefore the need to design a protocol for the treatment of recurrent attacks so as to include immunomodulators in the list may be helpful in remission.
Method: Study design: A prospective cohort study with a chance of reverting back to double blinded Randomized control trial in due course may be considered.
Study setting: SAT Hospital Trivandrum and a proposed teritiary care setting where the translational research could undertaken.
Study period: 18 May 2011 – Ongoing.
Study subjects: A part from the patient , the specific disease has shown to have a familial inheritance pattern, so the genotypic variance along with a pedigree analysis is mandatory in establishing the pathophysiological cause ,so the near and distant family members might be also a part in as study subjectsA semistructured questionare was devised at the onset of study and progress of the disease is currently documented.
Results: The brief outline of the natural history is being given as below. The patient of 9 years was brought to the orthopaedic Opd with first episode of recurrent pain and redness in the joint of the third interphalengial joint and in the knee joint of the upper tibia and the preliminary investigation done as of which is done in the teritiary care hospital . the routine Xray showed very well circumscribed callus around the lesion and the dull sclerosing opacities around the joint after which the it as subjected to open wide excision and sent for HP analysis. The result first showed inflammatory infiltrate and features suggestive of auto immune condition, it was also sent for AFB culture result of which came as negative.
On the provisional basis it was first diagnosis as garres osteomylitis but features of surrounding or well circumscribed marginal sclerosing lesions were present and as of which therapy was given with local antibiotics , the recurrent attacks in the due course made the the patient get refferd to pediatric OPD on complete review on an immunological basis . the ANA profile was done but the only sight fluctuations were seen in the markers. HLAB 27 was also performed which came as negative , rest of the immunological studies are currently proceded. The contrast enhanced MRI showe the features of suggestive of CRMO. As subjected to the low socioeconomic status of the patient higher investigations are pending, the current presisting symptom that the patient complain is the recurrent attacks of pain in at the lesion site. The two sites ie, the head of humerus and the and the tip of olecranon was not subjected to biopsy as it would hamper the epiphysial growth. The detailed presenting history, clinical examination, lab investigations and status of the subject cannot be revealed at present as the patient is only subjected to waiver of consent. As CRMO is diagnosis of Exclusion the next investigation most preferably would be A TPMT assay, laser cut biopsy of the specimen and detailed immunohistochemistry. the provisional diagnosis made were JIA, Chronic Non bacterial osteomylitis, Non Monogenic AIDs, systemic onsent of other autoimmune disease.
Discussion: A detailed history of the Specfic Autoinflamatoty disorder.
Disclosure of interest: None declared.

A226

P03-028 – Spectacular efficiency of isotretinoin in small doses in the sebopsoriasis treatment

M Mael-Ainin[1*], H Boudhir[1], A Saidi[2], K Senouci[1], B Hassam[1]
[1]Dermatologie - Vénérologie, Centre Hospitalier Universitaire IBN Sina, Morocco; [2]Centre of Anatomic Pathology, United Nations, Rabat, Morocco
Pediatric Rheumatology 2013, **11(Suppl 1):**A226

Introduction: Sebopsoriasis is a chronic erythematous scaly dermatitis that is inconveniencing due to its showing character and the frequent inefficiency of the various treatments used. We report through this case the efficiency of low-dose isotretinoin in this particular form of psoriasis.
Objectives: To emphasize on isotretinoin low-dose efficiency in sebopsoriasis treatment.
Methods: Miss H.B, 30 years old, with a history of a psoriasis in the father. The patient was followed up for two years for a histologically confirmed sebopsoriasis. She received various therapies, initially put on moisturizers and Imidazoles but with no improvement, then on dermocorticoids and vitamin D derivatives. Treatment with UVB phototherapy could not be achieved, given the professional constraints of the patient. She was put on Methotrexate at 12.5 mg/week dose for six months without improvement. Treatment based on 5mg Isotretinoin, three times per week, was recommended allowing the complete whitening of lesions two months after the start of treatment with the benefit of one year hindsight.
Results: The sebopsoriasis alters significantly the quality of life of patients. The treatment is mainly based on local treatments but those are often ineffective. Phototherapy or other systemic therapies are also used with varying results. These centro-facial effects during psoriasis respond well to small doses of isotretinoin at the rate of 5 mg three times per week or every day, which is well illustrated through this observation.
Conclusion: Our case shows a remarkable efficiency with a good tolerance of isotretinoin in sebopsoriasis treatment. This promising result should prompt us to use more this retinoid in the treatment of this particular form of psoriasis.
Disclosure of interest: None declared.

A227

PW03-001 - PFAPA syndrome in Turkish children

ED Batu[1*], F Kara Eroglu[2], Y Bilginer[2], S Ozen[2]
[1]Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey; [2]Pediatric Rheumatology, Hacettepe University Medical Faculty, Ankara, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A227

Introduction: Periodic fever, aphthous stomatitis, pharyngitis, and adenopathy (PFAPA) syndrome is a clinical entity of unknown etiology, characterized by periodic attacks of fever, associated with aphthous stomatitis, pharyngitis and cervical adenopathy.
Objectives: The aim of this study was to evaluate the demographic, clinical, laboratory and genetic characteristics of PFAPA patients, and to compare the parameters between MEFV carriers and noncarriers.
Methods: Seventy-one children were enrolled in the study diagnosed as PFAPA according to the previously published criteria and followed-up at the Pediatric Rheumatology Clinic of Hacettepe Children's Hospital. Mutation analysis included MEFV gene mutations. The ethical committee of our institute approved the study protocol.
Results: The median age of onset was 12 months, with a male-to-female ratio of 1,6:1. The length of fever attacks ranged between 2-8 days, and symptom-free intervals between 10-45 days. The most common associated symptoms were pharyngitis (100%), abdominal pain (45,1%), aphthous stomatitis (43,7%), and cervical lymphadenitis (32,4%). During fever flares, the leukocyte count and acute phase reactants (ESR and CRP) were significantly higher ($p<0,001$). Of 53 patients tested for MEFV mutations, 2 were homozygote, while 20 had a single MEFV mutation (9 of them with M694V mutation). Thus carrier frequency reached 1:2,6, higher than the overall carrier frequency in healthy Turkish population which is 1:5 ($p=0,037$). Also, nearly half of these patients had a M694V mutation (associated with higher clinical severity in FMF) which is around 15% in healthy Turkish carriers. No differences were found between carriers and noncarriers in demographic data, clinical and laboratory parameters and response to therapies. In 37 of the 38 who received steroids, attacks were abrupted. The attacks recurred in only 1 out of 10 patients who underwent tonsillectomy. We have also shown a positive family history of recurrent pharyngitis in 52,1% of patients.
Conclusion: The MEFV carrier frequency and M694V was significantly higher as compared to the healthy population. The high family history supports the heritability in PFAPA pathogenesis. PFAPA is a polygenic disease; the association with MEFV mutations in certain ethnic groups may suggest their possible role through the innate immune system. Studying the role of inflammasome in PFAPA may shed light on etiopathogenesis of PFAPA syndrome.
Disclosure of interest: None declared.

A228

PW03-002 – Calculating Gaslini diagnostic score in PFAPA

I Bosio[1], A Meini[1], P Cancarini[1], G Savoldi[2], M Berlucchi[3], M Cattalini[1*]
[1]Pediatric Clinic, University of Brescia, Italy; [2]Laboratorio di Genetica Pediatrica, Anatomia Patologica, Spedali Civili Brescia, Italy; [3]Pediatric Otorynolaryngology, Spedali Civili di Brescia, Brescia, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A228

Introduction: PFAPA syndrome is the most common cause of periodic fevers in children. In the clinical setting of children with periodic fever, there are a minority of patients in which to differentiate between PFAPA and monogenic periodic fevers is not immediate. The Gaslini score would provide a useful tool in this setting, calculating the probability to carry a mutation for monogenic periodic fevers
Objectives: We applied the Gaslini score in a cohort of patients with PFAPA syndrome to identify patients with a high Gaslini score. Once stratified our cohort for the Gaslini score, we compared the two populations to study which were the clinical characteristics determining the high risk score.
Methods: A total of 268 PFAPA patients were recruited and followed in a single referral centre. For the diagnosis Thomas criteria were applied, with the exception of the age at onset criteria. The Gaslini diagnostic score was calculated in each patient.
Results: Out of the 268 patients considered, 47 had a high Gaslini diagnostic score (>1.32). 30% of these patients were screened for mutations in the MVK, TNFRSF1A, and MEFV genes, but they were all negative. Since the clinical picture of all the remaining high risk patients was very coherent with the PFAPA diagnosis we did not performed any further genetic test. We also compared the two groups: age at the onset, family history, mean duration of the episodes, free interval, clinical features and mean age at resolutions were the variables considered. As expected patients with a high Gaslini diagnostic score had earlier age at onset (p = 0.002) and more frequently positive family history (χ^2 = 0.033). Similarly, the prevalence of abdominal pain and diarrhea was more consistent in patients with high Gaslini score (χ^2= 0.001) while -unexpectedly- aphthous stomatitis was similar. Cervical adenitis and prodroms were also more frequent in the high score population (χ^2 = 0.046 and χ^2= 0.022, respectively).
Conclusion: The Gaslini score represents a useful tool to identify patients with a high risk of carrying mutations for the monogenic periodic fevers. Even though our results were quite expected, since the differences between the two sub-populations were in the very same parameters considered in the Gaslini score, our study gives a clue on the fact that the score can fail, predicting a higher risk, in young PFAPA patients or in those with positive family history or clinical relevance of abdominal pain. Indeed this is a quite common scenario. Studying our cohort of patients we identified a subgroup of PFAPA children with high Gaslini score, young age at onset, clinical relevance of abdominal pain, cervical adenitis and prodromic symptoms before the fever onset. Whether this is due to a slightly different manifestation of PFAPA -based on the age of onset- or to the presence of two distinct clinical entities within "PFAPA" need to be addressed in future studies.
Disclosure of interest: None declared.

A229

PW03-003 – Altered neutrophil function in PFAPA

M Sundqvist[1], P Wekell[2,3], V Osla[1], J Bylund[1], K Christensson[1], K Sävman[3,4], A Fasth[3,4], S Berg[3,4], KL Brown[1,5], A Karlsson[1*]
[1]Dept Rheumatology and Inflammation Research, Gothenburg University, Gothenburg, Sweden; [2]Department of Pediatrics, NU-Hostpital Organization, Uddevalla, Sweden; [3]The Queen Silvia Children's Hospital, Gothenburg, Germany; [4]Department of Pediatrics, Gothenburg University, Gothenburg, Sweden; [5]Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada
Pediatric Rheumatology 2013, **11(Suppl 1):**A229

Introduction: The PFAPA syndrome is a non-mendelian autoinflammatory disease of unknown aetiology characterized by Periodic Fever, Aphthous stomatitis, Pharyngitis, and cervical Adenitis. In typical cases, attacks begin before the age of five and occur every 2-8 weeks, often with striking periodicity. We have previously profiled the blood cells and serum cytokine levels in a cohort study of patients with PFAPA (Brown et al, BMC Pediatric 2010). Since autoinflammatory diseases by definition are mediated by cells of the innate immune system, and the major innate immune cell, the neutrophil, is abundant in the circulation during PFAPA episodes we decided to investigate neutrophil function during the febrile and afebrile state in the same cohort.
Objectives: The purpose of this study was to characterize functional features of neutrophils, during different phases PFAPA syndrome, including priming, production of reactive oxygen species (ROS), and apoptosis.
Methods: Neutrophils (PMN) were isolated from blood collected from twelve PFAPA patients during febrile and afebrile phases (mean age 3.5 yrs), from 18 healthy control children (mean age 4.5 yrs), and from 4 febrile control children (mean age 4.8 yrs), with fever and abdominal pain due to infection or appendicitis. We assessed PMN apoptosis by annexin V staining, production of reactive oxygen species (ROS) by luminol/isoluminol-amplified chemiluminescence, MPO quantity by ELISA, and degranulation/priming as expression of CD11b and L-selectin by flow cytometry.
Results: We found a significantly increased intracellular (ic) production of ROS in response to PMA in PMN during the febrile phase, as compared to cells from the afebrile phase. No differences were found in MPO quantity between the samples indicating that the increase in icROS was due to increased NADPH-oxidase activation. We also found decreased spontaneous apoptosis in PMN during the febrile phase of PFAPA, while apoptosis was instead increased during the afebrile phase. Increased expression of CD11b indicated that the PMN were slightly primed during the febrile phase, supported by increased oxidative response to galectin-3, a priming-dependent process. Preliminary results indicate that the latter are not general features of PMN in children with fever, as the febrile control children were significantly lower in degranulation as well as galectin-3-induced ROS production.
Conclusion: Alterations in PMN function, e.g., icROS-production, priming and spontaneous apoptosis, may give mechanistic clues to the so-far undefined etiology of the PFAPA syndrome.
Disclosure of interest: None declared.

A230

PW03-004 - PFAPA patient's serum sensitizes monocytes to LPS

G Simon[1*], N Busso[1], A von Scheven-Gete[2], A So[1], M Hofer[2]
[1]CHUV / Rhumatology, Lausanne, Switzerland; [2]CHUV / Pediatrics, Lausanne, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A230

Introduction: PFAPA is a pediatric auto-inflammatory syndrome of unknown etiology, characterized by recurrent fever, aphthosis, pharyngitis and cervical adenitis. Dysregulated monocyte interleukin-1 beta (IL-1β) secretion is thought to play an important role in fever flares.
Objectives: We hypothesized that factor(s) present in the serum of PFAPA patients during a fever flare may induce monocytes to secrete IL-1β which prolongs symptoms.
Methods: Serum of three controls (CTRL) or PFAPA patients collected during (PFAPA-IN) and between (PFAPA-OUT) flares were incubated with monocytes isolated from healthy volunteers (n=3) and stimulated with ultra pure lipopolysaccharide (LPSup). IL-1β, TNF-α and IL-6 levels were measured by ELISA comparing serum stimulation alone and the impact of serum pre-incubation on LPSup induced cytokines.
Results: Serum-alone treatment of monocytes did not induce any detectable increase in IL-1β, TNF-α or IL-6 secretion. However, pre-treatment with PFAPA-IN serum did result in significantly more IL-1β secretion following LPSup stimulation as compared to PFAPA-OUT serum pretreatment (330.8 ± 181.5 and 173.5 ± 100.1 pg/ml respectively; n=9; $p<0.05$). There was no modulation in the levels of TNF-α and IL-6 under the present conditions, arguing for a process specific for the IL-1β pathway.
Conclusion: Incubation of healthy donor monocytes with serum collected during a PFAPA flare increases the capacity of monocytes to secrete IL-1β through TLR4 ligation. The identity of the molecule(s) and the mechanism of action of this observation still remain to be elucidated.
Disclosure of interest: None declared.

A231

PW03-005 - NLRP3-Q705K monocytes do not produce more IL-1B

G Simon[1*], N Busso[1], A von Scheven-Gete[2], I Moix[3], M Morris[3], A So[1], M Hofer[2]
[1]CHUV / Rhumatology, Lausanne, Switzerland; [2]CHUV / Pediatrics, Lausanne, Switzerland; [3]HUG / Service of Genetic Medicine, Geneva, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A231

Introduction: PFAPA is a pediatric auto-inflammatory syndrome of unknown etiology, characterized by recurrent fever, aphthosis, pharyngitis and cervical adenitis. Dysregulated monocyte interleukin-1 beta (IL-1β)

secretion is thought to play an important role in fever flares. Recently, it was published that Thp1 cells (a monocytic cell line) transduced with the Q705K variant of NLRP3 increased IL-1β secretion after alum (an adjuvant used in vaccines) stimulation.
Objectives: We hypothesized that monocytes isolated from healthy adults carrying the Q705K variant of NLRP3 secrete more IL-1β than monocytes from adults with a WT NLRP3 after ultra pure lipopolysaccharide (LPSup) stimulation.
Methods: Monocytes of six PFAPA families whereby only one of the two parents was carrying the Q705K variant were isolated by MACS and stimulated with LPSup. Levels of IL-1β, TNF-α and IL-6 produced by monocytes isolated from Q705K positive parents were compared to family members expressing WT NLRP3.
Results: The production of IL-1β, TNF-α or IL-6 is not significantly different between monocytes from Q705K positive and WT NLRP3 parents (Q705K NLRP3: 4583.7 ± 2671.1, 3110 ± 2904.6, and 49043.7 ± 37257.9 pg/ml; WT NLRP3: 3499.4 ± 2946.7, 935.6 ± 1259.4, and 45982 ± 18317.4 pg/ml respectively).
Conclusion: Our results show that the Q705K variant of NLRP3 do not lead to any modulation in cytokine production capacity following LPSup stimulation, as compared to WT controls.
Disclosure of interest: None declared.

A232

PW03-006 - IL-1-B inhibition in Schnitzler's syndrome

HD De Koning[1*], J Schalkwijk[2], J Jongekrijg[3], M Stoffels[3], JW van der Meer[3], A Simon[3]
[1]Dermatology, Radboud University Nijmegen Medical Centre y, Nijmegen, Netherlands; [2]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A232

Introduction: Schnitzler's syndrome is a chronic disabling autoinflammatory disorder, characterised by chronic urticaria, paraproteinemia and systemic inflammation. The interleukin (IL) 1 receptor antagonist anakinra is a very effective treatment, but requires daily injection and blocks both IL-1α and IL-1β. Canakinumab is a selective human monoclonal anti-IL-1β antibody with a long half-life.
Objectives: We investigated the long-term efficacy and safety of canakinumab in Schnitzler's syndrome.
Methods: In an open-label, single-treatment arm trial, eight patients with Schnitzler's syndrome received monthly injections with 150 mg canakinumab subcutaneously for 6 months, followed by a 3-month observation period. Primary outcome was complete or clinical remission at day 14. Secondary outcome measures included inflammatory markers, quality of life, time to relapse, safety and tolerability.
Results: After stopping anakinra, patients developed moderate to severe clinical symptoms. Canakinumab induced complete or clinical remission at day 14 in all eight patients. Median C-reactive protein concentrations decreased from 169 mg/l at baseline to less than 10 mg/l on day 14 and remained low or undetectable. One patient discontinued participation on day 39 because of return of symptoms while all others remained in complete or clinical remission during the 6-month treatment period. Relapse after last canakinumab dose occurred within 3 months in four patients. For two patients, remission continued several months post-study. Five patients reported at least one adverse event, predominantly mild upper respiratory tract infections. One patient died in a traffic accident.
Conclusion: In this 9-month study, monthly 150 mg canakinumab injection was an effective and well-tolerated treatment for Schnitzler's syndrome. Our data demonstrate that IL-1β plays a pivotal role in this disease.
Disclosure of interest: None declared.

A233

PW03-007 - NLRP3 genetic variants in Schnitzler's syndrome

HD De Koning[1*], J Schalkwijk[1], JW van der Meer[2], PL Zeeuwen[1], K Neveling[3], M van Gijn[4], A Simon[2]
[1]Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [4]Genetics, UMC Utrecht, Utrecht, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A233

Introduction: Schnitzler's syndrome (SchS) is an autoinflammatory disorder, characterized by chronic urticaria, fever, gammopathy and bone pain. The etiology is unknown, but interleukin-1 (IL-1) inhibition is very effective, like in the cryopyrin associated periodic syndrome (CAPS), that is caused by activating *NLRP3* mutations. Previously, a V198M mutation in *NLRP3* was reported in one patient with SchS, but this is a prevalent variation in the general healthy population.
Objectives: To study presence and significance of *NLRP3* genetic variants in SchS.
Methods: We performed exome screening on peripheral blood-derived DNA of three patients with SchS, and Sanger sequencing of *NLRP3* on peripheral blood-derived DNA of 9 patients with SchS. Patients were further clinically characterized and cytokine stimulation studies with peripheral blood mononuclear cells (PBMCs) were performed.
Results: We found *NLRP3* genetic variants in two patients. Exome screening revealed the known pathogenic CAPS-causing *NLRP3* c.1575C>G p.(P525L) mutation in one patient. Confirmation by Sanger sequencing on peripheral blood only showed a small aberrant peak at the corresponding location. In another patient, we found a hitherto unknown *NLRP3* variant c.1303A>G p.(K435E), of which the pathogenicity still needs to be determined. None of the patients had clinically affected family members. No V198M mutation in *NLRP3* was detected in our population of SchS.
The two patients with *NLRP3* variants fulfilled the criteria for SchS, and had the most severe clinical phenotype of the group. Also, both patients had IgG instead of IgM gammopathy, and both patients had the highest production of IL-1 and IL-6 upon stimulation of PBMCs with LPS.
Conclusion: In seven of nine patients with SchS, no *NLRP3* mutations were found. Two patients with IgG-type SchS with a severe phenotype carried a genetic variation in the *NLRP3* gene: in one, the novel variant K435E, and in the other one a known mutation P525L that was described in severe CAPS patients.
We hypothesize that somatic mosaicism or a less pathogenic effect of the novel mutation may explain the late onset of symptoms.
Disclosure of interest: None declared.

A234

PW03-008 – Mitochondrial disturbances in Schnitzler syndrome

A Sediva[1*], H Hansikova[2], J Sladkova[2], M Rodinova[2], Z Hajkova[2], J Zeman[2], P Szturz[3]
[1]Department of Immunology, Motol University Hospital, 2nd School of Medicine, Charles University, Prague, Czech Republic; [2]Department of Pediatrics, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic; [3]Clinic of Hematooncology, Faculty of Medicine, Masaryk University and University Hospital, Brno, Czech Republic
Pediatric Rheumatology 2013, **11(Suppl 1):**A234

Introduction: Schnitzler syndrome is an autoinflammatory disorder of unknown etiology. At least some of its clinical presentation is mediated through an activation of inflammasome and release of IL-1, as was repeatedly demonstrated by a prominent therapeutic effect of Il-1 blockade.
Objectives: Recent reports bring an evidence of an important role of mitochondria in inflammasome activation and in a pathogenesis of autoinflammatory diseases. We have therefore investigated mitochondrial function and structure in patients with Schnitzler syndrome.
Methods: Activity and amount of oxidative phosphorylation complexes (OXPHOS) were analysed by spectrophotometry, histochemistry and imunoelectrophoretic methods in fibroblast cell lines derived from skin biopsies of three adult male patients with Schnitzler syndrome. Ultrastructure of mitochondria, mitochondrial network and reactive oxygen species (ROS) were analysed by fluorescent and electron microscopy.
Results: The activities and amount of OXPHOS complexes I, III and IV were decreased in patients with Schnitzler syndrome. Interindividual differences in the degree of impairment (from severe to moderate) in analyzed mitochondrial parameters were found. Content of ROS, previously suggested as main inducers of inflammasome, were not significantly increased in cells with Schnitzler syndrome. We, however, did find

consistent and prominent changes in mitochondrial structure of all three patients. Disturbed mitochondrial network and mainly abnormal, partially swelling mitochondria with unusual and sparse cristae were characteristic for all patients. We did further notice marked accumulation of neutral lipids in all tested fibroblasts.
Conclusion: Severe structural damage of mitochondria associated with milder functional changes represented a consistent feature found in all tested Schnitzler syndrome patients. *Supported by RVO-VFN64165/2012*
Disclosure of interest: None declared.

A235

PW03-009 – Genetics of PFAPA syndrome

L Broderick[1*], D Carvalho[2], A Magit[2], W Jiang[2], S Leuin[2], M Bothwell[2], D Kearns[2], S Pransky[2], HM Hoffman[1,3,4]
[1]Medicine, University of California, San Diego, USA; [2]Surgery, University of California, San Diego, USA; [3]Ludwig Institute of Cancer Research, San Diego, USA; [4]Pediatrics, University of California, San Diego, San Diego, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A235

Introduction: Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis (PFAPA) syndrome is an autoinflammatory disorder of childhood and little is known about the underlying etiology. While mutations involving the IL-1 pathway have been identified in other recurrent fever disorders, including TNF-receptor associated periodic syndrome (TRAPS) and cryopyrin-associated periodic syndrome (CAPS), PFAPA syndrome is not traditionally considered to be a hereditary fever disorder.
Objectives: To evaluate pediatric patients with PFAPA for family histories suggestive of immune dysregulation and to correlate inheritability with immune phenotype.
Methods: Patient data and detailed family histories were collected for over 170 children with recurrent fevers including 70 patients with PFAPA to create a prospective cohort over a 4-year period. DNA was isolated from blood or tonsillar tissue from recurrent fever patients, and *NLRP3* and *TNFRSF1A* were sequenced. Quantitative real time PCR was used to evaluate *IL-36* transcripts in tonsils.
Results: Our cohort reflects the diversity of San Diego, without predilection for any specific ethnic background. Family histories revealed 21% of patients have a first degree relative with recurrent fevers and 12% with tonsillitis in childhood, with only 1.4% reporting a history of recurrent infections. We have identified over 30 families with 2-8 affected members. These patients do not possess mutations commonly seen in other autoinflammatory disorders such as CAPS or TRAPS, suggesting that a novel gene may be involved. Upregulation of IL-36 mRNA expression in tonsils identifies the IL-1 family member IL-36 as a candidate gene.
Conclusion: A substantial portion of our families with PFAPA report childhood histories of recurrent fevers that resolved either spontaneously or with tonsillectomy, indicating a possible dominantly inherited trait that impacts the developing immune system, including the tonsils.
Disclosure of interest: L. Broderick: None declared, D. Carvalho: None declared, A. Magit: None declared, W. Jiang: None declared, S. Leuin: None declared, M. Bothwell: None declared, D. Kearns: None declared, S. Pransky: None declared, H. Hoffman Consultant for: Regeneron, Novartis, and Sobi

A236

PW03-010 - MHC complexity in Behçet's disease

MJ Ombrello[1*], Y Kirino[2], P de Bakker[3,4], F Cosan[5], DL Kastner[6], A Gul[5], EF Remmers[6]
[1]Translational Genetics and Genomics Unit, NIAMS, Bethesda, USA; [2]Department of Internal Medicine and Clinical Immunology, Yokohama City University, Yokohama City, Japan; [3]Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, USA; [4]Departments of Medical Genetics and of Epidemiology, University Medical Center Utrecht, Utrecht, Netherlands; [5]Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [6]Inflammatory Disease Section, NHGRI, Bethesda, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A236

Introduction: Family studies support a genetic contribution to Behçet's disease (BD), with a sibling recurrence-risk ratio of 11-52. The class I MHC molecule, *HLA-B*51* (*B*51*), is the strongest known genetic risk factor for BD, however the gene immediately centromeric to *HLA-B*, *MICA*, has also been implicated in BD. Because of strong linkage disequilibrium (LD) between *HLA-B* and *MICA*, their respective contributions to BD susceptibility have been debated. A recent report has proposed that *B*51* is not a BD susceptibility allele, and several studies have identified *B*51*-independent association signals within the MHC.
Objectives: To clarify the relationship between *B*51* and BD, and to test for *B*51*-independent genetic variation within the MHC that influences BD susceptibility.
Methods: Using Illumina Human 370CNV SNP genotypes in a Turkish collection of 1244 BD patients and 1303 geographically-matched healthy subjects, we examined SNP haplotypes and LD patterns across the *HLA-B/MICA* region with Haploview. We performed SNP imputation of the MHC using IMPUTE2 and the 1000 Genomes Phase 1 dataset. We inferred classical HLA types and their amino acids using SNP2HLA. Association testing and regression analyses were performed using SNPTEST and SNP & Variation Suite 7.
Results: We identified a *B*51*(+) *HLA-B/MICA* haplotype that was strongly associated with BD (p=1.22E-46, OR 2.8). A *B*51*(-) version of the same haplotype occurred at equal frequencies in cases and controls, demonstrating that *B*51* is essential to the risk haplotype. Further, we found that rs2848713, a variant on the *MICA* end of the haplotype, conferred additional risk of BD in *B*51*(+) individuals. Through imputation, we generated a set of 32,689 imputed SNPs. The 2 most strongly associated SNPs were 4.8Kb centromeric of *HLA-B* (p_{min}=1.4E-50), but no SNP was more strongly associated with BD than was *B*51* itself (p=1.3E-55). Conditioning on *B*51* revealed an association near *HLA-A* (p_{min}=5.4E-9), and upon adding a representative *HLA-A* SNP to the regression model, we detected residual association centromeric of *HLA-B* (p=1.5E-5). Analysis of imputed HLA types supported these findings. In addition to the association of BD with *B*51* (p=2.2E-55), sequential regression of imputed HLA types identified associations of *HLA-A*03* (p=1E-8), *HLA-C*0701* (p=9.5E-4), and *HLA-B*15* (p=1.2E-4) with BD. Stepwise forward regression of imputed *HLA-B* amino acids identified 6 *HLA-B* residues that together fully accounted for the regional association at *HLA-B*.
Conclusion: This study affirms *B*51* as the strongest risk factor of BD. We have provided strong evidence opposing a *B*51*-independent role for *MICA* variants in BD susceptibility. We have identified significant effects of *HLA-A*03* and *HLA-C*0701*, which protect against BD, and *HLA-B*15*, which confers risk of BD. We have identified a group of *HLA-B* amino acids, most of which reside in the antigen binding groove, that together account for the entire association signal at the *HLA-B* locus.
Disclosure of interest: None declared.

A237

PW03-011 – New Behçet's loci and gene-gene interactions

EF Remmers[1*], Y Kirino[1,2], G Bertsias[1], MJ Ombrello[1], G Wood[1], Y Ishigatsubo[2], N Mizuki[3], I Tugal-Tutkun[4], E Seyahi[5], Y Ozyazgan[5], DL Kastner[1], A Gül[4]
[1]Inflammatory Dis Section/MGB, NHGRI, Bethesda, USA; [2]Int Med and Clin Immunol, Yokahama City University, Yokohama, Japan; [3]Ophthalmology and Visual Sci, Yokahama City University, Yokohama, Japan; [4]Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [5]Cerrahpaşa Faculty of Medicine, Istanbul University, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A237

Introduction: We previously identified disease-associated common variants in *IL10* and *IL23R*, as well as *HLA-B*51*, in a Behçet's disease (BD) genome-wide association study (GWAS) performed with 311,459 SNPs in 1,215 cases and 1,278 controls from Turkey, but the disease-associated variants in these genes do not fully account for the estimated genetic contribution to disease risk.
Objectives: To discover novel common BD susceptibility variants and to evaluate disease loci for evidence of gene-gene interactions.
Methods: We used the Turkish collection GWAS genotypes to impute genotypes of 779,000 markers in the GWAS subjects and then evaluated the imputed markers for disease association. We also searched for new disease associated loci by analyzing patients with uveitis and by specifying different genetic models. We replicated the new BD loci in additional Turkish samples (838 cases, 630 controls) and if polymorphic, in Japanese samples (612 cases, 740 controls). Gene-gene interactions were evaluated by testing the significance of a multiplicative interaction term in a logistic regression model.

Results: Imputation implicated three new BD susceptibility loci (*CCR1*, *STAT4*, and *KLRC4*). Validation, fine-mapping, and replication confirmed these associations and meta-analyses identified variants with genome-wide significance ($p<5x10^{-8}$) in each. The variants in *CCR1*, CC-chemokine receptor 1, and *STAT4*, signal transducer and activator of transcription 4, were associated with gene expression differences. PBMCs with the disease-associated *CCR1* variant exhibited reduced migration to the CCR1 ligand, MIP1a. Two disease-associated variants in *KLRC4*, which encodes an NK receptor family member, encoded missense changes (I29S and N104S). The BD-associated *HLA-B*51* haplotype includes *MICA*, an NK receptor ligand. A statistically significant interaction (p=0.03) was identified between *HLA-B*51* (presumably tagging *MICA* variation) and *KLRC4* N104S. Analysis of BD patients with uveitis identified two non-synonymous variants (D575N and R725Q) in *ERAP1* that recessively conferred BD risk ($p=4.7x10^{-11}$). ERAP1 is an endoplasmic reticulum-expressed aminopeptidase that trims peptides and loads them onto MHC Class I. We found strong evidence for an interaction between the BD-associated Class I allele HLA-B*51 and ERAP1 genotype ($p=9x10^{-4}$).
Conclusion: This study identified four new genetic loci (*CCR1*, *STAT4*, *KLRC4*, and *ERAP1*) and two gene-gene interactions (*ERAP1* with *HLA-B*51* and *KLRC4* with *HLA-B*51*, presumably via its LD with *MICA*) that contribute to BD susceptibility. Shared genetic associations of MHC Class I, *IL23R*, and *ERAP1*, and the strong interactive effect of the disease-associated Class I allele and *ERAP1* support an emerging concept that BD, ankylosing spondylitis, and psoriasis share pathogenic mechanisms.
Disclosure of interest: None declared.

A238

PW03-012 – Unmet need in Behçet's disease: remission is rare

F Alibaz-Oner[1*], G Mumcu[2], Z Kubilay[3], G Ozen[1], G Celik[3], A Karadeniz[1], M Can[1], S Yılmaz Oner[1], N Inanc[1], P Atagündüz[1], H Direskeneli[1]
[1]Rheumatology, Marmara University School of Medicine, Istanbul, Turkey; [2]Health Informatics and Technologies., Marmara University, Faculty of Health Sciences, Istanbul, Turkey; [3]Internal Medicine, Marmara University School Of Medicine, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A238

Introduction: The clinical course of Behcet's disease (BD) as a multi-systemic disorder with a remitting-relapsing nature is unsufficiently explored. As complete remission should be aimed in all inflammatory diseases, we investigated the frequency of complete remission in patients with BD in routine practice.
Methods: In this retrospective study, 258 patients with BD (F/M: 130/128, mean age: 41.1±11,5 years) classified according to ISG criteria were included. The demographic and clinical data for active organ manifestations and treatment protocols were evaluated, both for the current visit and in the last month. Patients having at least one of any disease manifestations were categorized as active.
Results: A total of 1757 visits of 258 patients were overviewed**.** Mean visit number was 6,8± 2,7 (range:1-10) and mean follow-up duration was 45.8 ±36.5 months (2-165). One hundred twenty-five patients (48.4%) were of mucocutaneus type, whereas 133 patients (51.6%) had major organ involvement. When all visits combined, 19.8-43.9% of the patients were using immunosuppressives (IS), whereas 35.3-59.3% was under non-IS therapies such as colchicine or NSAIDs. There was also a group of noncompliant patients (6.4-45%) without any treatment in some visits. Patients were clinically active in 67.2% (n=1182) of the total visits (n=1757). Frequency of clinical activity increased to 75.6% (68.1- 90.3) when the month before the visit was also included. The major cause of the activity was aphthous ulcers (39.4-63.2%) with other mucocutaneous manifestations also commonly present (Genital ulcer: 3.5-27.1 %, erythema nodosum: 8.2-22.5%, papulopustular lesions: 18.2-33.7%, arthritis: 21.3-33.5%, uveitis: 0.5-8.5% and vascular involvement: 2.5-10.8%). No difference was observed between the frequency of activity of patients having ISs or non-IS therapies.
Conclusion: Although complete remission is the current, primary target in inflammatory rheumatological diseases such as rheumatoid arthritis or vasculitides, it is fairly difficult to achieve complete remission in BD with current therapeutic regimens. The reluctance of the clinicians to be aggressive for some BD manifestations with low morbidity, such as mucocutaneous lesions, might be influencing the continuous, low-disease activity state in BD patients.
Disclosure of interest: None declared.

A239

PW03-013 - Behçet's disease: genotype-phenotype correlations

M Takeuchi[1,2*], T Kawagoe[1], A Meguro[1], Y Ishigatsubo[3], E Remmers[2], D Kastner[2], N Mizuki[1]
[1]Department of Ophthalmology, Yokohama City University, Yokohama, Japan; [2]Inflammatory Disease Section, Medical Genetics Branch, National Human Genome Research Institute, National Institute of Health, Bethesda, USA; [3]Department of Internal Medicine and Clinical Immunology, Yokohama City University, Yokohama, Japan
Pediatric Rheumatology 2013, **11(Suppl 1):**A239

Introduction: Although the therapy for Behçet's disease (BD) has improved since infliximab was approved for refractory retinochoroiditis therapy in Japan, the exact pathogenesis of BD remains unclear. Our recent genome-wide association study has identified the *IL10* and *IL23R-IL12RB2* loci as susceptibility genes for BD, in addition to *HLA-A*26* and *B*51*. rs1495965 is located in the intergenic region between *IL23R* and *IL12RB2* and rs1800872 is located in the promoter region of *IL10*. IL-10 is an anti-inflammatory cytokine that may have multiple effects in immunoregulation and inflammation. It is thought that regulatory T cell function related to IL-10 is an important factor in the disease pathogenesis.
Objectives: To examine the association of *IL23R-IL12RB2* and *IL10* gene polymorphisms and HLA typing with clinical presentation of BD, and to examine the expression levels of IL10 mRNA, IL23R mRNA, and IL12RB2 mRNA from PBMCs from each genotype in healthy controls.
Methods: A total of 464 patients with BD enrolled in our recent genome-wide association study were investigated for association between clinical manifestations and 4 susceptibility loci, rs1495965, rs1800872, *HLA-A*26*, and *HLA-B*51*. In our cohort, 196 patients had complete BD and 268 patients had incomplete BD. The expression levels of IL10 mRNA, IL23R mRNA, and IL12RB2 mRNA were examined in 33 healthy controls by real-time PCR.
Results: The frequency of complete BD was significantly increased in patients with the risk allele of rs1800872 under genotypic and recessive models (p=0.0004, 0.0003, respectively). The frequency of skin lesions, ocular lesions, and genital ulcers was also increased in patients homozygous for the risk allele of rs1800872 (p=0.02, 0.05, 0.05, respectively). *HLA-A*26, HLA-B*51*, and the rs1495965 risk allele showed no association with the frequencies of complete or incomplete BD or specific clinical findings. The frequency of patients with refractory chorioretinal uveitis treated with infliximab was significantly increased among the risk allele carriers of rs1495695 and rs1800872 (p=0.01, 0.02, respectively). The expression level of IL10 mRNA was significantly decreased in the homozygotes for the rs1800872 risk allele. There were no significant differences in the expression levels of IL23R mRNA and IL12RB2 mRNA among rs1495695 risk allele carriers.
Conclusion: This study suggests that the *IL10* polymorphism associates with complete BD and the *IL23R-IL12RB2* and *IL10* gene polymorphisms associate with the severity of uveitis. The latter association may be due to regulation of IL10 mRNA expression.
Disclosure of interest: None declared.

A240

PW03-014 - TLR4 and MEFV variants are Behçet's risk factors

Y Kirino[1,2*], Q Zhou[2], Y Ishigatsubo[1], N Mizuki[3], Y Kim[4], DL Kastner[2], A Gül[5], EF Remmers[2]
[1]Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [2]Inflammatory Disease Section, Medical Genetics Branch, NIH/NHGRI, Bethesda, USA; [3]Department of Ophthalmology and Visual Science, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [4]Genometrics Section, Inherited Disease Research Branch, NIH/NHGRI, Baltimore, USA; [5]Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul University, Istanbul, Turkey
Pediatric Rheumatology 2013, **11(Suppl 1):**A240

Introduction: Genome-wide association studies (GWAS) are a powerful means for identifying genes with disease-associated common variants, but they are not well-suited to detect genes with disease-associated rare or low-frequency variants. It has long been debated whether the innate immune system is involved in the pathogenesis of Behçet's disease (BD) but genetic evidence to support this hypothesis is sparse.
Objectives: To determine whether rare and low frequency variants in genes involved in innate immunity are associated with BD.
Methods: In the current study, non-synonymous variants (NSVs) identified by deep exonic resequencing of 10 genes found by GWAS (*IL10*, *IL23R*, *CCR1*, *STAT4*, *KLRK1*, *KLRC1*, *KLRC2*, *KLRC3*, *KLRC4*, and *ERAP1*) and 11 genes selected for their role in innate immunity (*IL1B*, *IL1R1*, *IL1RN*, *NLRP3*, *MEFV*, *TNFRSF1A*, *PSTPIP1*, *CASP1*, *PYCARD*, *NOD2*, and *TLR4*) were evaluated for BD association in Japanese and Turkish populations. A differential distribution of the rare and low frequency NSVs of each gene in 2461 BD cases compared with 2458 controls was evaluated by three different burden tests.
Results: By stringent criteria requiring at least one burden test with study-wide significance ($p < 0.0024$) and a corroborating test with at least nominal significance ($p < 0.05$), rare and low frequency NSVs in one GWAS-identified gene, *IL23R* ($p = 6.9 \times 10^{-5}$), and one gene involved in innate immunity, *TLR4* ($p = 8.0 \times 10^{-4}$), were associated with BD. In addition, damaging or rare damaging *NOD2* variants were nominally significant across all three burden tests applied ($p = 0.0063$ to 0.045). Furthermore, carriage of *MEFV*-M694V, but not other *MEFV* mutations known to cause recessively inherited familial Mediterranean fever, conferred BD risk in the Turkish population (OR = 2.65, $p = 1.8 \times 10^{-12}$).
Conclusion: Rare and low frequency NSVs of two novel BD-associated genes, *MEFV* and *TLR4*, implicate innate immune and bacterial sensing mechanisms in BD pathogenesis. Furthermore, disease-associated *IL23R* rare and low frequency NSVs add to the common variant GWAS evidence implicating this locus.
Disclosure of interest: None declared.

A241

PW03-014B - Gene-expression profiling study in FMF families

B Aygun[1], M Carrabba[2,3*], M Fabbri[1,4], L Gribaldo[1], M Zarantonello[3], G Fabio[2,3]
[1]European Commission - Joint Research Centre, Institute for Health and Consumer Protection – Molecular Biology and Genomics Unit, Ispra, Italy; [2]Internal Medicine Department, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy; [3]Medical Science and Community Health Department, Universita' degli Studi, Milan, Italy; [4]Experimental and Clinical Medicine Department, University of Insubria, Varese, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A241

Introduction: The inflammasome complex and the inflammatory pathway have been implicated in the pathogenesis of familial Mediterranean fever (FMF), and recently ASC (apoptosis-associated speck-like protein containing a CARD) mRNA expression was found to be up-regulated in patients carrying MEFV mutations independent of the mutation type. Gene-expression profiling has the capacity to reveal transcriptome profiles to discriminate disease "phenotypes" to understand a given disease and bring to light new diagnostic and predictive biomarkers.
Objectives: This pilot study focuses on comparative gene expression profiling in FMF patients with different phenotypes while bearing the same mutations in the search of signature patterns.
Methods: Members of two different families representing different mutation sets were subjects of the study. mRNA expression in carriers of the same MEFV mutation that exhibit different phenotypes was comparatively analyzed. Total mRNA was extracted from peripheral blood. Microarray analysis was run on Human 8X60 K Microarray slides and analyzed for whole genome expression levels using an Agilent Scanner.
Results: A within-group analysis of candidate genes for each family was carried out by comparison of gene expression levels in all family members with clinical FMF versus the member without FMF. Although variation in mRNA levels of inflammation related genes such as NLRP3, NLRP1, PYCARD, MEFV, NLRC4, CASP1, PSTPIP1 was observed it was not possible to identify a signature pattern of expression either within-family analysis or comparison between investigated families.
Subsequently, total gene expression was analysed using as threshold a log fold change of 1 or -1 and pathway enrichment was performed using DAVID (the Database for Annotation, Visualization and Integrated Discovery).
In the first family composed of the grandmother, her son and her nephew, all heterozygous for M694V, we compared the two subjects with clinical diagnosis of FMF (son and nephew), in complete remission under colchicine, to the healthy carrier (grandmother).We obtained a list of 73 genes regulated in the same direction. A significant enrichment in the pathways of regulation of amyloid precursor protein biosynthetic process, regulation of T-helper 2 type immune response, regulation of glycoprotein biosynthetic process was found.
In the second family, composed of the father, his son and his daughter all carrying the complex allele E148Q/R761H, we compared the two subjects with clinical diagnosis of FMF (son and daughter), in complete remission under colchicine, to the healthy carrier (father). We obtained a list of 48 genes regulated in the same direction. A significant enrichment was found in the pathways of negative regulation of apoptosis, inflammatory/defense response, cellular ion homeostasis, regulation of activated T cell proliferation.
Conclusion: This pilot study investigated pathways involved in genotype/phenotype correlation in two families manifesting FMF via mRNA gene-expression profiling. Different mutations seem to exert their effects involving different pathways. For any conclusive generalization higher number of patients and representative families are needed to be investigated.
Disclosure of interest: None declared.

A242

PW03-016 – Blau prospective cohort study: articular outcomes

CD Rose[1*], R Cimaz[2], C Thomee[3], R Khubchandani[4], G Espada[5], R Russo[6], M Harjacek[7], B Bader-Meunier[8], P Brissaud[9], N Wulffraat[10], S Vastert[10], R Merino[11], A Naranjo-Hernandez[12], S Oliveira-Knupp[13], F Mackensen[14], J Arostegui[15], J Anton[15], J Fernandez-Martin[16], C Wouters[17]
[1]Pediatrics, Nemours A. I. Dupont Hospital For Children, Wilmington, USA; [2]Universita degli Studi de Firenze, Firenze, Italy; [3]Centre Hospitalier de Luxemburg, Luxembourg, Luxembourg; [4]Jaslok Hospital, Mumbai, India; [5]Universidad de Buenos Aires, Buenos Aires, Argentina; [6]University of Buenos Aires, Buenos Aires, Argentina; [7]University of Zagreb, Zagreb, Croatia; [8]Hospital Necker Enfants malades, Paris, France; [9]None, Neully/Seine, France; [10]UMC Utrecht, Utrecht, Netherlands; [11]Hospital La Paz, Madrid, Spain; [12]Hospital Dr Negrin, Gran Canarias, Spain; [13]Universidad Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [14]Universitat Klinikum Heidelberg, Heidelberg, Germany; [15]Universidad de Barcelona, Barcelona; [16]Hospital do Meixoeiro, Vigo, Spain; [17]Katholieke Universiteit Leuven, Leuven, Belgium
Pediatric Rheumatology 2013, **11(Suppl 1):**A242

Introduction: Blau syndrome is an autosomal dominant monogenic granulomatous disease associated with gain of function mutations at or near the NACHT domain of NOD2; it is the only form of granulomatous arthritis with a known gene mutation. Although its phenotype has been amply described as a triad of arthritis, uveitis and dermatitis in case series and retrospective cohorts, prospective studies on natural history and outcome have not been done.
Objectives: To prospectively study in detail the phenotypic characteristics, functional articular and visual outcomes and radiographic progression of joint disease in patients with BS. Secondary goals are to investigate biomarkers of disease activity as well as to explore relevant pathogenic pathways and candidates for therapeutic targeting.
Methods: Participating centers of an international registry were invited to enroll patients with documented NOD2 mutation after IRB approval. This 3 year prospective study consists of one baseline and 3 yearly visits comprising a comprehensive clinical evaluation, functional assessment (CHAQ/HAQ), visual analogue scales, full ophthalmologic assessment and wrists/hand radiographs at baseline and at last evaluation. Poznansky and Sharp scores were utilized to analyze pediatric and adult X-rays respectively. Blood sampling was performed for follow up and exploratory for biomarkers. Drug therapy was recorded. Coded data were kept in a secured database at the coordinating center.
Results: We are reporting here baseline articular and functional data of the first 25 recruited patients. F: 8; M: 17. Ages:0-54 years;50% 0-15. More than half carried substitution R334W or R334Q. Onset of joint disease was 33 months (3-156). At evaluation arthritis duration was 15.7 yrs (1-53). Mean active joint count was 7 (0-24). Mean CHAQ/HAQ 0.42 (0-2). VAS-p 1.78

(0-8) and VAS-g 2.06 (0-8). A subgroup of patients with long duration (20-50 years) showed a mean joint count of 11.4 (1-24), HAQ of 0.9 (0-2), VAS-p of 4.9 (0-8), VAS-g of 4.1 (0-8). 11/25 required daily systemic prednisone with methotrexate and/or biologics. Significant destructive radiographic changes were documented over time. 50% of the entire group showed extra-triad manifestations with lymphadenopathy, fever, erythema nodosum and hypertension the most common.
Conclusion: This first prospective study on the natural history of BS demonstrates a relentlessly active and destructive articular involvement with significant functional morbidity, exhibiting high levels of disability and disease activity even after years of multiple therapies.
Disclosure of interest: None declared.

A243

PW03-017 – Combination TNF and IL-1 blockade in PAPA syndrome

P Hoffmann[1*], AK Ombrello[1], DL Stone[1], KS Barron[2], DL Kastner[1]
[1]NHGRI, National Institutes of Health, Bethesda, USA; [2]NIAID, National Institutes of Health, Bethesda, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A243

Introduction: The dominantly inherited PAPA syndrome is caused by mutations in *PSTPIP1*. It is one of the least understood monogenic autoinflammatory diseases, both from a pathogenic and treatment perspective. Patients often have persistent symptoms refractory to biologic therapies that necessitate prolonged exposure to high dose corticosteroids. Prior studies have reported increased production of IL-1b and TNF-α by peripheral blood leukocytes. There are Case reports documenting the efficacy of both IL-1 and TNF-α antagonists in treating PAPA but, to date, there are no reports of combination anti-TNF and anti-IL-1 use.
Objectives: To assess the safety and efficacy of combination TNF-α and IL-1b agents in four patients with refractory PAPA.
Methods: We studied 4 patients; 3 adults (ages 52, 26, 18) and one pediatric (age 16) patient. Two adults have the A230T mutation and one has the E250Q mutation. The fourth has a novel E257K mutation. Clinically, all 4 patients have pyoderma gangrenosum (PG) lesions and 3 of the 4 have arthritis. All 4 patients failed monotherapy with anti-IL-1 and anti-TNF agents. Initial single-agent treatments and dose ranges were: infliximab 5-10 mg/kg every 4-8 weeks, adalimumab 20-80 mg every 7-14 days, etanercept 25-50 mg every 7 days, and anakinra 100-500 mg daily. Three out of 4 patients required oral prednisone (15-60 mg daily) and IV methylprednisolone in addition to a biologic. Due to ineffective control of symptoms on single-agent treatment, the combination of an anti-TNF and an anti-IL-1 agent was introduced in all 4 patients. Patients with the A230T mutation received infliximab and anakinra and patients with E250Q and E257K received golimumab and anakinra.
Results: Four patients with PAPA syndrome were treated with the combination of anti-TNF and anti-IL-1 agents. In the 3 patients that required prednisone, after initiation of combination treatment, the dose was successfully decreased to 18-20 mg daily without requiring methylprednisolone boluses. Clinically, there was a significant decrease in frequency and severity of PG lesions and arthritis. Inflammatory markers on single biologic agents were ESR 57-86, CRP 29.5-140 mg/L and on combination treatment were ESR 2-40, CRP 0.62-14.1 mg/L. We did not observe an increase in the frequency of serious infections on combined biologics.
Conclusion: In patients with PAPA syndrome refractory to a single biologic agent, the combination of an anti-TNF and an anti-IL-1 agent is an effective alternative compared to escalating corticosteroids. Although careful monitoring is essential, in our patients, combination biologic therapy has not been associated with an increase in infections or other adverse events.
Disclosure of interest: None declared.

A244

PW03-018 – Efficacy of Anakinra in recurrent pericarditis

M Finetti[1*], A Insalaco[2], L Cantarini[3], M D'Alessandro[1], A Meini[4], L Breda[5], M Alessio[6], P Picco[1], A Martini[1], M Gattorno[1]
[1]Pediatric Reumathology Second Division, IRCCS G. Gaslini, Genoa, Italy; [2]Ospedale Pediatrico Bambino Gesù, Rome, Italy; [3]Policlinico Le Scotte, Università di Siena, Siena, Italy; [4]Spedali Civili di Brescia, Brescia, Italy; [5]Ospedale Policlinico di Chieti, Chieti, Italy; [6]Università di Napoli Federico II, Napoli, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A244

Introduction: Recurrent pericarditis represents an important complication of acute pericarditis. Therapeutic approach during recurrences consists of NSAID administration. However steroid is often necessary to control disease flares. IL-1 inhibitors efficacy has been anecdotally described as effective in the control of the disease in steroid-dependent and Colchicine-resistant patients.
Objectives: To evaluate the long term response to treatment with Anakinra (IL-1 receptor antagonist) in multicenter cohort of patients affected by idiopathic recurrent pericarditis.
Methods: Fifteen patients (12 pediatrics and 3 adults; M:F=11:4) affected by idiopathic recurrent pericarditis and followed by 6 different national referral centers were enrolled in the study. The mean age was 22 years (range 9-60 yrs); mean age at onset was 16 years (5-49 yrs), mean age at the beginning of treatment was 19 years (6-56 yrs). All patients received an initial dosage of 1-2 mg/Kg/die. All the patients presented steroid-dependence and 14 of them had received Colchicine during history disease. Outcomes evaluated in our study were i)response to Anakinra, defined as resolution of pericardial symptoms associated to normalization of laboratory-instrumental findings after first administration of the drug; ii)long term remission during IL-1 receptor antagonist regimen defined as absence of relapses during monotherapy; iii)resolution after Anakinra discontinuation.
Results: All the patients that received Anakinra during active disease (13 pts) presented a dramatic therapeutic response featured by a very rapid disappearance of precordial pain, fever, rub and normalization of acute phase reactants within a few hours from drug administration. Continuous therapy allowed rapid tapering and then discontinuation of steroid, Colchicine and NSAID administration. During continuous daily treatment (mean FU=11 months, range 5-17 months), no patient presented a relapse of the disease; 14 patients started tapering and 8 of them experienced a relapse (mean time since tapering start to relapse=9 months, range 2-17 months). In all patients, disease flare was successfully and quickly controlled by daily full-dose administration of Anakinra, without the requirement of any steroid treatment. A total of 10 flares have been observed in these 8 patients. In 5 patients Anakinra was successfully discontinued after 24 months of treatment (range 17-32 months). The mean time of remission since the withdrawn of the drug is now 12 months (range 2-24 months). At the last follow-up all patients were in remission. Two patients are still receiving daily administration of Anakinra as monotherapy. In 8 patients Anakinra tapering is ongoing.
Conclusion: The long term use of Anakinra in monotherapy is associated to a persistent control of clinical-laboratoristic-instrumental features of idiopathic recurrent pericarditis. In almost 50% of the patients reactivation of clinical manifestations during Anakinra tapering was observed.
Disclosure of interest: None declared.

A245

PW03-019 – Survey of off-label ANTI-IL1 treatments in France

L Rossi-Semerano[1*], B Fautrel[2], D Wendling[3], E Hachulla[4], A Meyer[5], S Ottaviani[6], C Galeotti[1], M Fouillet-Desjonqueres[7], O Richer[8], I Touitou[9], I Koné-Paut[1]
[1]Department of Paediatrics and Paediatric Rheumatology, Bicêtre Hospital, National Reference Centre for Auto-inflammatory Diseases, Le Kremlin Bicêtre, France; [2]Department of Rheumatology, Hôpital La Pitié Salpêtrière, Paris, France; [3]Departement of Rheumatology, CHU Besançon, Besançon, France; [4]Service de Médecine Interne, Centre de Référence des Maladies Auto-immunes et Maladies Systémiques rares, CHRU Lille, Lille, France; [5]Service de Rhumatologie, Hôpitaux universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France; [6]Service de Rhumatologie, CHU Bichat, Paris, France; [7]Service de Pédiatrie Générale et Néphrologie Pédiatrique, Hôpital Femme Mère Enfant, CHU Lyon, Lyon, France; [8]Service de Pédiatrie Médicale, CHU Bordeaux, Bordeaux, France; [9]Maladies Auto-Inflammatoires, Laboratoire de Génétique, Hôpital A de Villeneuve, CHRU Montpellier, Montpellier, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A245

Introduction: Despite their limited licensed indications, anti-IL1 agents are often used in real-life practice for an increasing number of diseases. A national survey to record their off-label use in France was started in January 2011. The survey is coordinated by the French National Reference Centre for Auto-inflammatory Diseases, under the aegis of the "Club Rhumatisme et Inflammation" (CRI).

Objectives: The survey aims to gather information concerning: the number of patients treated with anti-IL1 agents in France, the treated disease, the kind and the indication of the used anti-IL1 agents, their efficacy and safety.
Methods: We set up a physician-directed questionnaire available on the website of CRI since January 2011, covering the following areas: patient data, disease data, anti-IL1 agent, its efficacy, adverse events. We advertised the study on the occasion of French and European rheumatology congresses and by e-mail to French physicians that could be interested. Any adult or paediatric patient who had received an anti-IL1 agent after January 2005 in France could be included after medical informed consent.
Results: At two years 188 patients (99males, 88 females, mean age 35.2 years), from 37 centres have been included. Main diseases were: adult onset Still's disease (AoSD) (35), systemic onset juvenile idiopathic arthritis (SoJIA) (29), gout (26), anakinra-treated cryopirin associated periodic syndrome (CAPS) (21), mevalonate kinase deficiency (MKD) (14), familial Mediterranean fever (FMF) (12), SAPHO syndrome (9), Schnitzler's syndrome (7).
The main off-label used agent was anakinra, used at least once in 182 patients. Canakinumab was used in 23 patients. Rilonacept is not yet available in France. Anakinra shows partial to complete efficacy in most patients (90%); complete clinical response rates vary according to specific diseases, being higher in Schnitlzler's syndrome, gout, CAPS, AoSD and SoJIA. Fifty four percent of patients showed at least one adverse event (AE), mainly minor injection site reactions, and some showed a serious AE (SAE), mainly severe infection. Preliminary data of our survey suggest that canakinumab was generally well tolerated, without any SAE.
Conclusion: Two-year results of the survey confirm the wide use of anti-IL1 agents in clinical practice. The main off-label used agent was anakinra**,** which showed efficacy in the vast majority of patients. Patients with Schnitlzler's syndrome, gout, CAPS and AoSD showed the higher complete clinical response rate. A sizeable number of adverse events, namely injection site reactions, was reported in patients treated by anakinra, Canakinumab was generally well tolerated, without any SAE. The number of patients treated by canakinumab was too small to evaluate its efficacy.
Disclosure of interest: L. Rossi-Semerano: None declared, B. Fautrel: None declared, D. Wendling: None declared, E. Hachulla Consultant for: Consultant fee from Novartis, Sweedish Orphan Biovitrum, A. Meyer: None declared, S. Ottaviani: None declared, C. Galeotti: None declared, M. Fouillet-Desjonqueres: None declared, O. Richer: None declared, I. Touitou: None declared, I. Koné-Paut Grant / Research Support from: Educational and research grant from Sweedish Orphan Biovitrum, Consultant for: Consultant fee from Novartis.

A246

PW03-020 – A decade of ANTI-IL-1 therpay for CAPS in the UK

T Lane[1*], NM Stewart[1], A Bybee[1], DM Rowczenio[1], K Wynne[2], H Trojer[1], A Baginska[1], PA Brogan[2], PN Hawkins[1], HJ Lachmann[1]
[1]National Amyloidosis Centre, University College London, UK; [2]Rheumatology and Vasculitis, Great Ormond Strret Hospital NHS Trust, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A246

Introduction: In October 2002, the first patient with CAPS was treated successfully with the anti-IL-1 agent anakinra at our Centre in the UK, and in 2009 a nationally funded canakinumab treatment service initiated for CAPS in England. By the end of 2012, 82 symptomatic individuals have been assessed at our Centre.
Objectives: To describe our experience, and outcomes of 82 individuals with clinical CAPS, including treatment and natural history.
Methods: We examined all available medical and laboratory records.
Results: Of 82 patients with clinical CAPS a pathogenic sequence variant (PSV) was detected in 77 (94%); 17 PSVs were identified, the commonest were R260W (27%), A439V (23%), T348M (17%), V198M (5%). 5 patients had no PSV detected on Sanger sequencing: 3 children with CINCA and 2 patients with adult onset clinical MWS. 90% were white, 8.5% South Asian and 1% African. 46 (56%) gave a positive family history. There were 8 CINCA cases, 4 MWS/CINCA overlap (75% T348M), 59 MWS (62% A439V, 31% R260W, 19% T348M, 5% V198M) and 11 FCAS (36% A439V, 27% R260W, 9% V198M). Hearing impairment was present in 31 (38%); 12/14 patients (86%) with T348M, 1/19 (5%) of A439V, 3/22 (14%) of R260W and 3/4 (75%) of V198M.
51 patients are receiving canakinumab (20 having converted from anakinra). Over a median follow up (FU) period of 28 months (IQR 15-40), 43 (84%) have experienced complete remission (CR) of disease activity whilst the remainder (8, 16%) have experienced partial remission (PR), defined as good but incomplete resolution of symptoms or serum inflammatory markers. 15 (29%) patients are being treated with double the licensed dose – 4 CINCA (including 2 children), 4 MWS/CINCA overlap, 6MWS (including 2 children) and 1 FCAS with uveitis. 3 patients have discontinued canakinumab and are currently on no treatment (one each with A439V, T348M, R260W) due to: an episode of diverticulitis; pregnancy; a desire for a treatment break. Serious adverse events included infections (diverticulitis, UTI, tonsillitis).
24 patients are on anakinra and over a median FU of 47 months (IQR 12-72) 20 remain in CR and 4 in PR. 6 patients have previously tried canakinumab; 2 had CR but were converted due to: planned pregnancy; planned insertion of a ventricular peritoneal shunt. One adult CINCA patient with a good PR could not tolerate travel to our Centre. A female with mutation negative MWS previously in CR on anakinra opted for a trial of canakinumab, but developed a massive disease flare resulting in hospitalisation. She subsequently reverted to anakinra and is once again in CR. 2 males (A439V, T346I) discontinued canakinumab due to: lack of efficacy; development of major systemic inflammation and a morphea like rash. On anakinra the former remains in PR whilst the latter has experienced CR.
2 children with mild R260W disease have declined treatment.
Conclusion: In this series T348M underlies more severe disease than the other common mutations. A decade of IL-1 blockade confirms its efficacy and relative safety in CAPS across the clinical severity spectrum. Canakinumab is the more popular drug due to its long action; however, a small number of patients are unresponsive to this therapy, suggesting a possible role of IL-1α.
Disclosure of interest: None declared.

A247

PW03-021 - HSCT in mevalonate kinase deficiency

B Wolska-Kuśnierz[1*], B Mikołuć[2], R Motkowski[2], K Kałwak[3], E Bernatowska[1], D Rowczenio[4]
[1]Immunology, Children's Memorial Health Institute, Warsaw, Poland; [2]Pediatrics and Developmental Disorders of Children and Adolescents, Medical University, Białystok, Poland; [3]Pediatric Hematology and Oncology, Medical University, Wroclaw, Poland; [4]National Amyloidosis Centre, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A247

Introduction: Mevalonate kinase deficiency (MKD) has a wide spectrum and severity of clinical manifestation. Patients with mutations in MVK gene leading to complete lack of the enzyme, suffer from the most severe form of disease, also known as mevalonic acydosis, whereas defects with preserved, but insufficient enzyme activity present with autoinflammatory syndrome, also known as hiperIgD syndrome (HIDS). Both diagnosis and treatment of MKD is a great challenge for clinicians. Hematopoietic stem cell transplantation (HSCT) is the only available therapy that allows delivery to the tissues of the missing enzyme produced by healthy donor hematopoietic cells.
Case report: We present a case of 4-year-old girl with genetically confirmed mevalonic kinase deficiency.
She has suffered from recurrent fever, lymphadenopathy, hepatosplenomegaly, hepatitis, impaired growth since she was born. Constant mevalonic acyduria was the first key for diagnosis, then confirmed by genetical analysis of MVK and lack of MK enzyme activity in blood cells. The response to treatment with steroids and IL1-blocker was poor, with only partial resolution of autoinflammation and steroid-dependent adverse events. Due to worsening clinical condition, at the age of 2.5 years patient received bone marrow transplantation (BMT) from matched sibling donor. Due to decreasing donor chimerism, the girl required second transplantation from the same donor at the age of 3 years, followed by repeated donor lymphocyte infusions (DLI). Five months after last DLI the patient achieved stable full donor chimerism. At last follow up, 12 months after HSCT, the girl is in excellent clinical condition, without signs of autoinflammation for 7 last months, progress in psychomotorical and physical development and good immunological reconstitution was observed. Activity of mevalonate kinase is present after transplantation, although still below normal range and mevalonic

acyduria is still observed. To assess further improvement of laboratory markers and its clinical consequences longer follow up of the patient is required.
Discussion: Presented case encourages to qualify patients with severe course of MKD to hematopoietic stem cells transplantation.
Disclosure of interest: None declared.

A248

PW03-022 – Neutrophilic skin disease and inflammation

K Webb[1*], C Hlela[2], C Scott[1]
[1]Paediatric Rheumatology, Cape Town, South Africa; [2]University of Cape Town, Cape Town, South Africa
Pediatric Rheumatology 2013, **11(Suppl 1):**A248

Introduction: Robert Sweet first described a syndrome with a painful, erythematous nodular plaques, neutrophilic dermal infiltrates, fevers and peripheral neutrophilia. This cluster of syndromes became known as Sweet's syndrome. There have been many published cases in children of neutrophilic dermatoses and fever which are labeled as Sweet's syndrome. Recently, however, neutrophilic dermatoses have been associated with some autoimmune and autoinflammatory diseases .
Objectives: To present 3 cases of children with differing manifestations of neutrophilic skin disease and systemic inflammation and postulate on different possible autoinflammatory pathophysiological causes.
Methods: Retrospective case review was conducted.
Results: Patient 1 is a 3 year old child was referred from dermatology with recurrent intermittent episodes of annular erythematous lesions,arthralgias, fevers, red eyes and irritability. Clinically she had episcleritis, conjunctivitis, fevers, failure to thrive, markedly elevated inflammatory markers and a microcytic anaemia. Skin lesions were painful,annular erythematous plaques with cutis laxa. Histology demonstrated a neutrophilic dermatosis which responded to a course of steroids.
Patient 2 is a 6 month old girl who presented in 2012 with erythematous, nodular plaques on trunk, arms, legs and face since 6 weeks of age. These were accompanied by fever, raised white cells and raised inflammatory markers. She had been steroid dependant since 6 weeks of age. Histology showed a leukocytoclastic neutrophilic lobular panniculitis and dermatitis.
Patient 3 is a child with panniculitis (neutrophilic on histology) raised inflammatory markers, arthritis and lipodystrophy. She also presented with hepatitis, myositis, nephritis and macrophage activation syndrome. She was diagnosed with chronic atypical neutrophilic dermatosis, lipodystrophy and elevated temperature (CANDLE) syndrome, a recently described autoinflammatory condition.
Conclusion: We review the recent evidence that autoinflammation may play a role in neutrophilic skin diseases, including recent reports of therapy with IL1 inhibition.We propose that some conditions previously labelled Sweet's Syndrome could possibly represent a manifestation of autoinflammatory conditions.
Disclosure of interest: None declared.

A249

PW03-023 – Role of S100A4 in inflammatory disorders

M Grigorian[1*], MT Hansen[2], B Forst[2], D Sevumian[3], J Klingelhofer[1], H Hayrapetyan[3], T Sarkisian[3], N Ambartsumian[1]
[1]University of Copenhagen, Copenhagen, Denmark; [2]Danish Cancer Society, Copenhagen, Denmark; [3]Center of Medical Genetics and Primary Health Care, Yerevan, Armenia
Pediatric Rheumatology 2013, **11(Suppl 1):**A249

Introduction: Tight association of the pro-inflammatory events with the pathogenesis of a majority of serious human diseases (neoplasia, neurodegenetarions, cardiovascular and others) in addition to inflammatory and autoinflammatory diseases is firmly documented. S100A4 is a metastasis-promoting gene whose expression has been found implicated in chronic inflammation. Earlier we have shown an immense upregulation of S100A4 in affected tissues and plasma of patients suffering from rheumatoid arthritis, psoriasis, dermato/polymyositis [1-3], as well as in pro-inflammarory pathways in tumor metastasis [4,5]. However, its role in inflammation remains unclear.
Objectives: Objectives of the work were (1) to further explore the involvement of S100A4 in chronic inflammatory disorders in humans and (2) disclose the molecular mechanisms implicating S100A4 in the pathogenesis of inflammation- associated, particularly in cancer progression (metastasis).
Methods: We have used several approaches to explore the role of S100A4 in proinflammatory pathways stimulatimg cancer progression. These include in *vitro* (q-RT-PCR, Sandwich ELISA and others) , as well as *in vivo* mouse models for validation of the significance of pro-inflammatory factors in tumor metastasis
Results: We have obtained data demonstrating a significant increase of S100A4 in plasma of patients with Familial Mediterranean Fever (FMF) likely implicating implication of S100A4 in the pathogenesis of the disease. Correlation of the S100A4 concentration in plasma with a pattern of the genetic point mutations in MEFV gene has been analyzed.
Here we will also present data elucidating a putative link of S100A4 with inflammation on the model of cancer progression. We found that the acute phase response proteins Serum Amyloid A (SAA) 1 and SAA3 are transcriptional targets of S100A4 via TLR4/NF-κB signaling. Data obtained demonstrated SAA proteins stimulate their own transcription, as well as that of S100A8, S100A9, RANTES, G-CSF, MMP2, MMP3, MMP9 and MMP13. They strongly enhanced tumor cell adhesion to fibronectin, and stimulated chemotactic migration. Intravenously-injected S100A4 protein induced expression of SAA1 and SAA3 in an organ-specific manner. In a breast cancer animal model, ectopic expression of SAA1 or SAA3 in tumor cells potently promoted wide spread metastasis formation accompanied by a massive infiltration of immune cells.
Conclusion: These findings suggest that chronic inflammation mediated by S100A4 and SAA proteins can promote metastasis, and thus that therapeutic targeting of such pro-inflammatory pathways should be effective in combating metastatic disease.
Disclosure of interest: None declared.

A250

PW03-024 - A transgenic mouse model for variant procaspase-1

A Hermsdorf[1*], F Pessler[2], H Luksch[1], S Winkler[1], R Naumann[3], J Roesler[1], A Roers[4], A Rösen-Wolff[1]
[1]Department of Pediatrics, University Clinic Carl Gustav Carus, TU Dresden, Dresden, Germany; [2]TWINCORE Center for Clinical and Experimental Infection Research, Hannover, Germany; [3]Max Planck Institute of Molecular Cell Biology and Genetics, Germany; [4]Insitute of Immunology, TU Dresden, Dresden, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A250

Introduction: We have detected several genetic variants of *CASP1* in patients suffering from unexplained recurrent febrile episodes. Paradoxically, in vitro and in vivo analyses of patients' cells revealed decreased enzymatic activity of these caspase-1 variants leading to impaired cytokine production despite the proinflammatory phenotype of the patients. The pathophysiological processes associated with *CASP1* variants are still under investigation.
Objectives: In order to recapitulate the effects of the *CASP1* mutations found in the patients we tried to establish a bacterial artificial chromosome (BAC) transgenic mouse line expressing enzymatically inactive $Casp1^{C284A}$ under the control of the own promoter.
Methods: The purified BAC fragment containing Flag-tagged $Casp1^{C284A}$ ($Casp1^{C284AFlag}$) was injected into the pronuclei of fertilized C57Bl6 mouse oocytes, followed by transfer of these oocytes to pseudopregnant foster mothers. Pups born from these mothers were analyzed for the presence of full-length $Casp1^{C284AFlag}$ by screening with sequence specific PCR, Southern blot, and sequencing of the transgene. $Casp1^{C284AFlag}$ transgenic mice were crossed to conventional *Casp1* knock-out (KO) mice and the immunological phenotype of the progeny was analyzed by in vitro stimulation of BMDCs. Expression levels of the $Casp1^{C284AFlag}$ transgene were quantified by qRT-PCR and Western blots. Released cytokine levels were determined by cytometric bead arrays.
Results: From two independent pronucleus injections we received 180 pups. Only three of them harbored transgene sequences and only one female animal proved to harbor the complete $Casp1^{C284AFlag}$ transgene (TG). Crossing to *Casp1* KO mice yielded the following genotypes: *Casp1*WT/WT/TG, *Casp1*WT/KO/TG, and *Casp1*KO/KO/TG. qRT-PCR analyses revealed that

unstimulated *Casp1*^{C284AFlag} transcription was reduced to 0.1% of wild-type *Casp1*. Hence, protein expression could not be detected in unstimulated cells. However, stimulation with LPS upregulated transcription and low-level translation of Casp1^{C284AFlag} in BMDCs. Determination of released cytokines after LPS/ATP stimulation revealed increased release of IL-6 and TNF-a from *Casp1*WT/KO/TG mice with proven Casp1^{C284AFlag} expression.
Conclusion: These data indicate that even tiny amounts of Casp1^{C284AFlag} induced release of other proinflammatory cytokines and that this might contribute to the proinflammatory phenotype observed in our patients. Baseline expression of enzymatically inactive Casp1^{C284AFlag} may be embryonically lethal in mice since not a single mouse could be generated which expressed the transgene under unstimulated conditions. Hence, a conditional *Casp1*^{C284AFlag} knock-in mouse model is being established.
This study was supported by the Federal Ministry of Education and Research (BMBF; Deutsches Netzwerk für Primäre Immundefekte PID-NET) and by EU Marie Curie International Reintegration grant no. GA-2007-224894 (F.P.).
Disclosure of interest: None declared.

A251

PW03-025 - Procaspase-1 contributes to inflammation via NF-KB

M Heymann[1*], S Winkler[1], S Özen[2], E Yilmaz[2], T Kallinich[3], A Rösen-Wolff[1], J Roesler[1], S Hofmann[1]
[1]Dept of Pediatrics, University Hospital Dresden, Dresden, Germany; [2]Faculty of Medicine Ankara, Hacettepe University, Ankara, Turkey; [3]Dept for Pediatric Pneumology & Immunology, Charité Medical University of Berlin, Berlin, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A251

Introduction: Caspase-1 is a pro-inflammatory enzyme which gets activated by autoprocessing following the assembly of multiprotein complexes called inflammasomes. Mature caspase-1 is responsible for the activation of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18. Luksch and colleagues reported naturally occurring *CASP1* genetic variants in patients suffering from unexplained recurrent febrile episodes. Paradoxically, in vitro and in vivo analyses revealed decreased enzymatic activity of these caspase-1 variants leading to impaired cytokine production. A study of Lamkanfi and colleagues provides a possible explanation by indicating a link between enzymatically inactive procaspase-1 and activation of NF-κB, a pro-inflammatory transcription factor.
Objectives: We tried to solve the indicated paradox by analyzing NF-κB activation in the presence of the procaspase-1 variants found.
Methods: NF-κB activity was determined using a luciferase reporter assay system in transfected HEK 293T cells. RIP2 cleavage and ubiquitination studies were also performed in these cells. Protein/protein interactions of RIP2 and procaspase-1 were investigated in THP-1 cells by co-immunoprecipitation and in human monocyte derived macrophages by confocal fluorescence microscopy.
Results: Procaspase-1 variants with reduced enzymatic activity increased NF-κB activation by interacting with RIP2 (receptor interacting protein kinase 2). In contrast, wildtype (wt) procaspase-1 reduced NF-κB activity by cleaving RIP2 and decreasing RIP2 ubiquitination which is essential for NF-κB activation. In addition to transfection experiments, we showed RIP2/procaspase-1 interaction in the human monocyte cell line THP-1 and in human monocyte derived macrophages after stimulation with LPS in a time dependent manner.
Conclusion: Our results support the hypothesis that procaspase-1 variants with reduced enzymatic activity bind to RIP2 and thereby increase NF-κB activitation. This may contribute to pro-inflammatory signalling and thereby contribute to unexplained recurrent febrile episodes in the patients.
Disclosure of interest: None declared.

A252

PW03-026 - Caspase-1 variants involved in ER stress

H Luksch[*], V Schlipfenbacher, S Winkler, J Roesler, A Rösen-Wolff
Department of Pediatrics, University Hospital Carl Gustav Carus, Dresden, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A252

Introduction: Caspase-1 is a proinflammatory enzyme that is activated by the NLRP3 inflammasome in response to endoplasmic reticulum (ER) stress independent of the classical unfolded protein response. This finding linked ER stress to chronic inflammatory diseases. In patients suffering from unexplained recurrent febrile episodes we detected several genetic variants of *CASP1* leading to reduced enzymatic activity due to destabilization of the caspase-1 dimer interface.
Objectives: We investigated a possible association of reduced enzymatic activity of variant caspase-1 with impaired ER-stress responses.
Methods: We analyzed ER stress markers in THP-1 cells and lymphoblastoid cells lines (LCL, EBV transformed B cells) from healthy donors and individuals with *CASP1* variants. Additionally, we knocked down endogenous caspase-1 in THP-1 cells to analyze caspase-1 involvement in ER stress responses. We used quantitative real time RT-PCR to examine mRNA expression of genes involved in ER stress and Western blot detection of Bip (GRP78). Quantification of IL-1β, IL-8, and TNF-α secretion was performed by cytometric bead arrays.
Results: As expected, expression levels of spliced Xbp1, Bip, EDEM, and Chop were increased after induction of ER stress by Tunicamycin (inhibitor of protein glycosylation) in THP-1 cells and LCLs. In THP-1 cells such induction of ER stress lead to secretion of IL-1β, IL-8, and TNF-α. On the other hand, LPS induced activation of the NLRP3 inflammasome and increased expression of ER stress related genes. Furthermore, spliced XBP1 and Bip expression were significantly increased in unstimulated *CASP1* knock-down THP-1 cells and in native patients' LCLs expressing variant caspase-1 with reduced enzymatic activity.
Conclusion: These data indicate that ER stress induced activation of wildtype caspase-1 might be involved in a negative feed back reduction of ER stress and that impaired caspase-1 activity might allow for a perpetuation of ER stress thus contributing to the proinflammatory phenotype of our patients.
Disclosure of interest: None declared.
Acknowledgements: This study was supported by the German Research Foundation (DFG, KFO 249) and by a MeDDrive project (University of Technology, Medical Faculty) to HL.

A253

PW03-027 - CASP1 variants and live cell imaging

S Rabe[*], MC Heymann, S Russ, S Winkler, J Roesler, A Rösen-Wolff, SR Hofmann
Department of Pediatrics, Technical University Dresden, Medical Center Carl Gustav Carus, Dresden, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A253

Introduction: Patients with unexplained recurrent febrile episodes and *CASP1* variants suffer from systemic sterile inflammation despite reduced secretion of IL-1ß. As previously demonstrated by our group *CASP1* variants lead to reduced enzymatic activity of procaspase-1 by destabilizing the tertiary structure of the caspase-1 tetramer. A possible explanation for an alternative pro-inflammatory pathway has been provided by Lamkanfi and colleagues indicating an association between enzymatically inactive procaspase-1 and receptor interacting protein kinase 2 (RIP2) leading to NF-kB activation.
Objectives: The objective of this project is the identification of possible subcellular mechanisms how *CASP1* variants interfere with the IL-1β production or release and lead to the activation of alternative pro-inflammatory pathways.
Methods: Using confocal microscopy, in vivo live cell imaging and an in situ proximity ligation assay we analyzed the subcellular distribution of procaspase-1 wildtype and mutants as well as the interaction with RIP2 in naïve or virally transduced THP-1 cells.
Results: THP-1 cells were virally transduced with GFP- or mCherry fusion proteins of procaspase-1 wildtype and variant *CASP1-L265S*. Procaspase-1 activation, initiated by the assembly of multiprotein complexes (inflammasomes), was induced by stimulation with LPS and Nigericin. First results suggest disturbed microvesicle shedding from *CASP1-L265S* expressing cells after administration of Nigericin. In addition to live cell imaging, the interaction of procaspase-1 and RIP2 has been studied in vitro in naïve THP-1 cells using antibody labeling and proximity ligation assay showing a time dependency after LPS stimulation.
Conclusion: This result suggests a possible influence of procaspase-1 variants on plasma membrane properties, pyroptosis and the release of microvesicles.
Disclosure of interest: None declared.

A254

PW03-028 – Atypical presentation of CRMO in two children

F Vanoni*, A Von Scheven-Gête, M Hofer
Paediatric Rheumatology, Unité Romande de Rhumatologie Pédiatrique, Lausanne, Switzerland
Pediatric Rheumatology 2013, **11(Suppl 1):**A254

Introduction: Chronic recurrent multifocal osteomyelitis (CRMO) is an autoinflammatory bone disorder that manifests as recurrent flares of inflammatory bone pain, related to one or more foci of nonbacterial osteomyelitis. Patients may present with low grade fever and modest elevation of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell counts (WBC). We describe two cases of chronic recurrent multifocal osteomyelitis with high fever and pronounced elevation of inflammatory parameters.
Case report: The first case was an 8-year-old boy presenting to the hospital with polyarticular pain, limping, fever, decreased appetite, weight loss and fatigue. CRP was 171mg/l, ESR 81mmh, WBC 16.5 G/l with 85% of neutrophils. We could exclude an infectious origin and suspected systemic onset juvenile idiopathic arthritis (SoJIA). The patient responded well to NSAIDs, and after discontinuation he showed a stiff neck without history of trauma. Cervical MRI showed C2 and C4 vertebral compaction with bone oedema. Total body MRI showed right distal femoral, right distal fibular and left acetabular enhancement. CRMO was suspected and a fibular biopsy, performed to rule out a tumour, showed fibrous remodelling of the bone, supporting the diagnosis of CRMO. NSAIDs were restarted with progressive improvement. A follow up MRI 6 months later showed decrease of cervical vertebrae oedema.
The second case was a 7-year-old boy with 2 weeks of high fever, decreased appetite, and weight loss without perspiration or chills. Blood parameters showed CRP 105 mg/l, ESR 72 mm/h and thrombocytosis 651 G/l. WBC was normal. Infectious and onco-haematological origines were excluded. A few days later he complained about left wrist pain. An MRI showed a significant periosteal reaction of the two bones of the forearm, associated with soft tissue involvement. Bone scintigraphy revealed hypercaptation of both forearms suggesting CRMO. Bone biopsy showed no inflammation or other abnormalities. Under NSAIDs, the patient did not improve and the biological parameters remained elevated. Because of the persistence of high fever and significant systemic inflammation a treatment with Anakinra, interleukin-1 (IL-1) receptor antagonist, was started, and induced rapid improvement of both bone pain and fever. When Anakinra was discontinued, inflammatory parameters increased again, without fever or other symptoms. A total body MRI was performed and showed multiple symmetrical enhancements in different skeletal segments.
Discussion: The two cases described showed an atypical presentation of CRMO, with high fever and increased inflammatory parameters suggesting a SoJIA. Interestingly, the second case responded to IL-1 blockade, suggesting a role for this cytokine in the disease. Another genetic syndrome, DIRA (deficiency of interleukin-1 receptor antagonist), presents with a similar phenotype to CRMO and could suggest an overlapping of these diseases with a key role of IL-1 in disease pathogenesis.
Disclosure of interest: None declared.
References

1. Wipff J, Adamsbaum C, Kahan A, Job-Deslandre C: **Chronic recurrent multifocal osteomyelitis.** *Joint Bone Spine* 2011, **78(6)**:555-60, Review.
2. Beck C, Girschick HJ, Morbach H, Schwarz T, Yimam T, Frenkel J, van Gijn ME: **Mutation screening of the IL-1 receptor antagonist gene in chronic non-bacterial osteomyelitis of childhood and adolescence.** *Clin Exp Rheumatol* 2011, **29(6)**:1040-3.

A255

PW03-029 – Risk factors for AA-type amyloidosis in Germany

N Blank[1*], P Lohse[2], U Hegenbart[1], H-M Lorenz[1], SO Schoenland[1]
[1]Hematology, Oncology and Rheumatology, University of Heidelberg, Heidelberg, Germany; [2]Institute of Laboratory Medicine and Human Genetics, Singen, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A255

Introduction: Patients with autoinflammatory diseases or chronically active autoimmune disease are at risk for developing AA-type amyloidosis (AA).
Objectives: To identify risk factors for AA in patients with familial Mediterranean fever and chronic inflammatory autoimmune diseases living in Germany.
Methods: Seventy-three patients with FMF and 39 patients with active systemic inflammation due to autoimmune or idiopathic inflammation were evaluated in our reference center for amyloidosis. Mutations in MEFV and the SAA-1 exon 3 variants were analyzed for their contribution to AA risk. Amyloidosis was detected in all cases by congo red staining of kidney or bowel biopsy specimens and AA was classified by immunohistochemistry.
Results: Thirteen patients with FMF (18%) had AA which was diagnosed either before their first contact or during the initial presentation in our center. The relative risk (RR) for amyloidosis of two M694V mutations was 1.15, compared to a RR of 1.00 for a single M694V substitution and a RR of 0.46 in the absence of a M694V mutation in these patients. However, a homozygous SAA 1.1 genotype had a RR of 3.65 (p=0.003), compared to a SAA RR of 0.63 for SAA 1.1+other and to a RR of 0.32 for SAA other+other. FMF patients with AA were older at FMF diagnosis (31 vs 23 years, p=0.003), and AA was diagnosed at a median age of 34 years (95%CI 28.6-47.3). The delay of FMF diagnosis was 17 years in patients with AA and 9 years in patients without (p=0.064).
Twenty-three patients with rheumatic diseases or idiopathic inflammation had AA. A control group of 16 patients with chronically active systemic inflammation was recruited from consecutive patients in our rheumatology department. Twenty-two of 23 patients with AA (96%) had the SAA1.1+1.1 genotype compared to 5 of 16 patients (31%) in the control group, resulting in a RR of 2.05 for AA (p=0.054). Within this group, 7 patients with idiopathic inflammation could not be associated with any autoimmune or autoinflammatory disorder and were considered to be idiopathic AA. These patients with idiopathic AA were older at the onset of inflammation (51.0 vs 25.5 years, p=0.17) and were older at AA diagnosis (63.0 vs 53.5 years, p=0.9), but had a shorter duration of inflammation to AA diagnosis (16.0 vs 20.0 years, p=0.19).
Conclusion: Our data show that the SAA1.1+1.1 genotype is associated with a higher risk for AA than homozygosity for the pyrin M694V mutation. Although M694V+M694V is associated with a more severe disease course, our data show that SAA1.1+1.1 is the major risk factor for AA. In patients with chronically rheumatic diseases or idiopathic AA, homozygosity for SAA1.1 carries a RR of 3.65. Determination of the SAA polymorphism could be useful to assess the risk for AA in patients with chronic inflammatory conditions.
Disclosure of interest: None declared.

A256

PW03-030 – Collecting patients data to inform genetic studies

AK Bybee[1*], F Aslam[1], E Majdoub[2], D Rowczenio[1], A Baginkska[1], R Al-Nackkash[1], W Taylor[1], P Hawkins[1], H Lachmann[1]
[1]National Amyloidosis Centre, UCL, UK; [2]Software Development, Zsah Ltd., London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A256

Introduction: Our centre performed nearly 6000 genetic tests in 2011, in 11 genes, with a year on year increase of about 20% per year over 5 years. In half of the patients genetic testing was used as pre-screening before referral to clinic. A good patient history is essential for proper diagnosis and treatment of HRFS (hereditary recurrent fever syndromes), and genetic testing is a more useful adjunct when guided by relevant symptoms. In order to guide the testing strategy and provide more helpful interpretation of results, we asked the referring physicians to provide detailed information on the patient's family history, symptoms, etc. The approach to collecting this information has evolved over several years, and we describe our experience.
Objectives: To improve and streamline the collection of clinical data for correlation with genetic test results.
Methods: Questions included description of family history, ethnic origin, attack parameters, precipitants and age at onset, 13 symptom areas (including AA amyloidosis), measures of acute phase response, and treatments, dose and effect s. Initially (Stage 1) this information was elicited using a paper proforma, designed to fit on a single side of A4 paper, and was filled in by the referring physician. These were scanned into our records and were accessed as needed, but the information contained could neither be searched nor filtered, making finding correlations and trends difficult. Later (Stage 2), the referring doctor could access a web link, provided by us

on request, and could answer these questions online. The referring physician selected answers from drop-down boxes, with an option to write in some free text comments on the paper form to accompany the specimen. Further refinement was added in mid-2012 (Stage 3) with a web based approach, linked directly from the Centre website to allow the referring physician to request a test and answer the questions immediately. More detailed questions particularly about treatments were now included.
Results: Referring physicians were very cooperative but in Stage 1 often obtained forms well after the patient consultation, meaning many useful details may have been omitted. Initial impressions are that the smoother operation of the web-based approach (Stage 2 and 3) is reflected in the increasing use of the online submission method, and seems particularly popular with non-UK referrals. The specimen reception staff took over the operation of the system with little need to consult with scarce IT staff, and the data collected is easier to access and is searchable. Negative feedback has been limited to a few problems mainly with access local to the referring physicians.
Conclusion: Making the resulting data on the patient population searchable simplified correlation of the information provided with the genetic test findings, and has a immediate benefit of focussing scarce genetic testing resources to where they are most likely to be useful. In the future, technical advances may make genomic information much more plentiful; the concurrent development of good symptom curation using proper ontology will be an important tool for diagnosing and treating HRFS.
Disclosure of interest: None declared.

A257

PW03-031 – Activation-induced cell death of human monocytes

M Miranda-Garcia[1*], J Daebritz[1], G Varga[1], T Weinhage[1], J Ehrchen[2], K Barczyk[3], J Roth[3], D Foell[1]
[1]Pediatric Rheumatology and Immunology, Germany; [2]Dermatology, University Hospital Muenster, Muenster, Germany; [3]Institute of Immunology, University Hospital Muenster, Muenster, Germany
Pediatric Rheumatology 2013, **11(Suppl 1):**A257

Introduction: Monocytes are circulating cells with high plasticity. They respond to various stimuli with distinct activation and differentiation patterns, are able to secrete several humoral factors and they contribute to inflammation in the immune system, either by governing host defense response to invading pathogens or driving reactions to self-molecules in conditions of tissue-damage. Control of these mechanisms is necessary to ensure the self-limitation of inflammatory reactions and avoid perpetuated autoinflammation or autoimmunity. This aspect of immunoregulation is crucial and has been mainly associated with adaptive immunity. To date it is unclear how activated monocytes can regulate early cytokine signals promoting their survival or cell death.
Objectives: The goal of the study was to explore the role of IL-1b and TNFa in activation-induced cell death (AICD) in human monocytes.
Methods: Primary human monocytes were isolated and subjected to stimulation with GM-CSF and IFNg. Cell death was measured using Annexin V and propidium-iodide staining and analyzed by FACS. To explore the mechanism behind AICD of monocytes signaling pathways were analyzed by Western blot using the respective antibodies against phosphorylated and non-phosphorylated proteins. TNF-blockers were used to analyze the role of TNF in the process of AICD.
Results: In the present study we demonstrate in vitro, that simultaneous treatment with GM-CSF and IFNy promotes AICD of human monocytes. Analyzing the signaling pathways that lead to cell death revealed that pyronecrosis is induced by GM-CSF and IFNg. Pyronecrosis has morphological characteristics of necrosis, is caspase- and RIP kinase1-independent but cathepsin-B-dependent. GM-CSF/IFNy-induced cell death of monocytes involved IL-1ß and TNFa-hypersecretion. Furthermore, pyronecrosis was found to be dependent on TNFa and could specifically be inhibited by TNF-blockers such as etanercept.
Conclusion: Taken together, we identified AICD of monocytes as a novel mechanism, which could regulate inflammatory processes that may be altered in the context of autoinflammation. The involvement of different mediators and pathways in this process could have consequences on therapeutic strategies, e.g. for combination therapies involving TNF-blockers.
Disclosure of interest: None declared.

A258

PW03-032 – Periodic fevers in children

C Harper[1,2*], C Corlett[2], G Macdonald[1,2], G Worthington[1,2], D Porter[1,2], D Kelly[1,2], S Segal[1,2], AJ Pollard[1,2,3], S Atkinson[1,2]
[1]Paediatric Department, University of Oxford, UK; [2]Paediatric Department, Oxford Children's Hospital, UK; [3]NIHR , Oxford Biomedical Research Unit, Oxford, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A258

Introduction: This is a retrospective case review of patients presenting with periodic fevers to the paediatric infectious disease clinic at the Children's Hospital, Oxford, over a ten year period.
Objectives: The aim of the study was to characterise a cohort of children presenting with periodic fevers and to determine whether those with PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome) have unique features compared to other periodic fever syndromes.
Methods: Hospital records and biochemical data were obtained for patients presenting to the paediatric infectious disease clinic with periodic fever syndromes between 2001 and 2011. 68 patients were identified but 30 were excluded as they had a clear focus of infection or no history of recurrent fever on review of the medical records. The clinical diagnosis, patient demographics, aetiology, fever behaviour, presenting symptoms, biochemical features and response to treatment are described in the final cohort of 38 patients.
Results: PFAPA was the most prevalent periodic fever syndrome recognised (9 cases, 24%). Other diagnoses included Hyper IgD Syndrome, Familial Mediterranean Fever and Cryopyrin-Associated Periodic Syndrome; 17 (31%) patients remained undiagnosed. All patients were <5yrs at diagnosis and 23 (61%) patients were male. Common presenting symptoms included pharyngitis, cervical adenopathy and abdominal pain. Fever episodes lasted between 3 to 6 days and inflammatory markers were raised during fever episodes. Five (55%) PFAPA cases had a positive family history and six (71%) were treated successfully with tonsillectomy.
Conclusion: We characterised the clinical and biochemical features of patients presenting with periodic fever syndromes and found a high prevalence of PFAPA compared to other periodic syndromes though in many patients no firm diagnosis was made. The family history in a high proportion of cases strongly implies that genetic determinants of these syndromes should be identified.
Disclosure of interest: None declared.

A259

PW03-033 - SLC29A3 mutation: a new autoinflammatory condition

I Melki[1,2*], K Lambot[3], L Jonard[4], V Couloigner[5,6], P Quartier[1,5], B Neven[1,5,7], B Bader-Meunier[1,5,7]
[1]Unité d'Immunologie Hématologie et Rhumatologie Pédiatrique, Necker-Enfants Malades Hospital, Paris, France; [2]Service de Pédiatrie Générale, Robert Debré Hospital, France; [3]Department of pediatric radiology, Necker-Enfants Malades Hospital, France; [4]Department of genetics, Trouseau Hospital, France; [5]Institut IMAGINE, Paris Descartes University, Sorbonne Paris Cité, France; [6]Department of Microbiology, Necker Enfants Malades Hospital, France; [7]U768, INSERM, Paris, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A259

Introduction: Germline mutations in *SLC29A3* result in a range of clinically related, recessive syndromes: H syndrome, pigmented hypertrichosis with insulin-dependent diabetes mellitus (PHID) syndrome, Faisalabad histiocytosis (FHC), and sinus histiocytosis with massive lymphadenopathy (SHML). Main symptoms of these diseases are hyperpigmentation with hypertrichosis, sensorineural deafness, diabetes, short stature, uveitis and "Rosai-Dorfman-like" histiocytosis.
Case report: We report the case of an eleven-month-old boy with early-onset recurrent episodes of unprovoked fever lasting 7 to 10 days associated with pericardial effusion, abdominal pain, diarrhea, and inflammation. Physical examination revealed hyperpigmentation with hypertrichosis, dysmorphic features and a spleen and liver enlargement. Failure to thrive, sensorineural deafness, psychomotor development delay, and a "Rosai-Dorfman like" cheek lesion further developed. Febrile attacks

were not responsive to interleukin-1 and Tumor-Necrosis-Factor blocking agents. All known causes of genetic autoinflammatory syndromes were excluded by sequencing (*MEFV*, *NALP3*, *mevalonate kinase*, *NALP12*, *TNFRSF1*). Sequencing of *SLC29A3* gene revealed homozygous missense mutation c.1088G>A (p.Arg363Gln).
Discussion: This case is the first description of a patient with an auto-inflammatory disorder due to a mutation in *SLC29A3* gene. Genetic defect of *SLC29A3* should be considered in patients with recurrent febrile attacks associated with any symptoms reminiscent of *SLC29A3* broad spectrum of manifestations, especially hyperpigmentation with hypertrichosis.
Disclosure of interest: None declared.

A260

PW03-034 – How to classify autoinflammatory diseases?

G Grateau[1*], V Hentgen[2], K Stankovic[3], I Jéru[4], S Amselem[4], O Steichen[1]
[1]Université Pierre Et Marie Curie Paris 6, Paris, France; [2]Pédiatrie, Centre Hospitalier, Le Chesnay, France; [3]Hôpital Tenon, France; [4]Génétique, INSERM UMR 933, Paris, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A260

Introduction: Definitions and classifications of autoinflammatory diseases have been multiple. Their succession highlights the advances in our understanding of the innate immune system, especially the role of interleukin 1β and the inflammasome. However, these definitions and classifications face a number of structure and content issues.
Objectives: To propose a novel definition of autoinflammatory diseases and to challenge the global classification of inflammatory diseases.
Methods: We appeal to the desirable characteristics of classification systems (exhaustiveness, disjointness, naturalness, usefulness) and to a critical analysis of the notion of continuum.
Results: We propose a clinically-oriented definition: "autoinflammatory diseases are diseases with clinical signs of inflammation, associated with elevated acute phase reactants and due to a dysfunction in the innate immune system, genetically determined or triggered by an endogenous factor".
It is hard to find natural properties able to underlie a useful classification of autoinflammatory diseases, and inflammatory diseases as a whole, into disjoint and exhaustive categories. The notion of continuum is therefore appealing. However, a single continuum from purely autoinflammatory to purely autoimmune diseases oversimplifies, and even distorts, reality. How to locate, for instance, the disease caused by a deletion in PLCG2 (the gene encoding phospholipase Cγ2) that associates autoinflammatory symptoms to both common variable immunodeficiency and autoimmune features? Here we have an overactivation of both the innate and the adaptive immune system, associated with a deficiency of the adaptive immune system.
More than one dimension is needed to properly represent the immunological dysfunctions underlying inflammatory diseases. Furthermore, a classification of inflammatory diseases should also make sense of the clinical, pathological and biological phenotypes.
Conclusion: To be adequate and useful, a definition of autoinflammatory diseases and a classification of inflammatory diseases must take the multiple facets of reality into account, including clinical features. This can be done within a continuum only if it is multidimensional.
Disclosure of interest: None declared.

A261

PW03-035 – Autoinflammatory diseases diagnostic chart/tool

KL Durrant[1*], I Muscari[2], JI Aróstegui[3]
[1]Nursing Adjunct Faculty, Samuel Merritt University, Oakland, USA; [2]Medical & Surgical Sciences and Neuroscience, Institute of Rheumatology, Sienna, Italy; [3]Immunology Department, Hospital Clínic-IDIBAPS, Barcelona, Spain
Pediatric Rheumatology 2013, **11(Suppl 1):**A261

Introduction: There is great interest in having a reference chart to increase awareness and improve accurate diagnosis for systemic autoinflammatory diseases (SAID), which arealso classical primary immunodeficiency diseases. We have created this wall chart that includes all currently known SAIDs, and arranged the information so that a clinician could compare various symptoms, abnormal labs, genetics, inheritance pattern, affected ethnicity and other information between all of them. In addition, photographs of the main clinical features of many of these diseases have been included to help medical professionals to better understand these diseases, and consider them in the evaluation of candidate patients. As the understanding of autoinflammatory diseases is continuously changing, this chart will probably need to be updated over time. However, the basic information for these diseases will remain helpful for medical professionals.
Case report: In 2008, we developed a chart that compared the four main inherited SAID (cryopyrin-associated periodic syndromes [CAPS], TNF receptor-associated periodic syndrome, Familial Mediterranean Fever, and Hyper-IgD periodic fever syndrome). This chart was shared online, in print, and inside our CAPS guidebook available in print or online at nomidalliance.org. This chart has helped increase awareness about these diseases. We also received personal feedback from a large number of newly diagnosed patients and families, mostly with CAPS, about the usefulness of this comparative chart in helping their doctors to evaluate their symptoms, make a diagnosis and order genetic testing that later confirmed their disease. Previously undiagnosed families with CAPS in the United States, Australia and elsewhere, many with 6-18 affected family members spanning 3 generations that directly benefitted from downloading the chart from the website. They printed it out, and shared it with their doctor that led to their diagnosis, and prescribed treatment for CAPS. The chart also helped a number of other individuals, including many doctors that were seeking more about these diseases.
Discussion: We have developed this new, expanded chart including the main features of all currently known SAID. It will be distributed to doctors worldwide to help increase awareness, care and treatment for these diseases. The chart will be translated into various languages, for use online and in print, and will be printed in smaller batches. We can continually adapt it in the online version to keep it as current and accurate as possible. Our hope is that this tool helps to doctors to achieve an early diagnosis in these patients to gain access to treatment, especially at a young age, so that they can have the best chances for a healthy life with less disease complications.
Disclosure of interest: None declared.
References

1. Goldbach-Mansky R: **Immunology in clinic review series; focus on autoinflammatory diseases: update on monogenic autoinflammatory diseases: the role of interleukin (IL)-1 and an emerging role for cytokines beyond IL-1.** *Clin Exp Immunol* 2012, **167(3)**:391-404.
2. Masters SL, Simon A, Aksentijevich I, Kastner DL: **Horror Autoinflammaticus: The Molecular Pathophysiology of Autoinflammatory Disease.** *Ann Rev Immunol* 2009, **27**:621-668.
3. Hoffman H, Simon A: **Recurrent Febrile Syndromes– What a Rheumatologist Needs to Know.** *Nat Rev Rheumatol* 2009, 249-256.

A262

PW03-036 – Neutrophilic urticaria with systemic inflammation

H Belani, K Leslie[*]
Dermatology, UCSF, San Francisco, USA
Pediatric Rheumatology 2013, **11(Suppl 1):**A262

Introduction: Predominantly neutrophilic inflammatory infiltrates are seen in a subset of chronic urticaria patients, with lesions that tend to be less itchy and poorly responsive to antihistamine therapy.[1,2] There have been reports of patients with neutrophilic dermatoses presenting with extracutaneous inflammation.[3] We present two patients with neutrophilic urticaria and extracutaneous symptoms likely mediated by interleukin 1 (IL-1) and not associated with a known connective tissue disease. We propose the term "neutrophilic urticaria with systemic inflammation" (NUSI) to describe a spectrum of diseases, which includes neutrophilic urticaria, and highlights the role of IL-1 in driving this particular inflammatory process.
Case report: Patient 1: A 47-year old female presented with urticaria and associated night sweats, fevers and polyarticular arthritis. Acute phase reactants were elevated with worsening of symptoms. Initial treatment with topical and systemic corticosteroids, antihistamines, and immunosuppressants was unsuccessful. 100% clinical resolution was achieved with anakinra, an Il-1 receptor antagonist. Patient 2: A 26-year old female presented with urticaria and associated joint pain and swelling. Initial treatment included antihistamines, colchicine, and dapsone. Only colchicine provided moderate benefit, but was stopped due to significant

GI-discomfort. Anakinra was initiated; the patient now has 100% control on daily therapy.

Discussion: The cases described in this report represent a multi-systemic inflammatory entity: neutrophilic urticaria with systemic inflammation (NUSI). In NUSI, there is abrupt onset of neutrophilic urticaria occurring in tandem with inflammatory arthritis and other systemic symptoms, and complete control of symptoms is achieved with IL-1 blockade. NUSI likely lies on a spectrum with clinically similar autoinflammatory diseases, with IL-1 dysregulation and subsequent neutrophil-driven inflammation leading to cutaneous symptoms progressing from varied severity of neutrophilic urticaria to include Sweet's syndrome to possibly pyoderma gangrenosum. The diagnosis of NUSI is an important one to consider in patients who present with antihistamine-resistant urticaria in combination with systemic inflammatory symptoms, with IL-1 blockade a viable option for therapy.

Disclosure of interest: H. Belani: None declared, K. Leslie Consultant for: Novartis.

References

1. Toppe E, Haas N, Henz B: **Neutrophilic urticaria: clinical features, histological changes and possible mechanisms.** *Br J Dermatol* 1998, **138(2)**:248-53.
2. Tharp M: **Chronic urticaria: Pathophysiology and treatment approaches.** *J Allergy Clin Immunol* 1996, **98(6)**:325-330.
3. Kieffer C, Cribier B, Lipsker D: **Neutrophilic urticarial dermatosis: a variant of neutrophilic urticaria strongly associated with systemic disease. Report of 9 new cases and review of the literature.** *Medicine* 2009, **88(1)**:23-31.

A263

OR13-001 Loss-of-function mutations in CECR1, encoding adenosine deaminase 2 (ADA2), cause recurrent fevers and early onset strokes

Q Zhou[1], A Zavialov[2], J Chae[1], M Hershfield[3], R Sood[4], S Burgess[5], A Zavialov[2], D Chin[1], M Gadina[6], R Goldbach-Mansky[7], A Ombrello[1], D Stone[1], I Aksentijevich[1*], D Kastner[1]

[1]Inflammatory Disease Section, NHGRI, Bethesda, USA; [2]Turku Centre for Biotechnology, University of Turku, Turku, Finland; [3]Medicine, Duke University Medical Center, Durham, USA; [4]Zebrafish Core, USA; [5]Developmental Genomics Section, NHGRI, USA; [6]Office of Science and Technology, NIAMS, Bethesda, USA; [7]Translational Autoinflammatory Disease Section, NIAMS, Bethesda, USA

Pediatric Rheumatology 2013, **11(Suppl 1):**A263

Introduction: Here we describe a new genetic disease characterized by fevers and systemic inflammation, livedo reticularis, mild immunodeficiency, and early-onset recurrent lacunar strokes in 5 unrelated patients.

Objectives: To identify a possible genetic cause of this syndrome.

Methods: We used a combination of exome and Sanger sequencing, SNP arrays, Western blots (WB), ELISA assays, radiochemical-thin layer chromatography, and HPLC.

Results: The 5 unrelated patients shared 3 missense mutations in *CECR1*, encoding adenosine deaminase 2 (ADA2), with the genotypes A109D/Y453C, Y453C/G47A, G47A/H112Q, R169Q/Y453C, and R169Q/28kb genomic deletion encompassing the 5'UTR and first exon of *CECR1*. All of the mutations are either novel or present at low frequency (<0.001) in several large databases. Computer modeling based on the crystal structure of the human ADA2 protein suggests that *CECR1* mutations either disrupt protein stability or impair ADA2 enzyme activity. Whereas the ADA1 protein, which is mutated in some patients with severe combined immunodeficiency disease, is an intracellular protein that catalyzes the conversion of adenosine to inosine, ADA2 is expressed predominantly in myeloid cells and secreted into the blood, and has a lower affinity for adenosine than ADA1. Western blots showed a decrease in ADA2 protein in supernatants of PBMCs from patients relative to healthy controls. All patients had at least 10-fold diminished serum and plasma concentrations of ADA2 protein, and reduced ADA2-specific adenosine deaminase activity. However, whereas ADA1 deficiency leads to the accumulation of deoxyadenosine nucleotides and lymphocytotoxicity, such toxic metabolites were not found in the blood of patients with ADA2 deficiency. Animal models suggest that ADA2 is the prototype for a family of proteins with growth factor activity (adenosine deaminase growth factors, ADGF). There is no mouse homolog of *CECR1*, but there are 2 homologs in the zebrafish, *Cecr1a* and *Cecr1b*. While a zebrafish line with a hypomorphic retroviral insertion in *Cecr1a* did not exhibit an obvious phenotype, when we used 2 different morpholinos to knock down the expression of *Cecr1b*, at 48 hours post fertilization we observed intracranial hemorrhage in approximately 50% of zebrafish embryos, but in only 3% of control embryos. Utilizing zebrafish lines with GFP-tagged leukocytes, we found that *Cecr1b* knockdown also led to the near absence of neutrophils, but not monocytes. These observations suggest that the ADA2 homolog may be necessary both for vascular integrity and leukocyte development in the zebrafish, and that the near absence of ADA2 in humans may lead to strokes and autoinflammation by similar mechanisms.

Conclusion: We propose the term *fever with early onset stroke* (*FEOS*) to denote this condition. Although it is a rare disease of children, FEOS may provide important insights into the role of ADGFs in human disease, and may elucidate novel pathways underlying strokes in adults.

Disclosure of interest: None declared.

A264

OR13-002 Recessive mutations in CECR1, encoding adenosine deaminase 2 (ADA2), cause systemic and cutaneous polyarteritis nodosa (PAN)

E Levy-Lahad[1*], P Elkan-Navon[1†], R Segel[1†], SB Pierce[2†], T Walsh[2], J Barash[3], S Padeh[4], A Zlotogorski[5], YY Berkun[5], JJ Press[6], M Mukamel[6], PJ Hashkes[1], LL Harel[6], M Tekin[7,8], F Yalcinkaya[7], O Kasapcopur[9], EF Emirogullari[8], MK Lee[2], RE Klevit[2], PF Renbaum[1], A Weinberg-Shukron[1], S Zeligson[1], D Marek-Yagel[4], M Shohat[10], A Singer[11], E Pras[4], AA Rubinow[5], Y Anikster[4], M-C King[2]

[1]Shaare Zedek Medical Center, Jerusalem, Israel; [2]University of Washington, Seattle, USA; [3]Kaplan Medical Center, Rehovoth, Israel; [4]Sheba Medical Center, Tel Hashomer, Israel; [5]Hadassah Medical Center, Jerusalem, Israel; [6]Schneider Children's Medical Center, Petach Tikvah, Israel; [7]Ankara University, Ankara, Turkey; [8]University of Miami, Miami, USA; [9]Istanbul University, Istanbul, Turkey; [10]Rabin Medical Center, Beilinson Hospital, Petach Tikvah, Israel; [11]Barzilai Medical Center, Ashkelon, Israel

Pediatric Rheumatology 2013, **11(Suppl 1):**A264

Introduction: Polyarteritis nodosa (PAN) is a systemic necrotizing vasculitis of middle-sized arteries, found in both adults and children. Disease pathogenesis is poorly understood. We identified multiple cases of systemic PAN and cutaneous PAN in families and individuals of Georgian-Jewish ancestry, consistent with autosomal recessive inheritance. While most cases (17/20) had childhood onset, cutaneous PAN could also initiate in middle age.

Objectives: To determine the genetic basis of monogenic PAN.

Methods: Exome sequencing of 4 affected individuals from 2 families was followed by targeted sequencing of 16 additional Georgian-Jewish cases and 6 Turkish pediatric cases of PAN. Mutations were assayed by protein structure analysis, expression in mammalian cells, biophysical analysis of purified protein, and enzymatic activity in patient sera.

Results: Missense mutation CECR1 p.G47R (c.139G>A), in the gene encoding ADA2, was the only damaging variant homozygous in all 4 exomes. Of the 20 Georgian-Jewish patients, 19 were homozygous for this mutation and one was compound heterozygous for G47R and H391Y. One Turkish patient was compound heterozygous for G47V and W264S. In the Georgian-Jewish population, the frequency of G47R was 0.05, reflecting the high prevalence of PAN in this endogamous community. The other mutations were absent from ethnically matched controls.

ADA2 activity was significantly reduced in patient sera. Expression of mutant proteins in HEK293T cells yielded significantly reduced levels of secreted ADA2 and biophysical assays indicated reduced protein stability.

Conclusion: We report mutations in the gene encoding ADA2 as the first genetic cause of a systemic vasculitis. ADA2 is the major extra-cellular ADA, so blood vessels may be particularly vulnerable to loss of its catalytic and immune growth factor activity.

Disclosure of interest: None declared.

A265

OR13-003 - TNFRSF11A molecular defects cause autoinflammatory disorders

I Jéru[1,2,3*], E Cochet[3], P Duquesnoy[1], V Hentgen[4], B Copin[1], M Mitjavila-Garcia[5], S Sheykholeslami[1], G Le Borgne[1,2], F Dastot[1,3], S Karabina[1,2], M Mahevas[6], S Chantot-Bastaraud[1], L Faivre[7], S Amselem[1,2,3]

[1]UMR_S933, INSERM, France; [2]Université Pierre et Marie Curie-Paris, France; [3]Génétique et d'Embryologie Médicales, Hôpital Trousseau, Paris, France; [4]Centre de Référence des Maladies AutoInflammatoires (CeRéMAI), Centre

Hospitalier de Versailles, Le Chesnay, France; [5]U.935, INSERM, Villejuif, France; [6]Médecine Interne, Hôpital Henri Mondor, Créteil, France; [7]Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Hôpital d'Enfants, Dijon, France
Pediatric Rheumatology 2013, **11(Suppl 1):**A265

Introduction: Hereditary recurrent fevers (HRF) are autoinflammatory disorders whose etiology remains unknown in many cases.
Objectives: To identify a new HRF gene
Methods: Comparative genomic hybridization (CGH, 385K array) was performed in the proband. *TNFRSF11A* was screened by Sanger sequencing in other patients. *TNFRS11A* expression was quantified by fluorescence-activated cell sorter analysis (FACS). NF-k B activation was assessed using a luciferase assay in HEK293 cells transfected with plasmids encoding wild-type and mutated TNFRSF11A.
Results: Array-CGH analysis performed in a patient with multiple congenital anomalies and a recurrent fever syndrome revealed a de novo heterozygous chromosomal rearrangement encompassing a duplication of *TNFRSF11A*. This transmembrane receptor binds the TNFSF11 cytokine, activates NF-k B signaling, and regulates fever in rodents, consistent with a possible role in HRF. *TNFRSF11A* screening in other patients with genetically-unexplained HRF revealed a heterozygous frameshift mutation in a patient and her affected mother. The mutated protein is expressed at similar levels as the normal receptor on leukocytes. Most importantly, this mutation results in a gain of function on NF-k B signaling, since the mutated protein is more responsive to TNFSF11 stimulation than the wild-type receptor. Since *TNFRSF11A* (also known as *RANK*) was previously known for its key role in osteoclastogenesis, the medical history of our patients was reassessed and revealed minor symptoms also found in patients with *TNFRSF11A*-associated bone disorders.
Conclusion: The implication of *TNFRSF11A* in HRF reveals a key role of this receptor in autoinflammation and opens up new fields of research at the crossroads between bone metabolism and innate immunity.
Disclosure of interest: None declared.

A266

OR13-004 – Evidence-based clinical classification criteria for periodic fevers

S Federici*, S Ozen, I Koné-Paut, H Lachmann, P Woo, L Cantarini, G Amaryan, A Insalaco, J Kuemmerle-Deschner, B Neven, N Dewarrat, Y Uziel, D Rigante, T Herlin, S Martino, A Simon, S Stojanov, H Ozdogan, J Frenkel, N Ruperto, A Martini, M Sormani, M Hofer, M Gattorno
GASLINI, Genova, Italy
Pediatric Rheumatology 2013, **11(Suppl 1):**A266

Introduction: No evidence-based classification criteria are so far available the majority of autoinflammatory diseases
Objectives: To elaborate and validate a set of clinical criteria able to correctly classify patients affected with the most common periodic fevers
Methods: All FMF, TRAPS, MKD and CAPS patients enrolled in the Eurofever registry until March 2013 were evaluated. For each disease gold standards were considered according to the following criteria: i) clinical validation by centers and disease-principal investigator, ii) confirmative molecular analysis (2 mutations for MEFV with at least one mutation in exon 10, 2 mutations of MVK gene, 1 mutation of TNFRFS1A with exclusion of low-penetrance variants, 1 mutation of NLRP3 with exclusion of low-penetrance variants), iii) PFAPA patients validated by disease-principal investigator and confirmed by the centers on the basis of the follow-up. Clinical criteria was formulated on the basis of a univariate and multivariate analysis in a first group of patients (training set) and validated in an independent set of patients (validation set).
Results: A total of 1204 consecutive patients with periodic fevers were enrolled in the registry. Among them 743 consecutive gold standard patients (288 FMF, 73 MKD, 96 TRAPS, 87 CAPS, 199 PFAPA) were evaluated (440 in the training set and 303 in the validation set).The multivariate analysis identified the clinical variables (either as presence or absence).
The classification score was than tested in an independent set of patients (validation set) revealing a sensitivity of 93% and specificity of 89% for FMF; a sensitivity of 100% and specificity of 74% for TRAPS; a sensitivity of 80% and specificity of 90% for MKD and sensitivity of 97% and specificity of 92% for CAPS; sensitivity of 99% and specificity of 96% for PFAPA. The performance in non-gold standard patients (i.e. heterozygous patients in autosomal recessive diseases or patients with low-penetrance mutations) revealed a variable percentage of patients (70% FMF, 75% TRAPS, 41% MKD and 94% CAPS) positive for the respective criteria.
independently correlated to for each disease with their specific weight. The cut off value of the classification score was chosen on the ROC curve in order to guarantee the highest sensitivity and specificity.
Conclusion: Evidence-based clinical criteria for the classification of patients with inherited periodic fevers have been elaborated. These clinical criteria could be used in association with molecular analysis and other variables (i.e. metabolic examinations, response to specific treatments) for patients classification.
Disclosure of interest: None declared.

A267

OR13-005 – Investigation of clinical and laboratory significance of TNFRSF1A intron by reverse-phase protein microarray

E Drewe[1*], O Negm[2], W Abduljabbar[2], P Hawkins[3], L Fairclough[2], I Todd[2], P Tighe[2]
[1]Clinical Immunology and Allergy, Nottingham University Hospitals NHS Trust, UK; [2]Molecular Medical Science, University of Nottingham, Nottingham, UK; [3]National Amyloidosis Centre, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A267

Introduction: Genetic analysis for autoinflammatory disease may reveal sequence changes of uncertain clinical significance. We describe investigation of a 26 year old with unexplained inflammatory symptoms with a novel intronic change in TNFRSF1A (intron 4 c.472+88 C>A) by examining reverse-phase protein microarray. In addition we describe clinical symptoms and response to anakinra and etanercept.
Objectives: To assess whether this subject with intronic change had laboratory and clinical features which would confirm a diagnosis of TRAPS.
Methods: Clinical history was documented along with clinical response to prednisolone, anakinra and etanercept. Intracellular signaling pathways that govern inflammation associated with TNFRSF1A signaling pathways were examined in peripheral blood mononuclear cells (PBMC) using reverse-phase protein microarray. Profiles were compared between the subject with intronic change, C33Y TRAPS subjects and matched healthy control.
Results: Clinical history confirmed some clinical features of TRAPS with fevers up to 40 degrees C from childhood and frequent episodes of myalgia, vomiting and abdominal pain lasting up to 4 weeks. On many occasions, however, symptoms were associated with normal CRP and SAA. Symptoms responded to oral prednisolone but no benefit occurred with anakinra. Subsequent treatment with etanercept resulted in less frequent and severe attacks.
Reverse phase protein microarray technology of signaling pathways has previously demonstrated that PBMC from patients with C33Y TRAPS show subtle upregulation of NF-kB, p38, MEK/ERK and JNK MAP kinase pathways, Phosphinositide 3 kinase, STAT3, JAK2/c-Src, GSK-3beta and transcription factors including ATF, Elk and Jun. The microarray from subject with intronic change did not show any significant variations compared to healthy control and did not resemble a C33Y TRAPS profile. However some proteins such as pERK1/2, pP38 and TRAF6 were downregulated in subject intron compared to control.
Conclusion: This subject with intronic change lacks some typical clinical features of TRAPS such as lack of acute phase response and responsiveness to anakinra. In addition protein array profile did not resemble that of C33Y TRAPS patient. This subject does however have a chronic recurrent febrile illness associated with TNFRSF1A intronic change and improvement on etanercept, suggesting other mechanisms could be implicated.
Disclosure of interest: None declared.

A268

OR14-001 – Tocilizumab in autoinflammation and AA amyloidosis

T Lane*, JD Gillmore, AD Wechalekar, NM Stewart, JA Gilbertson, S Sachchithanantham, DM Rowczenio, S Banypersad, JH Pinney, S Mahmood, HJ Lachmann, PN Hawkins
National Amyloidosis Centre, University College London, London, UK
Pediatric Rheumatology 2013, **11(Suppl 1):**A268

Introduction: The value of IL-6 blockade has been established in rheumatoid arthritis (RA) and systemic onset juvenile idiopathic arthritis

(SOJIA), but its wider utility in patients with AA amyloidosis and autoinflammatory diseases has been little studied.
Objectives: To assess clinical and serological responses to tocilizumab therapy in adult patients with AA amyloidosis and various autoinflammatory disorders.
Methods: 16 patients at the UK National Amyloidosis Centre with AA amyloidosis and various autoinflammatory disorders that were refractory to various treatments underwent therapeutic trials of tocilizumab. Disease activity and treatment response were monitored by symptoms, serial SAA and CRP measurements and a comprehensive range of standard blood and urine analyses. Amyloid load was evaluated and monitored by SAP scintigraphy.
Results: 13 (81%) patients had AA amyloidosis (7 RA, 3 JIA, 1 hyper IgD and periodic fever syndrome [HIDS], 1 presumed Castleman's disease, and 1 unclassified autoinflammatory disorder), and 3 had severe, longstanding refractory autoinflammatory disorders (1 HIDS and 2 unclassified autoinflammatory disorders). 10 (63%) patients were male. Median age at presentation to our clinic was 48 years (inter-quartile range, IQR, 23-52). All patients had received at least one previous unsuccessful treatment with anti-cytokine or other disease modifying anti-rheumatic therapies prior to receiving tocilizumab.
Median SAA concentration prior to tocilizumab treatment, calculated as the median of all SAA measurements for each individual in the 12 months preceding initiation of therapy, ranged from 10 – 414mg/L; serum SAA concentration following introduction of tocilizumab was a median of 3mg/L for the entire cohort (IQR 3 – 6), i.e. indicating complete normalisation of SAA (Mann Whitney test $p<0.0001$). All patients reported improvement in symptoms. During median follow-up of 21 months (IQR 11–43), SAA values remained normal/near normal in 14 patients; median SAA concentration was 11mg/L and 18mg/L in the two remaining patients, representing substantial improvement from pre-treatment medians of 58mg/L and 198mg/L respectively. 8 patients, all of whom responded completely to tocilizumab, have had follow-up SAP scans, demonstrating regression of amyloid in 6 (75%) and stable deposits in 2 cases.
Conclusion: Treatment with tocilizumab has been successful in producing sustained suppression of refractory inflammatory disease, both serologically and symptomatically, in 14 of 16 (88%) of patients treated under our care, with accompanying regression of amyloid deposits in 6 (75%) of these.
Disclosure of interest: None declared.

A269

OR14-002 - ANTI IL-1 therapies and pregnancy outcome

HJ Lachmann[1*], H Ozdogan[2], A Simon[3], N Stewart[1], DM Rowczenio[1], S Ugurlu[2], T Lane[1], PN Hawkins[1]
[1]National Amyloidosis Centre, University College London Medical School, London, UK; [2]Department of Rheumatology, Cerrahpasa Medical School, Istanbul, Turkey; [3]General Internal Medicine, Radboud University , Nijmegen, Netherlands
Pediatric Rheumatology 2013, **11(Suppl 1):**A269

Introduction: Young women with autoinflammatory diseases on long term IL-1 blockade are increasingly asking about the feasibility, safety and outcomes of pregnancy but few data are available. The FDA classes anakinra as pregnancy risk grade B and rilonacept and canakinumab as grade C. The manufacturers advise that there are no data on the outcome of pregnancy or on excretion into breast milk and that anakinra and canakinumab should only be taken if the benefits outweigh risk; for rilonacept the advice is to avoid in pregnancy. The literature contains only 3 reported cases of use of anakinra in pregnancy – all in adult onset Stills disease (AOSD) and with successful outcomes.
Objectives: To assess pregnancy outcomes in women who had received anti IL-1 therapies in pregnancy.
Methods: We indentified women who have been exposed to anti IL-1 agents in completed or planned to complete pregnancies under our care. Data were collected on medication, pregnancy outcome, breast feeding and development.
Results: 7 cases were identified; 5 completed, 1 first and 1 second trimester pregnancies. The underlying diseases were: 3 CAPS, 1 TRAPS, 1 FMF, 1 idiopathic pericarditis and 1 AOSD. 3 completed pregnancies (CAPS, TRAPS, pericarditis) were on anakinra from preconception throughout the pregnancy, the 2 current pregnancies (CAPS) were on canakinumab pre conception, 1 switched to anakinra 8 weeks pre conception, the other stopped canakinumab 8 weeks after conception and currently (12 weeks later) is on no treatment. The patient with FMF also had multiple sclerosis with presumed prolonged febrile myalgia refractory to colchicine for which she received 12 weeks of anakinra from 22 weeks. The AOSD patient received anakinra and prednisolone from 22 to 33 weeks. Median maternal age was 30 years (25-38), all were first pregnancies. The 5 completed pregnancies resulted in 5 healthy boys; median gestation 38 weeks (35 to 41). One baby was delivered by caesarean section at 36 weeks for vaginal bleeding (FMF), the others were vaginal deliveries (2 induced); median birth weight 2.59 kg (2.02-3.94), 1 minute APGAR score was 8 in 1 case and 9 in the rest. All were normal on neonatal checks, one had evidence of unilateral reduced hearing at 6 weeks. One was breast fed, the others bottle fed. Follow up data is available on 3 beyond 6 months and their development remains normal.
Conclusion: These 5 successful pregnancies more than double the number of known outcomes in anakinra treated mothers and provide reassurance to physicians caring for young women. Nonetheless the numbers remain very small and each pregnancy should be assessed and the risks and benefits of continued therapy individually discussed with the potential parents.
Disclosure of interest: None declared.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Lachmann *et al.*: OR14-002 - ANTI IL-1 therapies and pregnancy outcome. *Pediatric Rheumatology* 2013, 11(Suppl 1):A269