oral communication

**Amyloidosis**

(abstract 148)

**Expression of ASC in renal tissues of FMF patients with amyloidosis; postulating a role for ASC in AA type amyloid deposition**


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Familial Mediterranean fever is characterized by recurrent attacks of fever and serositis. The most serious complication is deposition of AA type amyloid, mainly in the kidneys. Treatment with colchicine reduces the frequency and severity of FMF attacks and prevents amyloidosis, though the mechanism behind these effects is unknown. The FMF gene, MEFV, encodes pyrin which interacts with several proteins, including ASC, a key molecule in both apoptotic and inflammatory processes. ASC forms aggregates called specks in cells. Given the ability of pyrin to modulate ASC speck formation and subsequent apoptosis, and the known beneficial effects of colchicine in preventing FMF attacks, the initial aim of this study was to determine the effect of FMF-causing mutations in pyrin and the impact of nocodazole (a microtubule toxin with effects similar to those of colchicine) on the process of speck formation. In this study we demonstrate that specks are generally cytosolic, and are most often located near the microtubule organizing center of cells. Furthermore, administration of nocodazole prevents speck formation. In the course of these experiments, we occasionally noted specks that appeared to be outside of cells. In addition, some dying cells appeared to extrude a speck. We further investigate the expression pattern of ASC in the kidney, and find that ASC is expressed in renal glomeruli of FMF patients but not control patients. To investigate whether ASC expression was correlated with amyloid deposition, renal biopsies from FMF patients were analyzed after congo red staining. Sequential sections were stained to determine the localization of ASC. There was a 100% correlation between ASC expression and the presence of amyloid deposits. On the basis of these data, we hypothesize that an inflammatory spectrum unique to FMF patients results in ASC expression in renal glomeruli. High local ASC expression may result in speck formation in these glomerular cells, a process that will lead to apoptosis of these cells. If specks from dying cells survive in the extracellular space, as they clearly do in cultured cells, it is possible that such extracellular specks (perhaps in association with pyrin) could act as nucleating centers for amyloid deposition. The fact that speck formation requires an intact microtubule network as shown here could potentially account for the ability of prophylactic colchicine administration to prevent and even reverse amyloidosis in FMF patients.

(abstract 204)
Tumor Necrosis Factor alpha Antagonists in the Treatment of Secondary Amyloidosis

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OBJECTIVE: To evaluate the efficacy and safety of TNFα antagonists in secondary amyloidosis. METHODS: 26 patients with secondary amyloidosis were treated with TNF antagonists between April 2001 and December 2007. Among these, 22 patients (13 men, 9 women, mean age 36.1 ± 12.3 years) who were treated with TNF antagonists for at least 6 months were surveyed. Patient charts were surveyed for disease and treatment duration, concomitant medications, serum creatinine and proteinuria levels during treatment and adverse events. RESULTS: Primary diagnoses were JIA in 6 patients, FMF in 1 patient, FMF and JIA in 1 patient, AS in 6 patients, RA in 4 patients, Crohn’s disease in 2 patients, Behçet’s syndrome in 1 patient and adult onset Still’s disease in 1 patient. 9 were prescribed infliximab, 5 etanercept, 6 patients used initially infliximab and later etanercept and 2 patients received all 3 TNF antagonists successively. Concomitant medications were corticosteroids in 15 patients, DMARDs in 14, colchicine in 9, and eprodisate in 1 patient. Mean duration of treatment with TNF antagonists was 16.6±11.9 (6-41) months. Mean creatinine level increased from 1.1±0.6 to 1.4±1.5 mg/dl (p=0.35) and proteinuria decreased from 2.9±3.6 g/day to 1.6±1.8 g/day (p=0.03). Serum creatinine level increased in 2/22 patients, was stable in 11/22 decreased in 9/22. 20/22 patients had proteinuria when TNF antagonists were started and in 6 patients the proteinuria was in the nephrotic range. Proteinuria disappeared in 7/20, decreased in 8/20, remained stable in 3/20 and increased in 2/20 patients. Dialysis was started in 3 patients. 6/26 patients died. Causes of death were definite sepsis in 1, probable sepsis in 3, massive bleeding from bladder in 1 patient 6 months after she stopped infliximab, and bleeding following fondaparinux use after hip prosthesis surgery in 1 patient, 26 months after she stopped infliximab. Other serious adverse events were retinal vein thrombosis, urinary tract infection with vancomycin resistant enterococci, pneumonia, gluteal abscess, a psoriasis-like eruption, anaphylactic reaction, deep vein thrombosis and popliteal artery occlusion. CONCLUSION: Treatment with TNF antagonists over one year seems to reduce proteinuria and stabilize serum creatinine levels. However caution is needed due to a high number of deaths and other serious adverse events. Whether these are related to the disease or to the use of TNF antagonists remains to be studied.

Expanding spectrum of autoinflammatory diseases and new diseases

(abstract 142)

Longer form of CCTG microsatellite repeat in the promoter of the CD2BP1/PSTPIP1 gene is associated with aseptic abscesses and with Crohn disease in French patients


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Aseptic abscesses syndrome (AA) is an inflammatory disease in which non-infectious deep abscesses develop. These are mainly located in the abdomen and in particular in the spleen. AA is often associated with fever and a raised leukocyte count, and respond quickly to corticosteroids. AA is associated with Crohn disease (CD) in 57% of cases and with neutrophilic dermatosis (ND) in 20% of cases. Pyoderma gangrenosum is usually a sporadic ND. An hereditary autosomal dominant syndromic kind of pyoderma gangrenosum, PAPA (Pyogenic Arthritis Pyoderma gangrenosum Acne) syndrome, is linked to mutations in the CD2BP1/PSTPIP1 gene. Some patients with PAPA syndrome have sterile abscesses at sites of parenteral injections. We hypothesized that PSTPIP1 might be involved in the development of AA. We systematically screened the exons and their flanking sequences of the PSTPIP1 gene in AA patients to examine whether sequence variants of the PSTPIP1 gene play a pathophysiological role on AA. No single point mutation was identified, but numerous single nucleotide variants, a novel 3 bps deletion in the 3’-downstream region and one microsatellite in the 5’-upstream region were found. We demonstrated that this microsatellite motif is located in the promoter of PSTPIP1 gene and has 3 alleles. Among AA patients, the longer allele (CCTG)7 was significantly more frequent than among French controls (p=0.0154). We further compared the distribution of these alleles in French patients with CD and French controls and found also an association of (CCTG)7 allele with CD (p=0.0351). This association was not found in the Indian population. These results demonstrate that the CCTG repeat in the PSTPIP1 promoter may play a role in the pathogenesis of AA and of CD. Further investigations are required to demonstrate the possible modulation of gene expression by the (CCTG)n motif and the 3’-downstream deletion.

A Mutation in Exon 8 of the Cherubism Gene is Associated with a Novel Autoinflammatory Phenotype that Affects the Skin and the Bone

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Chronic recurrent multifocal osteomyelitis (CRMO) and cherubism are autoinflammatory disorders that affect the bone. CRMO is characterized by recurrent episodes of sterile osteomyelitis, often accompanied by psoriasis or inflammatory bowel disease. Mutations in LPIN2 and pstpip2 have been shown to cause chronic multifocal osteomyelitis in a human and murine model, respectively. Cherubism is a rare autosomal dominant disorder due to mutations in the SH3 binding protein 2 gene (SH3BP2). Cherubism presents with jaw enlargement that begins shortly after the primary teeth begin to erupt. Once all permanent teeth are present, the process often remits. There is no reported skin phenotype in cherubism. We identified a family with a unique autoinflammatory phenotype including sterile, local pyogenic reaction after immunization, childhood onset nodular inflammatory rash, and, in one, recurrent sterile osteomyelitis of the mandible; a phenotype distinct from both CRMO and cherubism. The skin and bone lesions were not responsive to antibiotics but improved with daily or every other day oral corticosteroids and bi-weekly interferon-alpha injections. After informed consent, we obtained DNA from all members of the kindred. The pedigree suggests autosomal dominant inheritance with incomplete penetrance. There were 3 affected individuals including the proband, his brother and his daughter; his parents and his other daughter are unaffected. Given the small size of the pedigree, we utilized a candidate gene approach to try to identify the causative gene. No mutations in the coding regions or splice sites of PSTPIP1, PSTPIP2 or LPIN2 were found. Then, because of the jaw involvement in one of the individuals, the cherubism gene, SH3BP2, was sequenced. A heterozygous SNP was identified in exon 8 (c.1274A>C), which changes a histidine to a proline at amino acid 338 (H338P) and is present in all
Mutations in NALP12 cause periodic fever syndromes


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NALP proteins, also known as NLRPs, belong to the CATERPILLER protein family involved, like Toll-like receptors, in the recognition of microbial molecules and subsequent activation of inflammatory and immune responses. Current advances in the function of NALPs support the recently proposed model of a disease continuum bridging autoimmune and autoinflammatory disorders. Among them, hereditary periodic fevers (HPFs) are Mendelian disorders associated with sequence variations in very few genes; these variations are mostly missense mutations whose deleterious effect, which is particularly difficult to assess, is often questionable. The growing number of identified sporadic cases of periodic fever syndrome, together with the lack of discriminatory clinical criteria, has greatly hampered the identification of new disease-causing genes, a step that is however essential for appropriate management of these disorders. Using a candidate gene approach, we identified non-ambiguous mutations in NALP12 (i.e., nonsense and splice site) in two families with periodic fever syndromes. As shown by means of functional studies, these two NALP12 mutations have deleterious effect on NF-kB signaling. Overall, these data identify a novel group of HPFs defined by molecular defects in NALP12, opening up new ways for management of these disorders. The identification of these first NALP12 mutations in patients with autoinflammatory disorder also clearly demonstrates the crucial role of NALP12 in inflammatory signaling pathways, thereby assigning a precise function to this particular member of an emerging family of proteins whose putative biological properties are currently inferred essentially through in vitro means.
There is a disease reported the first by Nakajo in 1939 and next by Nishimura et al in 1950 (both reports were written in Japanese), and further by Kitano et al in 1975 as “a syndrome with nodular erythema, elongated and thickened fingers, and emaciation” in the Archives of Dermatology. This disease is very rare and only 20 cases has been reported so far. This disease has only been reported from Japan, however, the same disease can be discovered in databases as the name "Nakajo syndrome" (MIM#256040, ORPHA#1953) or "Nakajo-Nishimura syndrome" (ORPHA#2615). This disease seems to be inherited in an autosomal recessive manner, because of frequent parental consanguinity and appearance in sibships. It begins with pernio-like eruptions in early-infancy and gets worse in winter. The patients periodically develop high fever and nodular erythemas over entire body. Lipomusculoatrophy, without significant power loss, and joint contracture gradually progress mainly in upper part of the body to cause the characteristic facial appearance with big eyes and elongated clubbed fingers. Inflammatory changes, such as elevated ESR, high serum CRP, amyloid A protein and gamma-globulinemia are observed without appearance of distinct autoantibody. Skin biopsy shows infiltration of inflammatory cells in the whole dermis and subcutaneous tissue, as well as vasculitis. Abnormal findings on electrocardiogram increase with age and some cases suddenly died. Notably, most cases are concentrated in the distinct Kansai and Tohoku areas of Japan, including Wakayama prefecture. Here we report the summary of 8 cases, which we have experienced in our Department, and show the detailed clinical course of 5 cases of 3 families, 4 of which were born in consanguineous parents. Based on our observations, we propose to designate the disease as “familial Japanese fever”, in clear contrast with familial Mediterranean fever, as a new entity of hereditary autoinflammatory syndrome with periodic fever restricted in Japan. As the genetic cause of this disease, LPIN gene can be a candidate, considering that a LPIN mutation is responsible for fatty liver dystrophy (fld) mice and that the homologous LPIN2 is the responsible gene for a rare autoinflammatory disease, Majeed syndrome. Finally, we also report the result of mutational analysis of LPIN gene in two of our patients.

(abstract 198)

**Clinical phenotype and CARD15 gene mutation with Blau Syndrome in Chinese children and their parents**

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Background: Granulomatous arthritis of childhood designates a chronic inflammatory disease characterized by granulomatous polyarthritis, uveitis and rash with a typical onset before 5 years. It can present in a familial form, called Blau Syndrome (BS), and a sporadic from, known as early onset sarcoidosis. The prevalence is unknown, but it is a rare auto-inflammatory disease.

Objectives: We summarized the article to find the clinical features of our patients and analyze CARD15 gene mutation of the patients and their parents. Design, setting and method: Retrospective review of cases of Blau Syndrome in Beijing Children’s Hospital from the year of 2006 to 2007. Results: Eight cases Blau Syndrome patients were diagnosed. There were 5 boys and 3 girls. The age was from 1.5 to 13 years, the onset age was from 1 month to 5 years. The disease course before diagnosed as Blau syndrome was 1 year to 11 years. Three of them were misdiagnosed as JIA and Takayasu's arteritis respectively. One case had family history, no family history in others. All patients has had typical rash, it was the first symptom in all patients. The second clinical feature was joints problem. All the patients had polyarticular symmetrical cyst-like synovitis in large and
small joints. Bilateral pan-uveitis was in all the patients. Additional, two patients had hearing lose, four patients had Takayasu's arteritis with hypertension, and two of them had renal artery stenosis with severe hypertension and aortitis. Histologically, there was synovial and dermis proliferation with non-caseating giant cell granulomas in all of the patients. Mild anemia, elevated sedimentation rate were in all the patients. Chest radiographs were normal in all. We analyzed 6 patients and their parents’ NOD2/CARD15 gene. We have found six mutations in them. R334W and R334Q were reported previously abroad, E383D, R471C and R587C are new mutations, 1761T/G is also a new mutation but a same sense mutation. In addition, the parents of a patient also have mutations and his father with the disease. In the treatment, all of them received NSAIDS, steroid treatment, one of them also with TNF blockers. All of them were efficiency. Conclusions: Blau syndrome is a rare auto-inflammatory disease. We diagnosed 8 patients in Chinese Children. That indicate Blau syndrome also can involve Chinese population.

(abstract 137)

**Systemic and tissutal IL-18 expression in adult onset Still’s disease**


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High serum concentration of IL-18 is described in adult onset Still’s disease (AOSD) although its tissue origin is unknown. We evaluated the expression of IL-18 in two lymph nodes (LN) of AOSD and in a liver biopsy obtained by a an AOSD patient with persistent increase in transaminases. As control we examined non-specific lymphadenitis (NSL), normal LN and a liver biopsy obtained from a control patient (pt). Sequential sections were stained using a monoclonal antibodies (abs) against IL-18 (IgG1 clone 2D3B6, MD Biosciences, CH) and also against CD68 (clone PG-M1; DAKO A/S, Cambridge, UK clone KP1 Dako, DK) to identify the presence of macrophages. Moreover, we collected serum samples from 9 consecutive cases of AOSD for the measurement of IL-18 levels before therapy. As controls, we used sera from 70 pts with other connective tissue diseases (CTD) and 21 healthy subjects. Higher expression of IL-18 was observed in AOSD LNs compared to controls. IL-18 was particularly over-expressed in hyperplastic, dysmorphic germinal centres. IL-18 co-localized almost exclusively with CD68 positive cells, indicative of a monocyte-macrophage lineage origin in LN. AOSD liver biopsy showed moderate infiltrate of lympho/monocytes, discrete hepatocyte apoptosis and moderate microvacuolar steatosis with a prominent infiltrates of CD68+ macrophages in particular in the periportal area. IL-18 strong expression was detected within the AOSD liver parenchyma, and not in the control liver, by Kupfer cells, hepatocytes and by infiltrating cells characterized by wide cytoplasms and large nuclei detected in the CD68+ areas. Double staining performed with anti IL-18 and anti CD68 abs clearly showed co-localization of IL-18 with the macrophage marker. Serum levels of IL-18 were significantly increased in AOSD pts compared with levels detected in patients affected by CTD and healthy subjects. We here describe for the first time over-expression of IL-18 in two cases of AOSD LNs and in a case of AOSD-associated hepatitis compared to NSL, normal LN and liver control. The increased systemic production of IL-18 may reflect the high local expression of this cytokine in the LN as an important site for such activation. This possibility is supported by our demonstration that IL-18 co-localized with CD68, a marker of monocyte-macrophage lineage cells which are known to be over-activated in AOSD. IL-18 might be locally involved in the generation of the
Mutated, structurally altered caspase-1 with decreased enzymatic and increased RIP2 mediated inflammatory activity leads to a new type of periodic fever (ICE fever)


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Caspase-1 (interleukin-1 beta converting enzyme, ICE) is a proinflammatory caspase that plays a pivotal role in many inflammatory disorders such as infections, tissue damage, autoimmune and autoinflammatory diseases including different types of periodic fevers. Inflammation is mediated by enzymatic activation of interleukins especially IL-1 beta, but also via CARD/CARD interactions between the CARD domains of pro-caspase-1 and RIP2 that engage RIP2 to activate NFkappa-B and MAPK pathways. We found CASP1 mutations in five patients with recurrent severe febrile episodes accompanied by arthralgia, exanthema, and strongly enhanced parameters of systemic inflammation. These mutations (R240Q/R240Q, one male patient; N263S/wt, two unrelated female patients; L265S/wt, two related female patients) destabilized the caspase-1 tetramer interface bonding as shown by crystal structure analysis. In accordance with these structural data the recombinant N263S-caspase-1 and R240Q-caspase-1 showed reduced enzymatic activity that further decreased considerably with rising temperature, whereas activity of the wildtype form increased at higher temperatures. The recombinant L265S-caspase-1 did not form tetramers at all. Hence, all mutations detected led to a decreased enzymatic activity and increased availability of unprocessed pro-caspase-1. Unexpectedly, in spite of decreased enzymatic activity and decreased IL-1 beta production, the mutations led to enhanced proinflammatory signaling via RIP2 mediated increased activation of NFkappa-B and MAPK p38 phosphorylation in a transfection model. All mutated forms of pro-caspase-1, including an artificial mutation at the active site (C285A), activated NFkappa-B significantly stronger than the wildtype. NFkappa B activation induced by overexpression of RIP2 could be abolished by co-transfection with wildtype pro-caspase-1 but not by the mutated forms. In this context, we show for the first time a RIP2 down-regulating activity of wildtype pro-caspase-1 by enzymatic cleavage of RIP2 that is lacking or significantly diminished in the mutant forms thus leading to the enhanced NFkappa-B activation via RIP2. Enhanced NFkappa-B activity was also found in non-transfected macrophages that had only been treated with YVAD-CHO, a caspase-1 and pro-caspase-1 inhibitor, indicating that our findings were not restricted to an artificial transfection model. In summary, our results suggested that the patients’ CASP1 mutations induced or substantially contributed to the severe febrile episodes defining a new type of periodic fever (ICE fever).

Familial Mediterranean Fever

(abstract 121)

Novel insights into the inheritance and diagnosis of familial Mediterranean fever (FMF)

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Since the genetic testing of MEFV began, it has been observed that a substantial number of FMF patients possess only one demonstrable mutation, and recent reports have also raised the question of possible dominant inheritance. These single-variant patients often have a typical disease history and respond well to colchicine. One explanation for this observation may be a lack of sensitivity in screening techniques. We report an extensive search for a second MEFV mutation in 41 patients clinically diagnosed with FMF and carrying only one structural MEFV mutation. Utilizing standard capillary electrophoresis sequencing, this cohort of patients was screened for a second disease-associated mutation in 10 exons of MEFV and a second mutation was not identified. A subset of 10 patients was then sequenced for the entire 15kb MEFV genomic region using a hybridization-based chip technology (Callida Genomics, CA). We identified 44 SNPs, including 22 novel SNPs, predominantly in introns 1, 2, 3, 4, and 6. Each patient was heterozygous for at least one of many SNPs in the MEFV genomic region, arguing against the presence of large genomic deletions. Haplotype analysis of patients with positive family history did not identify the common haplotype that should be associated with the transmission of the second FMF allele. Allelic expression using total PBMC-RNA, showed that both MEFV transcripts were expressed. qRT-PCR was performed to compare the expression levels of the group of single-variant patients with patients possessing two FMF-associated mutations and we did not observe a significant difference between single and double variant patient groups. We considered the possibility of a digenic model of inheritance by examining candidate genes encoding proteins known to interact with pyrin. Five proteins were chosen for genetic analysis in 10 patients: ASC, SIVA, PSTPIP1, POP1, and POP2. Two novel variants were identified in ASC and SIVA, but the SIVA substitution was established to be a polymorphism in a panel of Ashkenazi Jewish controls. The ASC variant had a frequency of 0.004 in a panel of Caucasian and Ashkenazi Jewish controls and awaits further characterization. Our data underscore the existence of a significant subset of FMF patients who are carriers for only one mutation in the MEFV gene. Thus, FMF may not be a simple monogenic inflammatory disease and FMF phenotype may occur in patients with only one MEFV mutation in the presence of other permissive alleles.

(abstract 144)

Can Toll-like receptor 2 polymorphism affect the phenotype of heterozygous?


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FMF is the most common of the monogenic autoinflammatory diseases caused by mutations in the MEFV gene. Although FMF is an autosomal recessive disease patients with one mutation do sometimes display the phenotype. Unidentified MEFV mutations or other genetic factors are thought to be involved. Toll like receptor 2 plays a major role in innate immune activation through recognition of microbes. The aim of this study was to search whether Arg753Gln TLR 2 polymorphism had an impact on the development of the clinical manifestations of FMF in the children heterozygous for MEFV mutations. We studied 24 children diagnosed clinically as FMF with mutation in one of their alleles. 116 healthy controls were studied as the control group. The TLR 2 Arg753Gln polymorphisms were analyzed with PCR-RFLP based method. There was a significant difference between the frequencies of TLR-2 Arg753Gln polymorphism in healthy controls (6%) and heterozygotes (25%) (p=0.01). We suggest that this polymorphism may be one of the factors affecting the phenotypic appearance in heterozygote patients in areas where there is a high infection rate. These results need to be confirmed after these patients are tested for mutations in other autoinflammatory genes.
Differences In The Severity Of The Phenotype Of Children and Adolescents With Familial Mediterranean Fever Residing In Turkey and Germany


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Familial Mediterranean Fever (FMF) is worldwide the most common autoinflammatory disease. It has been long known that environmental factors affect the phenotype since patients in the United States are not expected to develop the complication of secondary amyloidosis. To substantiate this hypothesis we compared the disease-severity in Turkish FMF patients living in Turkey and Germany, based on a modified score for children. A total of 53 Turkish children living in Turkey were compared to 45 Turkish children born and raised in Germany. All were under the age of 18 years, mean age among the group from Turkey and Germany was 42.2 (range 2-120 months) and 44.29 (range 3-178 months) months, respectively. No sex difference was present between the two groups. M694V was the leading mutation in both groups. There was no significant difference between the last visit mean CRP and ESR levels of the group from Turkey (mean CRP 0.83 mg/dl, mean ESR 16.9 mm/hr) and Germany (mean CRP 0.5 mg/dl, and mean ESR 16.2 mm/hr). The score developed by Livneh et al was modified by the integration of the recommended age-related doses, previously published by us. Additionally, disease severity was determined by the use of the scoring system developed by Pras et al. There was no correlation between the disease severity defined by the different scoring systems and the acute phase reactants. According to the modified Livneh score, 78.2 % of patients from the group living in Turkey had a severe course compared to 34.1% from the group living in Germany. Pras scores were also higher in the patients born and grown up in Turkey (34.5%) compared to patients living in Germany (15.4%). The difference between the two groups for both scoring systems were statistically significant (p< 0.05 for both). We suggest that environmental factors may affect the severity of FMF even if they were coming from the same ancestors.

Incidence of MEFV exon 10 and exon 2 polymorphisms in multiple Asian, European and African countries and a novel test for geographically restricted positive selection pressure


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To address the paucity of data on the incidence of mutations in exon 10 of MEFV and the E148Q exon 2 polymorphism in different regional populations, as well as the geographic distribution of these polymorphisms, we sequenced exon 10 and genotyped the E148Q polymorphism in 26 groups from 21 countries in Europe, North America, Asia and Africa (n = 3,237). The dataset comprised samples from Armenia, the Armenian Diaspora (U. K. and U.S.A.), Azerbaijan, Cameroon, Ethiopia (five ethnic groups: Afar, Annuak, Amhara, Maale and Oromo), China, Georgia, Greece,
Iran, Italy, Malawi, Mongolia, Morocco (Berber), Mozambique, Papua New Guinea, Sudan, Sweden (Saami), Syria, Turkey, Ukraine and United Kingdom. We report that the exon 10 mutation resulting in the amino acid change A744S associated with Familial Mediterranean Fever was observed in four of the five Ethiopian groups and was greater than 6% in the Maale. We then developed a permuted odds ratio test that compared the frequencies of synonymous and non synonymous mutations with the frequencies of un-mutated sites to detect evidence of positive selection acting on exon 10 in the Armenian region but not in other geographic areas. The results of the geographic survey and test for selection are presented.

(abstract 199)

**Dissecting Inflammatory and Chemotactic Pathways in Familial Mediterranean Fever**


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Background: Familial Mediterranean fever (FMF) is a heritable autoinflammatory disease characterized by substantial neutrophil influx at sites of serosal and synovial involvement. Inflammatory attacks are usually prevented by prophylaxis with colchicine, a microtubule inhibitor. Pyrin, the FMF protein, colocalizes with microtubules and may impact leukocyte cytoskeletal functions such as adhesion and migration. We therefore sought to further investigate leukocyte migration in FMF. Methods: Peripheral blood samples were obtained from 25 treated patients, 10 untreated patients, and 16 controls. Subsets of patients were studied in several assays. Chemokines produced from cultured supernatants and from serum samples were evaluated with the Luminex immunoassay. Chemotaxis assays included a transwell system and a live imaging protocol with granulocytes stimulated to various chemoattractants, including MIP-1 alpha, IL-8 and fMLP. Analysis of gene expression sequences with the Affymetrix system was utilized in 8-paired patient samples who underwent colchicine withdrawal. Results: Microarray analysis revealed a “chemotactic signature” induced by colchicine withdrawal with upregulation of major chemotactic genes including CRK, BCL6, ADAMTS 10, and downregulation of genes involving chemokine receptors and cell adhesion molecules such as CCR 7 and CXCR3. At 72 hours off colchicine, monocytes from FMF patients stimulated with LPS produced more TNF-alpha and IL-12p70. Moreover, neutrophil activation was increased, as seen with the downregulation of the L-selectin marker, CD 62L. Patients off colchicine demonstrated significant hyperresponsive cell movement compared with treated patients and controls, although such movement was found to be random (chemokinetic) and less directed (chemotactic) when visualized. FMF patients on colchicine showed elevated serum levels of the chemokines, MIP-1 alpha and beta, as compared to controls. Conclusion: A cascade of intracellular events involving chemokine signaling can impact leukocyte migration in FMF. Current studies are focused on determining the extent to which these effects represent the underlying pathophysiology of FMF, independent of colchicine, by studying colchicine naïve patients.

(abstract 135)

**Validation of a diagnostic score for molecular analysis of hereditary autoinflammatory syndromes in children with periodic fever.**

Objective: Among children with periodic fever of unknown origin, only 20-25% are affected by one of the known hereditary autoinflammatory diseases, namely familial Mediterranean fever (FMF), TNF receptor associated periodic syndrome (TRAPS), and mevalonate kinase deficiency (MKD). Aim of the study was to validate a set of clinical parameters able to predict gene mutations in hereditary autoinflammatory diseases associated to periodic fever. Patients & Methods: 234 consecutive patients with a clinical history of periodic fever were screened for mutations of MVK, TNFRSF1A and MEFV genes and detailed clinical information was collected. A Diagnostic score was formulated on the basis of a univariate and multivariate analysis in genetically positive and negative patients (training set). The Diagnostic score was validated using an independent set of 76 patients (validation set). Results: Age at onset (OR=0.94, p=0.003), positive familiar history (OR=4.1, p=0.039), thoracic (OR=4.6, p=0.05) and abdominal pain (OR=33.1, p<0.001), diarrhea (OR=3.3, p=0.028) and oral aphthosis (OR=0.2, p=0.007) were the variables independently correlated to the positivity at the genetic testing. These variables were combined in a linear score whose ability to predict the risk of positive results at the genetic testing was validated on an independent dataset. The Diagnostic score in the validation set revealed high sensitivity (82%) and specificity (72%) in discriminating positive and negative patients. A regression tree analysis was able to provide, for patients with a high risk to be positive at the genetic test, the most reasonable order of the genes to be screened. Conclusions: The proposed approach in patients with periodic fever will increase the risk in obtaining a positive results in genetic testing with good specificity and sensitivity. Our results further suggest the order of the genes to be screened. To the work has also contributed the Italian group of Autoinflammatory diseases consisting of A. Meini (University of Brescia), F. Zulian (University of Padua), L. Obici (University of Pavia), L. Breda (University of Chieti), S. Martino (University of Turin) and A. Tommasini (University of Trieste).

(abstract 201)

The MEFV Gene 3’-UTR Alu Repeat Polymorphisms in Patients with Familial Mediterranean Fever


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Objective. The MEFV gene mutations can be detected in the majority of FMF patients, but there is an important proportion of patients with FMF phenotype who carry a single or no coding region mutation. This study aimed to investigate the promoter region and 3’-UTR polymorphisms of the MEFV gene in a group of FMF patients with no coding region mutations to identify variations with a possible role in the regulation of MEFV expression. Methods. The study group consisted of 289 patients with FMF and 103 ethnically-matched healthy individuals of Turkish origin. All
individuals were first genotyped for five most commonly observed mutations (M694V, M680I, V726A, E148Q and M694I). Then, the coding regions of the MEFV gene in patients carrying none of the 5 mutations were amplified and screened by using single-stranded conformation polymorphism and DNA sequencing. After the exclusion of patients with mutations in exons, the promoter and 3'-UTR regions of the MEFV gene were investigated in the remainders. For the haplotype analysis, all study group genoyped for two of the 3'-UTR single nucleotide polymorphisms (SNP). Results. Genotyping for five mutations revealed 186 patients (64.4%) with two mutations, 61 patients (21.1%) with one mutation, and 42 patients (14.5%) with no mutation. The carrier rate for healthy controls was found to be 10%. After the screening of the all 10 exons in patients with none of the 5 mutations, we identified 36 patients (12.5%) as having no coding region mutations. Analysis of the 3'-UTR region showed two Alu repeats (AluSx and AluSq), which were located in the 3'-UTR of the reference mRNA sequence. Sequencing of the 3'-UTR of the MEFV gene showed several SNPs, which were clustered in 2 haplotypes. With the genotyping of all study group for two of the 3'-UTR SNPs (rs2741918 and rs450021), we observed a significant increase in the frequency of heterozygotes for 3'-UTR haplotypes in FMF patients with no coding region polymorphisms compared to healthy controls (75% versus 48.5%, P = 0.006, OR = 3.2, 95% CI 1.4-7.4). Conclusion. This study showed a group of 3'-UTR polymorphisms in the MEFV gene, which are clustered in two haplotypes, and a genetic association was observed between the 3'-UTR polymorphisms and FMF patients with no coding region mutations. These findings may suggest a role for 3'-UTR sequences in the regulation of the MEFV gene expression.

(abstract 147)

S-100A12 as a sensitive marker for the detection of inflammation in children and adolescents with Familial Mediterranean Fever

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S100A12 is a member of the Damage-Associated Molecular Pattern molecules (DAMP). It is expressed by activated granulocytes and exhibits its proinflammatory capacity by binding to RAGE, a receptor found on endothelium and various cells of the immune system. Its role as marker for inflammation in Familial Mediterranean Fever (FMF) is unknown. In a cross-sectional study 51 children and adolescents with the clinical and/or genetical diagnosis of FMF were followed up over a period of 18 month (mean age 10.5 yrs [3.2 – 20.4]; 19 females, 32 males, total number of visits 192). Patients presented to the pediatric outpatient clinic of the Charité, Berlin. Patients were categorized into 4 groups: (1) pats. treated with colchicine not exhibiting any attacks (n=28); (2) pats. treated with colchicine exhibiting attacks at some time during the 18 months (n=20); (3) pats. with the genetical diagnosis of FMF never having any clinical signs and not being treated (n=5) and (4) pats. newly diagnosed having FMF (n=7). During the clinical visits CrP, ESR and serum amyloid A protein (SAA) were determined. Concentrations of S100A12 were analyzed using an ELISA system established in our laboratory. In active FMF S100A12 is highly increased, in some cases concentrations were > 25000 ng/ml (upper limit of healthy controls 120 ng/ml). Mean concentration in group 1 was 490 ng/ml ± 80, which was significantly lower as compared to all other groups (vs. group 1 p< 0.001; group 2 p< 0.001; group 3 p< 0.011). In group 2 mean concentration was 5380 ng/ml ± 1200 and in group 3 2540 ng/ml ± 1210 (no statistical difference). In group 4 mean concentration was 30000 ng/ml ± 18260, thus being statistically increased compared to the other groups (vs. group 1 p< 0.001; group 2 p=0.06; group 3 p=0.06). In general S100A12 correlated with CrP (r=0.47, p< 0.001) and ESR (r=0.29; p< 0.01). But when comparing
group 1 to group 3, S100A12 was the only increased inflammation marker (vs. group 1 p< 0.011).
High S100A12 concentration correlated with the occurrence of a homogygous M694V mutation. In conclusion, S100A12 is a sensitive surrogate marker for disease and inflammation monitoring in FMF. In asymptomatic subjects with two mutations within the MEFV gene it is more sensitive in the detection of subclinical inflammation compared to the standard inflammation markers. In untreated patients high concentrations of S100A12 can be helpful to differentiate FMF from other febrile conditions.

(abstract 170)

ROLE OF SMALL INTESTINAL BACTERIAL OVERGROWTH IN COLCHICINE NON-RESPONDERS


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Familial Mediterranean Fever is an autosomal recessive disease due to a genetic unbalance of innate immune response. The periodic attacks could derive from interplay of genetic and environmental factors. It was described that some infections as Helicobacter pylori can increase the severity and frequency of Familial Mediterranean Fever attacks. Small intestinal bacterial overgrowth could be associated with a releasing of antigens or metabolic productions. We hypothesized this condition could trigger Familial Mediterranean Fever attacks and consequently could lead to unresponsiveness to colchicine. Twenty Familial Mediterranean Fever non-responders, with a small intestinal bacterial overgrowth diagnosed by a H2 glucose breath test, were enrolled in our study. We evaluated laboratory and clinical features before and after three months from the eradication. A questionnaire regarding subjective severity of disease was administered. We demonstrated that the eradication of the small intestinal bacterial overgrowth leads to a significant improvement both of laboratory and clinical features. The production of bacterial antigens could induce Familial Mediterranean Fever attacks and consequently could simulate a colchicine unresponsiveness. We suggest that patients with unresponsiveness to colchicine treatment should be investigated for small bowel bacterial overgrowth by a fair and simple test as H2 glucose breath.

(abstract 222)

TRENDS IN COLCHICINE TREATMENT IN FAMILIAL MEDITERRANEAN FEVER (FMF)

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35 yrs after the colchicine (Co) introduction in FMF treatment, many issues, such as co-resistance (CoRs) or intolerance (CoI), still remain unresolved. To evaluate the current trends, we sent a questionnaire to clinical FMF centers worldwide. We received 24 completed questionnaires from 8 countries, covering a total of 4563 treated patients (pts), 1230 < 10 y.o. and 2473 >20 y.o. Interviewed physicians have had experience with Co for 15.5 yrs on average. 83% of them perform a diagnostic test with Co (43% regularly). In adults, 71% of them use 1 mg/d initial dosage, 86% 1-
1.5 mg/d maximum dosage, 57% 2 mg/d as the highest tolerated dosage. In children < 5 yrs of age, 93% of them use ≤0.5 mg/d initial dosage, 80% ≤1 mg/d maximum dosage and only 33% reached 1.5-2 mg/d; in children >5 yrs of age, 60% of them use ≤0.5 mg/d initial dosage, 93% ≤1 mg/d maximum dosage, 47% reached 2.5 mg/d. All the physicians monitor Co dose on the disappearance of attacks, 64% also on reduced acute phase reactants (APR). 76% think that protracted arthritis is not linked to Co, 53% leg pain. 73% perform a gradual increase of Co dose; 65% went over 2 mg/d mainly in adults. 95 % of them use ≥2 mg/d in Co resistsants, 75% use 1-1.5 mg/d in intolerants, 75% of them don’t increase Co before menses but 83% increase it in proteinuric pts. 85% keep the same dose in pregnancy; adverse effects with statins and macrolides were observed. Up to 33% of pts reduced and 1-10% stopped Co because of side effects (gastrointestinal 90%). 50% of the physicians suggest to test response and tolerance every 6 months: all ask for urinalysis, 87% WBC, 74% ESR and 69% liver tests and CRP, 39% muscle enzymes. Few cases of allergy were reported. 45.5% suggest lactose withdrawal. 83% consider diarrhea as CoI, 56% myopathy, 48% neuropathy. 91% define a CoRs on the basis of observation time, 83% on the basis of attack frequency, 56% of Co dose, 48% of abnormal APR during attack-free period. In terms of the criteria for CoRs, 94% of physicians refer to attacks, 55% to persistent high APR, only 16% to onset or worsening of renal disease and 11% to Co dose. 84% suggest diarrhea as sign of CoI, 35% neuropathy, 48% myopathy, leucopenia, only 16% refer to Co dose. Only 39% tried an alternative treatment (anti-TNF-a, IFN or thalidomide). This study shows a large variance in Co use for FMF worldwide and confirms the need of standardized definitions of Co resistance, intolerance and guidelines.

Clinical Disease among FMF Heterozygotes


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diagnosed with FMF we could show that the affected siblings inherited a different MEFV allele from the parent without the MEFV mutation. In the 20 patients with one mutation the most prominent features were fever and abdominal pain. Arthritis and ELE were rare and amyloidosis was not found in any of them. The heterozygous patients tend to have a relatively mild disease but can not be distinguished on a clinical basis from homozygotes. The findings presented above are highly consistent with the existence of a clinical phenotype among some FMF heterozygotes and could explain vertical transmission in some families. Although rare reports on disease occurring in the context of one mutation have been previously published, our data suggests that the extent of this phenomenon may be much more common than previously thought.

(abstract 124)

Altered Development and Function of CD11c+ Cells in Pyrin-Null Mice

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The familial Mediterranean fever (FMF) protein, pyrin, is encoded by the MEFV gene and expressed in myeloid/monocytic cells upon lipopolysaccharide (LPS) stimulation. Mutated pyrin causes FMF, a recessively inherited autoinflammatory syndrome, presenting in humans with recurrent episodes of fever, polyserositis and subsequent development of amyloidosis. Although pyrin is known to modulate inflammation and apoptosis, its overall function has not been completely elucidated. To better understand the physiologic role of pyrin in vivo, we have successfully generated pyrin-null mice (pyrin -/-) by disrupting exons 1 and 2 of murine Mefv. These animals were produced at the expected Mendelian frequency, developed normally, and reproduced as well as wild-type (WT) littermates. Resident peritoneal cells from both pyrin -/- and WT mice were harvested, adherent cells were treated with LPS ex vivo, and the mRNA was compared by microarray analysis. A large functional group of differentially expressed genes was found to be involved in inflammation and the immune response. Of these genes, CD11c – the prototypic marker of murine dendritic cells (DCs) – was downregulated in pyrin -/- mice relative to WT animals, which was confirmed by quantitative real-time PCR. Flow cytometric analysis of peritoneal cells and splenic leukocytes revealed a 20% and 15%, respectively, reduction of the percentage of CD11c+ cells in pyrin -/- compared to WT mice. Following the ex vivo generation of DCs from the bone marrow, a similar difference of CD11c+ cells between pyrin -/- and WT mice was found, which was mainly caused by a reduced percentage of CD11c+ precursor cells in pyrin -/- relative to WT animals. Severe abdominal symptoms, as peritonitis and diarrhea, often accompany the febrile episodes of FMF. This prompted us to further analyse the intestinal CD11c+ cell distribution and function in pyrin -/- and WT mice. Whereas the percentages of CD11c+ cells were comparable between pyrin -/- and WT animals, intestinal CD11c+ lamina propria cells of pyrin -/- mice appeared to show an aberrant pattern of cytokine expression, which was not seen for splenic CD11c+ cells of these mice. Based on these results, we hypothesize that pyrin is involved in the development of dendritic cells from their myeloid precursors and may exert a tissue-specific functional deficit potentially leading to a dysregulated intestinal immune homeostasis.
RNA interference of MEFV in THP.1 cells reveals a role for endogenous pyrin in Toll-like receptor signaling (TLR) that is mediated by the transcription factor IRF2

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The gene causing familial Mediterranean fever (FMF), MEFV, encodes a protein, pyrin that is expressed at high levels in granulocytes, monocytes, dendritic cells and in some human myeloid leukemia cell lines, such as THP.1. Although pyrin appears to play a role in the regulation of IL-1? and NF?B activation, its function has not been completely defined. To provide molecular insight into pyrin function, RNAi technique was employed to compare gene expression profiles between THP.1 cells expressing endogenous pyrin (SC) and cells in which the gene had been knocked down (siMEFV). Using Affymetrix cDNA microarray analysis to identify potential novel pyrin-dependent pathways, we identified over 300 genes differentially expressed in siMEFV treated cells compared to control. Based on Gene Ontology Classification, subsets of down regulated genes (CD36, CD14, MD2, TIRAP and MyD88) were found to be involved in Toll-like receptor (TLR) signaling. Western blot analysis confirmed reduction of protein for CD36 and MyD88. Flow cytometry analysis of CD14 demonstrated a 2-fold reduction in siMEFV treated cells compared to SC. It has been previously shown that CD36 functions as a co-receptor involved in the recognition of LTA via the TLR2/6 pathway. Functional analysis for CD36 showed inhibition of TNF? production in siMEFV treated cells after LTA stimulation. Consistent with the down regulation of the genes identified, stimulation of TLR agonists revealed a reduction in TLR2/1, TLR2/6 and TLR4 signaling. NF?B activation with E. coli LPS was also suppressed. Phagocytosis assays demonstrated a reduction in the ability of siMEFV treated cells to internalize E. coli bioparticles. Interferon regulatory factor 2 (IRF2) was found to be down regulated in siMEFV treated cells. To investigate the possibility of IRF2 as a common regulator of the TLR genes investigated, promoter analysis was used. We found that downregulated TLR genes showed enrichment of binding sites for the interferon regulator factor family (IRFF). Expression profile screening of IRFF members revealed IRF2 as the most significantly changed IRFF. IRF2 was validated by qRT-PCR and Western blot. FMF patients on and off colchicine had reduced IRF2 mRNA relative to controls, indicating that mutations in MEFV may in some cases act like knockdown in THP.1 cells. These data suggest that, directly or indirectly, endogenous pyrin facilitates signaling in part by its effect on IRF2.

A NEW SET OF CRITERIA FOR THE DIAGNOSIS OF FAMILIAL MEDITERRANEAN FEVER IN CHILDHOOD


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Objective. Although the gene of familial Mediterranean fever (FMF) was identified a decade ago the diagnosis is still based on clinical criteria. Existing criteria have been developed mainly for adult patients. However, as the episodes of FMF typically appear in childhood, these criteria need validation in children. The purpose of the present study is to establish a new set of criteria for use in childhood. Methods. The study group consisted of recently diagnosed FMF patients who had mutations at both alleles and who were initially examined in one of the four main centers for pediatric nephrology and rheumatology. 170 consecutive FMF patients (88 males, 82 females) between August 2007 and January 2008 were interviewed by one of the experienced physicians about the presence of 35 features and manifestations of FMF at the time of diagnosis. Controls were consecutive patients without FMF (n: 83) who had episodes of fever and clinical features mimicking that of FMF. The diagnostic performance of the candidate features was assessed by multiple logistic regression analysis. Outcome variable (FMF or Controls) was cross-classified with a predicted group variable whose values were derived from the estimated logistic probabilities. To obtain the derived predicted variable, a cut-point (0.50) was defined and compared each estimated logistic probability to 0.50. If the estimated probability of a patient exceeds the 0.50 then the patient was considered as a FMF, otherwise the patient was considered as a control. Results. The multiple logistic regression analysis showed that 6 of the 85 candidate criteria discriminate FMF from controls with a sensitivity of 92% and specificity of 94%. These 6 criteria were: fever (over 38°C, 6-72 hours of duration), abdominal pain (6-72 hours of duration), chest pain (6-72 hours of duration, unilateral), arthritis (6-72 hours of duration, oligoarthritis), exertional leg pain and family history of FMF. The presence of two or more of these six criteria diagnosed FMF with a sensitivity of 91% and a specificity of 88%. An equation for probability was also developed for calculating the likelihood of FMF that may guide clinicians to seek genetic analysis. Conclusion. The proposed diagnostic criteria were found highly sensitive and specific and may be used to diagnose FMF and to distinguish it from other periodic fever diseases in childhood.

**FCAS, Muckle-Wells, CINCA/NOMID**

(abstract 214)

**Characterization of the human CIAS1 promoter**

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Mutations in the CIAS1 (NLRP3) gene have been identified in a continuum of inflammatory disorders including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID). These disorders are collectively referred to as cryopyrinopathies because CIAS1 codes for the protein Cryopyrin. However, there are several patients with classic cryopyrinopathy phenotypes that do no have readily detectable mutations in CIAS1 coding regions. It is known that CIAS1 expression is highly regulated, but there has been no formal characterization of the CIAS1 promoter. We hypothesized that variations in the CIAS1 promoter sequence may have significant effects on disease state in patients with cryopyrinopathies, as well as more common inflammatory diseases since variants in non-coding regulatory sequence can have significant effects on gene expression or function, and in some cases may be associated with disease. We first determined the transcriptional start site of CIAS1 and analyzed the DNA region upstream of the transcriptional start site for potential transcription factor binding sites. Luciferase reporter plasmid constructs were created to assay the
promoter activity of several areas of the promoter region and confirm the regulatory function of specific binding sites. Finally, we sequenced the promoter regions from cryopyrinopathy patients and normal controls without coding region mutations, looking for variations unique to these patients. Three different alternative splice forms for the 5' end of the gene were confirmed and two distinct regions with significant promoter activity were found. Within these distinct regions several potential transcription factor binding sites were identified. We also identified several unreported sequence variations in the promoter region in normal controls, and found one unique single nucleotide polymorphism (SNP) near a transcriptional factor binding site in a mutation negative FCAS patient that was not identified in over 200 matched controls. Cloning of this unique SNP into luciferase reporter plasmid constructs resulted in greater than two fold increased promoter activity, suggesting that this variant may play a role in disease in this patient. From this report additional studies can now be done to further characterize the CIAS1 promoter and sequence variants, which will lead to a better understanding of the regulation of CIAS1 expression and its role in disease.

Microarray-based gene expression studies of systemic inflammation in patients with cryopyrin-associated periodic syndromes (CAPS)


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CAPS are rare autoinflammatory diseases characterized by various degrees of systemic inflammation. Most CAPS patients have gain of function, missense mutations in the CIAS1 gene (encodes cryopyrin), which mediates the activation of IL-1β and IL-18. CAPS patients respond dramatically to treatment with anakinra. To better understand the pathogenesis of CAPS and identify new candidate genes, we searched for differentially expressed genes (DEGs) in CAPS patients vs. controls and in NOMID patients before and after treatment with anakinra. Gene expression-based models were developed to differentiate CAPS patients from healthy and from individuals with other autoinflammatory disorders. We collected PBMCs from 22 CAPS patients, 30 TRAPS patients, 8 HIDS patients, 6 PAPA patients, and 34 healthy controls. These samples were hybridized to Affymetrix HG_U133A 2.0 microarrays. The statistics were corrected for multiple testing and used to identify DEGs between the various groups of samples. Ontological classification and pathway analysis using Ingenuity Pathway Analysis (IPA) software was utilized to understand relationships between the DEGs. Linear discriminant analysis (LDA) was used to build models that are predictive of CAPS. These models were evaluated by cross validation and by testing independent CAPS samples. We identified 1,880 DEG’s in CAPS patients vs. controls. In our analysis of 16 NOMID pre vs. post anakinra samples, 263 DEGs were identified. To enhance confidence in the list of anakinra-responsive genes, the 2 gene lists were merged and we identified the overlapping set of 173 transcripts. 127/173 genes were down-regulated by anakinra. Among interesting genes were SNCA, SOD2, BCL2L1, STAT3, several integrins and heat shock proteins. 23/173 anakinra-responsive genes were validated by qRT-PCR. 1707/1880 DEGs identified in the CAPS patient vs. control analysis were not responsive to anakinra. These genes may be part of pathways that are not directly regulated by IL-1β or they may not response to dose of anakinra given. Finally, the genes, which underlie the CAPS specific models, may be used to understand the mechanism of disease since they are able successfully to classify samples from NOMID patients both on and off anakinra.
Initial Characterization of a Mouse Model of Cryopyrinopathy


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Mutations in the human CIAS1 gene are responsible for a continuum of autoinflammatory disorders, including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID). CIAS1 (NLRP3) codes for the protein cryopyrin (NALP3), which interacts with other proteins in a complex called the inflammasome, and is involved in interleukin-1 (IL-1) production. There is evidence suggesting that mutations in CIAS1 lead to a gain of function for cryopyrin resulting in increased IL-1 and systemic inflammation. In order to further study disease pathogenesis, knockin mice were generated with mutations corresponding to L353P (observed in FCAS) and A352V (observed in MWS). The DNA construct used included a floxed neomycin resistance cassette, which prevented expression of the mutant allele. Mice were mated to Cre Zp3 (oocyte expression) and Cre Lysozyme (monocyte and neutrophil expression) to remove the cassette. Serum was obtained for cytokine analysis by Luminex. Tissues were fixed with formalin and stained for histologic analysis. Gross phenotype becomes apparent at day 2 with obvious growth retardation and skin abnormalities including pustules, scaling, and absent hair growth that progress during the first week of life. Death occurs within 8 or 9 days. Multiple serum cytokines were markedly elevated in the mutant mice compared to wild type littermate controls, including proinflammatory cytokines such as IL-1 and IL-6, chemokines such as KC (IL-8), and regulatory cytokines such as IL-17 and IL-10. IL-1 was markedly decreased relative to controls. Pathologic analysis revealed patchy subcutaneous and epidermal neutrophilic infiltrates in the skin. In addition, neutrophilic infiltrates were observed in conjunctiva, joints, and muscle. Perivascular neutrophils were also detected in the meningeal lining. Although the knockin mouse phenotype appears to be more severe than what is observed in MWS and FCAS patients, the character and distribution of neutrophilic tissue inflammation and profiles of serum cytokines are similar, suggesting that this model can be used to study the pathogenesis of cryopyrinopathies and explore novel therapeutic interventions.

Mechanisms of IL-1 secretion

Impaired isoprenoid biosynthesis induces caspase-1 activation in a Rac1/PI3kinase/PIKB dependent fashion: implications for the Hyper IgD syndrome.

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Background: Mevalonate kinase deficiency (MKD) is an autosomal recessive disorder characterized by recurring episodes of fever and inflammation. Peripheral blood mononuclear cells from MKD patients secrete high levels of IL-1β when stimulated with lipopolysaccharide due to the presence of hyperactive caspase-1. This readily secreted IL-1β is thought to be a primary cause of the recurring inflammation. However, the molecular mechanism of mevalonate kinase deficiency-induced caspase-1 activation remains unclear. Methods: To investigate this, we incubated monocytic cells
(THP-1) with simvastatin to artificially impair the isoprenoid biosynthesis pathway, mimicking MKD, after which cells are were stimulated with LPS. Results: Simvastatin-treated THP-1 cells stimulated with LPS demonstrated enhanced release of IL-1β. LPS enhanced transcription of IL-1β, which was shown to be partially dependent on p38 MAP kinase-mediated activation of NF-κB. Simvastatin-mediated effects were shown to be mediated by phosphatidylinositol 3 kinase (PI3K) and protein kinase B (PKB/c-Akt). Inhibition of PI3K strongly reduced IL-1β secretion, whereas introducing constitutively active PKB enhanced it. In addition, simvastatin-induced IL-1β secretion required the small GTPase Rac1. Simvastatin treatment increased GTP-bound Rac1 levels and inhibition of Rac1 reduced simvastatin-mediated IL-1β secretion. Rac1 functioned upstream of PKB, since Rac1 inhibition abolished simvastatin-induced phosphorylation of PKB. Simvastatin-mediated activation of the Rac1/PI3K/PKB pathway enhanced IL-1β secretion through activation of caspase-1, since inhibition of both Rac1 and PI3K blocked the release of active caspase-1 subunits. The importance of Rac1 in MKD was confirmed when a specific Rac1 inhibitor was shown to inhibit spontaneous IL-1beta release by MKD PBMC. Conclusion: Rac1, PI3K and PKB are involved in simvastatin-induced secretion of IL-1beta through regulation of caspase-1 activity. Hence, Rac1 is a potential new therapeutic target in MKD.

(abstract 62)

ATP released following activation of various pathogen-sensing receptors autocrinally induces IL-1β and IL-18 secretion by monocytes


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IL-1β is a multifunctional cytokine and a major soluble mediator of inflammation. Despite its extracellular localization and function, IL-1β lacks a secretory signal peptide and does not follow the classical ER-Golgi pathway of secretion. IL-1β is synthesized in the cytosol upon activation by inflammatory stimuli as a 35-kDa precursor (proIL-1β), and then proteolytically processed to the mature active form of 17-kDa by caspase-1/IL-1beta converting enzyme (ICE). The processing pathway is arranged in two steps. First, Toll-like receptor ligands, such as lipopolysaccharide (LPS) induce gene expression and synthesis of the inactive IL-1β precursor. Then a second stimulus is necessary to induce IL-1β processing and secretion. Among the second stimuli, exogenous ATP that strongly enhances the proteolytic maturation and secretion of IL-1β is the best characterized. A crucial role in IL-1β processing is played by the inflammasome, a multiprotein complex responsible for the activation of caspase-1, which, in turn, converts proIL-1β to the mature IL-1β. As the cleavage of the inactive preIL-1β is immediately followed by the release of the mature cytokine, processing and secretion appear to be linked. The complexity of the process is further increased by the observation that inflammasomes harbouring diverse molecular components exist. Also IL-18, a pleiotropic cytokine involved in the early events of the defensive innate immune reaction lacks a secretory signal peptide and like IL-1β requires cleavage by caspase-1 to be secreted in its active form. A defective control of their release may cause serious inflammatory diseases. Here we show that in primary human monocytes, several microbial components (pathogen associated molecular patterns, PAMPs) as well as molecules released by injured tissue, called danger associated molecular patterns (DAMPs) acting on different pathogen-sensing receptors (PPRs) are all competent to induce synthesis, maturation and secretion of IL-1β through a process that requires extracellular release of endogenous ATP, K+ efflux and activation of phospholipase A2. Moreover, all molecules inducing IL-1β also trigger processing and secretion of IL-18, indicating that, like for IL-1β, different signalling pathways converge on
caspase-1 activation and IL-18 secretion. However, the amount of IL-18 secreted was very low compared to IL-1beta. Antagonists of the surface ATP purinergic receptor P2X7, or treatment with the ATPase apyrase, prevent IL-1beta and IL-18 maturation and secretion triggered by the different stimuli on healthy monocytes. Differently, IL-1beta maturation and secretion induced by the ionophore nigericin, that elicits K+ efflux without activation of pathogen-sensing receptors, is not inhibited by blockers of P2X7 receptors. Remarkably, P2X7 inhibitors did not affect PAMPs-induced IL-1beta secretion by monocytes from CINCA patients carrying a mutated NALP3, confirming our previous observation that inflammasome in CINCA patients does not require exogenous ATP for activation. These data indicate that in human monocytes from healthy individuals the autocrine stimulation of P2X7 receptors by the externalized ATP is required for IL-1beta and IL-18 processing and release. Thus, stimuli acting on different pathogen-sensing receptors converge on a common pathway where secretion of ATP is the first step in the cascade of events leading to inflammasome activation and IL-1beta and IL-18 secretion.

**Mevalonate-kinase deficency**

(abstract 133)

Follow-up, clinical features, and quality of life in 103 patients with HyperImmunoglobulin D syndrome

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Introduction: The Hyper Immunoglobulin D and periodic fever syndrome (HIDS), one of the auto-inflammatory syndromes, is caused by mutations in the gene coding for Mevalonate Kinase (MVK). A patient registry database was founded in 1994 by the international HIDS study group. The aim of our current study was to asses the genetic, laboratory and clinical features as well as complications and course of disease in patients with mutation-positive HIDS. In addition we studied the quality of life and course of life in a selection of patients. Patients and methods: Follow-up data was acquired by a questionnaire that was sent to all submitting physicians. In addition, the course of life and quality of life (QoL) was assessed in Dutch patients >16 years using validated QoL instruments.

Results: Follow-up data was obtained from 103 patients (81.6% of total patients in HIDS registry) from 16 different countries. The median age of first attack was 6 months (range 0-120). Mean diagnostic delay was 14.8 years; this did not decrease in recent years. Most frequent symptoms that accompanied attacks of fever include lymphadenopathy (87.3%), abdominal pain (85.3%), arthralgia (83.3%), vomiting (71.6%), diarrhoea (72.3%), skin lesions (68.8%), and aphthous ulcers (52%). Amyloidosis is an infrequent complication (2.9%). The median highest serum IgD level was 409 U/ml. IgD levels were normal in 24% of patients. The four most prevalent mutations (V377I, I268T, H20P/N, P167L) account for 71.5% of mutations found. There was no association between age of onset, number of attacks, or clinical features and presence of a specific mutation. Frequency of attacks decreases with age: in the first decade of life 43.5% of patients have more than 12 attacks per years, in the second decade of life 23.9%, and 17.8% in patients >20 years. Many drugs have been tried in HIDS. Some patients respond to high dose prednisone (25% good response). Anakinra and etanercept can also be effective (good response 36% and 25%). HIDS impaired several aspects of QoL. Social functioning, general health perception, and vitality are significantly lower than
controls, as is autonomy and social development. Conclusion: HIDS patients have an early onset of disease, with often a large delay in diagnosis. The attacks gradually decrease during life, but persist in many. HIDS significantly impairs the quality of life.

New therapies

(abstract 119)

Safety and efficacy of Infliximab and Rituximab in patients with refractory Behcet disease
Safety and efficacy of infliximab and rituximab in patients with refractory Behcet disease


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Background: Behcet disease (BD), a systemic vasculitis, has a wide variety of clinical manifestations resulting from ubiquitous small-vessels inflammation. A defect in immune regulation has been proposed, and one or several infectious agents may trigger the immune system. In patients with BD, CD4+ T lymphocytes are found in the inflammatory infiltrates; and Th1 response predominates (producing IL-1, IL-2, IL-6, IL-12, INF gamma and TNF). BD treatment includes colchicines, corticosteroids, and/or cyclophosphamide. In refractory disease, methotrexate or mycophenolate mofetil are used as well as anti-TNF agents. Objective: To study safety and efficacy of biological therapies in patients with refractory BD. Methods: We studied 9 patients with BD refractory to corticosteroids and conventional immunosuppressive agents. Results: Nine patients, 5 female and 4 male, mean age was 34.3 years (17-53), and mean time disease evolution of 10.67 years (6-13) at the end of the study. Their clinical manifestations included: genital and oral ulcerations (100%); neurological manifestations (55.5%); mucocutaneous (33.3%); uveitis (33.3%), arthritis (55.5%); motor diarrhea (33.3%), Raynaud phenomena (22.2%); thrombosis and pathergy phenomena (22.2%). These patients were previously and unsuccessfully treated with: high-dose corticosteroid (>20mg/day of prednisone) and colchicine (100%); cyclosporine (55.5%); azathioprine (44.4%); cyclophosphamide (33.3%); chloroquine (11.1%); methotrexate (66.6%); and salazopirine (11.1%). All the patients discontinued using these treatments because of severe side effects or inefficacy. We decided to prescribe them infliximab 5 mg/kg/infusion as Rheumatoid arthritis protocol. Nine patients received infliximab during a mean time of 1.25 years (SD: 2.05), 5 patients improved their symptoms and the remaining 4 patients had to stop infliximab infusion because of allergic reaction (1) and inefficacy (3:repetitive neurological manifestations, mucocutaneous lesions and arthritis). The 4 non-responder patients were switched to rituximab therapy. Patients on rituximab are free of symptoms after a mean duration of 10.6 months (SD: 8.9), and continued only with low-dose of corticosteroid. Conclusions: Infliximab, a well tolerated and safe treatment, is a therapeutic alternative in patients with refractory BD. Those patients, who may not respond to infliximab, should get benefit from an alternative and safe option as rituximab therapy may offer.

(abstract 160)

Long-lasting response to ACZ885 (a new human IgG1 anti-IL-1β monoclonal antibody) in patients with Muckle-Wells Syndrome (MWS)

MWS is a rare autoinflammatory disease caused by mutations in the CIAS1 gene. The current treatment is anakinra, a soluble IL-1 Ra; however, frequent and high-dose injections are not well tolerated. Herein, we report a single-centre interim analysis of an open-label study to investigate safety and efficacy of ACZ885. Patients with documented CIAS1 mutation and active disease were recruited. Patients received one ACZ885 s.c. injection (adults 150 mg/children 2 mg/kg) followed by an observation period and re-dosing upon relapse. Complete response was defined by physician’s global assessment of disease activity (PGADA) and assessment of skin disease ≤2 on a 5-point scale (1=absent, 2=minimal, 3=mild, 4=moderate, 5=severe) and normal serum values (below 10 mg/L) of C-reactive protein (CRP) and/or serum amyloid A (SAA). ACZ885 5 mg/kg was administered i.v. to patients with incomplete response within 7 days. Patients overall rating of disease severity was recorded on a 5-point scale (0–4). Of 12 pts (median age: 27.6 [4.3–47.4]; 4 children below 14 yrs), 9 pts had a medical history of treatment with anakinra up to 8 mg/Kg. All 12 pts treated with ACZ885 achieved complete and rapid clinical and serological response. Data presented below show the median of 12 pts at baseline, 8 and 38 days after treatment, 9 pts at Day 68 and 3 pts at Day 98 after treatment. PGADA was 4 at baseline and rapidly decreased to 1 at Day 8 and 38 and increased to 3 and 4 at Day 68 and 98. Patients rated disease severity as 2, 0, 1 and 2 at baseline, Day 8, 38, 68 and 98, respectively. CRP decreased to normal values during the study. SAA (mg/L) decreased from baseline (16.05) to normal values at Day 8, 38, and 68, and increased to 17 at Day 98. 2 children and 1 adult received i.v. injection. The median time to re-dosing was 92 days (9 pts) after the first and 66 days (6 pts) after the second treatment cycle. 2 pts remained relapse free for 106 days. ACZ885 was well tolerated. AEs were upper respiratory tract infections (7 pts) and elevated pancreatic amylase and lipase (2 pts). 1 pt experienced an SAE (vertigo). In conclusion, ACZ885 achieved a complete and long-lasting response in 12 pts with MWS. Long treatment-free intervals of up to 106 days is a great advantage in patients who tolerate daily injections poorly. The first predictor of disease progression was clinical deterioration (PGDA assessment), while the rise in inflammatory markers was observed at a later timepoint.

(abstract 171)

Treatment of cryopyrin associated periodic fever syndrome with a fully human anti-IL-1beta monoclonal antibody (ACZ885): results from a subcutaneous administration study.


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Background NLRP3/cryopyrin plays a vital regulatory role in caspase 1 mediated processing of pro IL-1, and mutations in the gene for this protein cause the autoinflammatory disease cryopyrin associated periodic fever syndrome (CAPS). The pivotal role of IL-1 in the pathogenesis of CAPS has been confirmed by complete remission following treatment with short acting recombinant IL-1Ra (anakinra). Objectives: To assess the clinical efficacy, safety, pharmacokinetics and pharmacodynamics of a fully human anti-IL-1 monoclonal antibody (ACZ885) given by subcutaneous (s.c.) administration in CAPS. Methods: Eight adult patients with CAPS associated with NLRP3 gene mutations were recruited and gave informed consent. Entry criteria were elevated CRP and SAA concentration and moderate to severe symptoms of CAPS. All patients were treated with ACZ885 150 mg s.c. and clinical and laboratory outcomes were measured. A further doses was administered at each relapse. Clinical remission was defined as: absence of fever, rash,
conjunctivitis and joint or muscle pain; CRP and SAA within the normal range of < 10 mg/L; and normal leukocyte count. Relapse or incomplete remission was defined as: return of at least two symptoms associated with CRP and/or SAA values >30 mg/L. Results: 5 women and 3 men (median age 35 years) received s.c. injections of ACZ885 for a median of 18.5 months. The drug was uniformly well tolerated and resulted in improvement of clinical symptoms within 1 day and complete clinical remission within 7 days. Each clinical remission lasted a median of 115 days (IQ range 91-127 days). The CRP and SAA fell to healthy values within one week of dosing. Modelling using data on ACZ885 pharmacokinetics and clinical symptoms indicated ACZ885 had a plasma half life of 29 days and that the critical drug concentration where there was a 50:50 probability of clinical relapse was 1.1 mcg/ml. Simulations from the model predicted that regular dosing of 150 mg every 8 weeks should maintain sufficient drug concentration to sustain disease control in patients over 40 kg. Conclusion: In 8 patients treated for up to 22 months, blockade of IL-1 with the monoclonal antibody ACZ885 was extremely well tolerated and produced complete clinical and biochemical remission of CAPS disease activity in all cases. Phase 3 placebo controlled studies are underway.

(abstract 65)

Effective Treatment with IV Pamidronate in Chronic Recurrent Multifocal Osteomyelitis (CRMO)-Resolution of Pain, Normalization of Radiologic Abnormalities, and Improvement of Elevated Urine-N-Telopeptide


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Objectives: To report clinical, magnetic resonance imaging (MRI), and bone turnover response to intravenous pamidronate (IVP), in pediatric patients with chronic recurrent multifocal osteomyelitis (CRMO). Methods: A prospective open label study was conducted of all CRMO patients (pts)treated with IVP between 2003 and 2007. 10 patients (5M:5F) received IVP. Mean age at CRMO diagnosis was 11.4 (range 4.5-16.3) years. Mean duration of symptoms pre-IVP was 23.9 (range 2-36) months. Involved sites included spine, femur, tibia, clavicle, sacroiliac joints (2,3,2,1,1 patients, respectively). All pts had failed NSAIDs and reported 10/10 on visual analog scale (VAS) for pain. Plain x-rays, bone-scan, MRI, and histology were compatible for CRMO for all pts. All pts had T1 signal hypointensity and T2 signal hyperintensity of affected bones on pre-IVP MRI, consistent with inflammation. Pamidronate was administered as intravenous cycles (IVP), 1mg/kg/day. All pts received an initial 3-day cycle, and subsequently either 1-day IVP monthly, or 3-day IVP every 3 months, with maximum dose < 11.5mg/kg/year. VAS for pain, and urine N-telopeptide/urine creatinine (uNTX/uCr) ratio (a marker for bone resorption), were measured at baseline and at monthly intervals during IVP treatment. MRI(s) of affected sites were obtained at baseline, every 2 months, and at suspected CRMO recurrence. The primary endpoint was pain response, the secondary endpoint MRI signal resolution. Results: VAS decreased from 10/10 to 0-3/10 by end of first IVP for all pts. MRI findings of bone marrow edema resolved by mean of 5.2 (range 2-10) months and IVP was discontinued. The mean number of IVP treatments was 5.2 (range 2-10); mean dose was 6.2 (range 2.5-9.5) mg/kg/year. Mean pre-IVP uNTX/uCr was 722 nmol/mmol/creatinine; with 63.3% reduction after first IVP (range 16-88%). Mean follow-up after initiation of IVP was 24.9 (range 12-54) months. 4 pts had MRI confirmed CRMO relapse at 12-18 months after IVP. All 4 pts responded clinically and by MRI to 1-day IVP re-treatment. UNTX/uCr increased by mean of 184% with flare. Conclusions: 1. IV Pamidronate resulted in rapid
and sustained pain relief in these patients with severe CRMO. Only 1-day retreatment was required for flares. 2. MRI signal resolved more gradually than pain. 3. UNTX/uCr was elevated with active CRMO, decreased with IVP, and increased with CRMO flare. 4. IVP is an effective 2nd line agent for severe CRMO.

(abstract 175)

**Placebo-controlled Pilot Study of Rilonacept (IL-1 Trap), A Long Acting IL-1 Inhibitor, In Refractory Chronic Active Gouty Arthritis**


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Preclinical studies and a clinical case series suggests that blockade of the NLRP-3 (cryopyrin) inflammasome IL-1 pathway may offer a new treatment strategy for gout. Rilonacept, a soluble receptor-Fc fusion protein that blocks IL-1 showed rapid and sustained benefits in a Phase 3 study of subjects with cryopyrin-associated periodic syndrome (CAPS) arising from NLRP-3 mutations. This pilot study explored the potential utility of rilonacept in chronic active gout. Objective: To assess the safety profile and change in disease activity in subjects with chronic, active gouty arthritis after treatment with rilonacept. Methods: This was a multi-center, non-randomized, single-blind, placebo-controlled, monosequence crossover study. The study population included subjects with a diagnosis of ≥6 months of chronic gouty arthritis, ≥1 active joint for ≥4 weeks, and a self-reported pain visual analogue scale (VAS) of ≥3. A run-in period of 2 weekly subcutaneous (SC) injections of placebo (PBO) was followed by 6 weekly injections of rilonacept. Gout activity was assessed by Subject Pain VAS, Subject and Physician Global VAS, joint count, and hs-CRP. Results: Ten subjects (8M/2F) with a mean age of 62 years (50-78), mean disease duration of 13 years (6-26), and Day Subject Pain VAS of 5.1/5.0 (mean/median) were enrolled. There were no reported deaths or SAEs, and drug-related AEs were most often associated with mild-to-moderate ISRs. Mean/median changes in Subject Pain VAS rating from Day to Wk 2 with PBO treatment were 0.25/-0.25 (ns), respectively, and -3.2/-2.25 (p=0.02) with rilonacept treatment from Wk 2 to Wk 8. During this period, seven of 10 subjects on rilonacept showed at least 50% improvement in Subject Pain VAS (p<0.0001) and six of 10 subjects showed at least 75% improvement (p=0.0001), while no subjects showed improvement in this parameter while on PBO. Hs-CRP median decreased 59% (p=0.004) by Wk 8 after rilonacept therapy. At Wk 14 (6 weeks after last dose of rilonacept) a trend towards baseline hs-CRP levels was observed. Conclusion: Rilonacept was generally well tolerated. Substantial decrease in both clinical activity and hs-CRP was seen after blinded switch from treatment with PBO to rilonacept. These results support the hypothesis that IL-1 blockade may offer an important new therapeutic option in a subset of gout patients with long-standing arthritis that can not be managed with other treatments. Further studies are planned.

(abstract 208)

**mIL-1 Trap Reduces Pain and Inflammation in Animal Models of Gout**

Gout is a common and very painful arthritic disease with increasing prevalence in the United States. Monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals induce interleukin-1 (IL-1) release through the inflammasome, and this release is thought to contribute to the pain and inflammation associated with gout and pseudogout. However, consequences of IL-1 blockade have not been explored with regard to clinically relevant joint pain and inflammation readouts in gout animal models. In this study, we employ IL-1R1 knockout mice and mIL-1 Trap, a high-affinity receptor-Fc fusion protein blocker of mouse IL-1, to assess the involvement of this cytokine in the pain and inflammation observed in mouse models of gout. We developed a new murine joint pain and inflammation model of gout in which endotoxin-free MSU crystals were injected intra-articularly into the ankle joint followed by measurement of thermal hyperalgesia and weight bearing distribution of the affected hind paw and ankle inflammation over a 4 days period. The effects of genetic (IL-1R1-null mice) and pharmacological (mIL-1 Trap) blockade of IL-1 signaling was studied in this model as well as in crystal-induced peritonitis and subcutaneous air pouch mouse models of gout and pseudogout inflammation. We show that blocking IL-1 leads to reduced neutrophil influx in both the MSU and CPPD crystal-induced peritonitis and subcutaneous air pouch models. mIL-1 Trap’s efficacy in preventing neutrophil infiltration in the MSU crystal-induced peritonitis model was equivalent to that achieved with the highest tolerated dose of the gout medication colchicine. In the ankle joint pain model, inhibiting IL-1 lead to significant reductions in thermal hyperalgesia, weight bearing redistribution, ankle inflammation, and SAA levels. mIL-1 Trap was also effective in relieving established pain and ankle swelling when administered 1 day after ankle MSU injection. mIL-1 Trap was as effective as the highest tolerated dose of colchicine. Our data demonstrate that the mIL-1 Trap is able to decrease both pain and inflammation in mouse gout models. These studies suggest that IL-1 Trap (rilonacept) may be a promising therapeutic candidate for preventing and treating acute gout attacks in human disease. Rilonacept may provide a new mechanism of action treatment option for patients in which currently available therapeutics are not well-tolerated or not sufficiently efficacious.

(abstract 138)

Rilonacept in Patients with Cryopyrin-Associated Periodic Syndromes (CAPS): The Durability of Response over 48 weeks
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Background: IL-1B inhibition with rilonacept has been shown to rapidly improve the clinical and laboratory signs and symptoms associated with Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS). Short term (6 wk) treatment of FCAS/MWS patients (Pts) with rilonacept 160 mg weekly demonstrated marked (85%) decreases in the 21 day mean key symptom score (KSS) (0-10 scale of rash, fatigue, feeling of fever/chills, joint pain and eye redness/pain) and normalized serum amyloid A (SAA) and C-Reactive Protein (CRP) compared to placebo. The study described here assessed the efficacy of rilonacept over 48 weeks. Methods: Pts completing an initial 24-week blinded treatment period (44/47) enrolled in a 24-week open-label rilonacept treatment extension (OLE) in which all received rilonacept 160 mg sc weekly. Symptoms were assessed daily (pt report), CRP and SAA were assessed at day and wk 1, 3, 6, 12, 18 and 24. Results: Of the 47 pts from the initial 24-week blinded study, 43 completed through the subsequent
24-week OLE. Results are ordered by the following groups: rilonacept-DB-week 6-(n=23), Placebo-DB-week-6 (n=24) and OLE-week-48-(n=44). Baseline mean KSS 3.1, 2.4, 2.8 Mean reduction in KSS from baseline -2.6 (84%), -0.3 (13%), -2.2 (79%) Baseline mean number of disease flare days 8.6, 6.2, 7.6 Mean reduction in disease flare days from baseline -8.4 (98%), -1.0 (16%), -6.9 (91%) Baseline mean SAA 60.4, 109.9, 86.6 Mean reduction in SAA from baseline -56.6 (94%), -0.1 (0.1%), -71.9 (83%) Baseline mean CRP 22.5, 29.7, 26.8 Mean reduction in CRP from baseline -20.1 (92%), -1.4 (8%), -20.9 (78%) All symptom-based parameters were based on 21-day observation period. all rilonacept groups were statistical significant from placebo; p< 0.03 In all analyses, the improvement in signs and symptoms of FCAS/MWS in pts treated with rilonacept was similar, and sustained for the duration of treatment, up to 48 weeks. The most common adverse events were injection site reactions and upper respiratory tract infections similar to those observed in the initial blinded studies. Conclusions: The marked improvements in clinical and laboratory signs and symptoms of FCAS/MWS seen with rilonacept treatment are maintained during long-term therapy.

Systemic-Onset Juvenile Idiopathic Arthritis
(abstract 169)

The pattern of response to anti IL-1 treatment distinguish two subset of patients with systemic onset juvenile idiopathic arthritis (SoJIA).


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Aim: to assess the clinical response to IL-1 blockade in patients with SoJIA treated with the IL-1 receptor antagonist (Anakinra). Patients and Methods: twenty-two SoJIA patients (11 F, 11M) were selected for the treatment with IL-1 receptor antagonist (Anakinra). All patients required prolonged cortico-steroid therapy, mostly in association with one or more second line agents. Response to treatment was evaluated according to the behaviour of a number of clinical (fever, rash, number of active joints) and laboratory (ERS, CRP, WBC, Hb) parameters during the follow-up. Complete response was defined as the absence of systemic and joint manifestations and complete normalization of acute phase reactants at follow up. Other patients were considered as incomplete or non responders. Results: our study shows that SoJIA can be divided into two subsets according to the type of response to Anakinra. A subset (complete responders), accounting for the 40 % of patients, had a dramatic and persistent response to IL-1 blockade that allowed the rapid discontinuation of any other treatment. In the other group of patients (incomplete or non responders), the treatment, although effective on systemic manifestations, was unable to control arthritis and inflammation and was either withdrawn or continued in association with second line agents or steroids. At baseline, complete responders displayed different pattern of joint involvement with a significant lower number of active joints (median 3.5, range 1-10) in respect to incomplete and non responders (median 7, range 3-55, p=0.02). Conversely, no significant differences were observed in the systemic features (i.e. presence of fever, rash, hepatosplenomegaly, serositis). Among laboratory parameters at baseline, no differences were observed for acute phase reactants (CRP, ESR, fibrinogen, ferritin) or haemoglobin levels between the two groups. Conversely, complete responders had a significant higher number of circulating neutrophils (median 19.3x103/mm3, range 6.1-30.9) as compared with incomplete and non-responders (median 9.1 x103/mm3, range 7.3-19.7) (p=0.02) Conclusions: we have shown that two subsets of SoJIA patients with distinct clinical features can be identified according to their response to IL-1 blockade. Our study
TNF-associated periodic syndrome

(abstract 182)

Abnormal TNFR1 cell surface expression and NF-KappaB activation in TNFR1-associated periodic fever syndrome (TRAPS)


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Objective: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory condition caused by mutations in the TNFRSF1A gene. The cellular mechanisms by which mutations in this gene trigger inflammation are currently unclear. As NF-kappaB is the major intracellular signaling component inducing secretion of pro-inflammatory cytokines, we sought to determine whether differences in the clinical phenotype of patients with TRAPS may be due to variable effects of TNFRSF1A mutations on TNFR1 expression, localization, or NF-κB activity. Methods: Peripheral blood mononuclear cells were obtained from patients with informed consent, and cellular nuclear and cytosolic fractions generated by subcellular fractionation. IkappaB alpha and NF-kappaB localization was determined by Western blotting of the resultant fractions. NF-kappaB subunit activity was determined by ELISA analysis and confirmed by EMSA. Subcellular localization of TNFR1 was determined by immunofluorescence confocal microscopy, or by immunoblotting following affinity-isolation of plasma membrane by subcellular fractionation. Results: Cells from patients with the fully penetrant C73R mutation have marked activation of the pro-inflammatory p65 subunit of NF-kappaB. By contrast, cells from patients with the low penetrant R92Q mutation displayed high levels of DNA-binding by the p50 subunit, an interaction previously linked to repression of inflammation. Interestingly, while C73R cells have no TNFR1 shedding defect there was nonetheless an unusually high concentration of functional TNFR1 at the plasma membrane. Conclusion: This study shows for the first time that the R92Q mutation associated with low penetrance TRAPS likely results from p50-p50 NF-kappaB homodimer repression. The P46L mutation however does not alter p50 signalling, but instead renders TNFR1 non-functional. By contrast, the fully penetrant C73R mutation results in persistent elevated localization of functional TNFR1 at the cell surface, and is associated with increased TNF induction of the pro-inflammatory intracellular NF-kappaB pathway. Thus variation in NF-κB activity in PBMCs of patients with different TNFR1 genotypes provides an explanation for the observed variation in clinical phenotype.
Identification and analysis of gene-expression signatures in peripheral blood leukocytes of patients with TRAPS.

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Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is a rare autoinflammatory syndrome characterized by self-limiting episodes of recurrent fevers, abdominal pain and systemic inflammation. We undertook microarray analysis in an effort to characterize the molecular mechanisms involved in regulating the inflammatory response in these patients. Peripheral blood mononuclear cells were obtained from 29 patients with structural (high penetrance) TRAPS-associated mutations and 34 age and sex matched controls. cRNA was hybridized to Affymetrix U133A 2.0 microarrays and data processed using MAS5 algorithm. P-values were corrected for multiple testing using the false discovery rate (FDR). Genes were validated for the degree of expression by quantitative real-time PCR. Ingenuity Pathway Analysis (IPA) software was used to assess the relevance of the differentially expressed genes (DEG) to known biological pathways. Of the 29 TRAPS patients included, seven had clinical symptoms and biochemical evidence consistent with active disease. A further 7 patients had an elevated C-reactive protein or erythrocyte sedimentation rate consistent with subclinical inflammation. Sixteen patients were treated with etanercept, of these 2 patients were maintained on transplant immunosuppression and 1 required anakinra therapy. At an FDR of 10% and fold change (FC) of ≥1.4, 255 transcripts encoding 187 unique genes were identified. The most significantly DEGs were hemoglobin delta (FC 3.4) and SNF1-like kinase (-1.78). IPA analysis generated multiple networks of genes, with the most highly ranked centered on nuclear factor kappa B. Analysis of symptomatic patients identified a larger number of DEGs with high fold change values including 15 with a FC >5, enrichment of erythroid (delta-aminolevulinate synthase (FC 50)) and antigen presenting genes were notable. DEGs in common with symptomatic cryopyrinopathy (CAPS) patients included alpha-synuclein (5.3), selenium binding protein 1 (9) and carbonic anhydrase I (11.1). Validation of genes of interest using real-time PCR techniques is ongoing. This study identified a number of DEGs in patients with TRAPS, with particularly marked changes noted in symptomatic patients. Similarities with the DEGs identified in CAPS patients and a relative lack of DEGs downstream of TNF may support the ‘ligand independent’ theory of disease pathogenesis, and implicates a role for IL-1 dependent genes in TRAPS.

poster

Amyloidosis

Expression of ASC in renal tissues of FMF patients with amyloidosis; postulating a role for ASC in AA type amyloid deposition


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Familial Mediterranean fever is characterized by recurrent attacks of fever and serositis. The most serious complication is deposition of AA type amyloid, mainly in the kidneys. Treatment with colchicine reduces the frequency and severity of FMF attacks and prevents amyloidosis, though the mechanism behind these effects is unknown. The FMF gene, MEFV, encodes pyrin which interacts with several proteins, including ASC, a key molecule in both apoptotic and inflammatory processes. ASC forms aggregates called specks in cells. Given the ability of pyrin to modulate ASC speck formation and subsequent apoptosis, and the known beneficial effects of colchicine in preventing FMF attacks, the initial aim of this study was to determine the effect of FMF-causing mutations in pyrin and the impact of nocodazole (a microtubule toxin with effects similar to those of colchicine) on the process of speck formation. In this study we demonstrate that specks are generally cytosolic, and are most often located near the microtubule organizing center of cells. Furthermore, administration of nocodazole prevents speck formation. In the course of these experiments, we occasionally noted specks that appeared to be outside of cells. In addition, some dying cells appeared to extrude a speck. We further investigate the expression pattern of ASC in the kidney, and find that ASC is expressed in renal glomeruli of FMF patients but not control patients. To investigate whether ASC expression was correlated with amyloid deposition, renal biopsies from FMF patients were analyzed after congo red staining. Sequential sections were stained to determine the localization of ASC. There was a 100% correlation between ASC expression and the presence of amyloid deposits. On the basis of these data, we hypothesize that an inflammatory spectrum unique to FMF patients results in ASC expression in renal glomeruli. High local ASC expression may result in speck formation in these glomerular cells, a process that will lead to apoptosis of these cells. If specks from dying cells survive in the extracellular space, as they clearly do in cultured cells, it is possible that such extracellular specks (perhaps in association with pyrin) could act as nucleating centers for amyloid deposition. The fact that speck formation requires an intact microtubule network as shown here could potentially account for the ability of prophylactic colchicine administration to prevent and even reverse amyloidosis in FMF patients.

(abstract 204)

**Tumor Necrosis Factor alpha Antagonists in the Treatment of Secondary Amyloidosis**

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OBJECTIVE: To evaluate the efficacy and safety of TNFα antagonists in secondary amyloidosis. METHODS: 26 patients with secondary amyloidosis were treated with TNF antagonists between April 2001 and December 2007. Among these, 22 patients (13 men, 9 women, mean age 36.1 ± 12.3 years) who were treated with TNF antagonists for at least 6 months were surveyed. Patient charts were surveyed for disease and treatment duration, concomitant medications, serum creatinine and proteinuria levels during treatment and adverse events. RESULTS: Primary diagnoses were JIA in 6 patients, FMF in 1 patient, FMF and JIA in 1 patient, AS in 6 patients, RA in 4 patients, Crohn’s disease in 2 patients, Behçet’s syndrome in 1 patient and adult onset Still’s disease in 1 patient. 9 were prescribed infliximab, 5 etanercept, 6 patients used initially infliximab and later etanercept and 2 patients received all 3 TNF antagonists successively. Concomitant medications
were corticosteroids in 15 patients, DMARDs in 14, colchicine in 9, and eprodisate in 1 patient. Mean duration of treatment with TNF antagonists was 16.6±11.9 (6-41) months. Mean creatinine level increased from 1.1±0.6 to 1.4±1.5mg/dl (p=0.35) and proteinuria decreased from 2.9±3.6 g/day to 1.6±1.8 g/day (p=0.03). Serum creatinine level increased in 2/22 patients, was stable in 11/22 decreased in 9/22. 20/22 patients had proteinuria when TNF antagonists were started and in 6 patients the proteinuria was in the nephrotic range. Proteinuria dissappeared in 7/20, decreased in 8/20, remained stable in 3/20 and increased in 2/20 patients. Dialysis was started in 3 patients. 6/26 patients died. Causes of death were definite sepsis in 1, probable sepsis in 3, massive bleeding from bladder in 1 patient 6 months after she stopped infliximab, and bleeding following fondaparinux use after hip prosthesis surgery in 1 patient, 26 months after she stopped infliximab. Other serious adverse events were retinal vein thrombosis, urinary tract infection with vancomycin resistant enterococci, pneumonia, gluteal abcess, a psoriasis-like eruption, anaphylactic reaction, deep vein thrombosis and popliteal artery occlusion. CONCLUSION: Treatment with TNF antagonists over one year seems to reduce proteinuria and stabilize serum creatinine levels. However caution is needed due to a high number of deaths and other serious adverse events. Whether these are related to the disease or to the use of TNF antagonists remains to be studied.

(abstract 46)

Tissue specific rate and amount of amyloid deposition in induced and natural amyloidosis.


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Background: Contrary to AL amyloidosis, in AA amyloidosis a relatively invariable course of protein deposition is maintained, uniform to all patients, which in humans appears to preferentially involve the kidneys and only then to affect other organs. Local tissue factors, particularly cell type, composition of cytokines and chemokines and enzyme activity may govern the rate and quantity of amyloid A deposition in certain tissues. Aim: To determine organ differences in the amount and rate of AA amyloid sedimentation in mouse and hamster models of amyloidosis. Methods: The amount and rate of amyloid deposition was studied in spleens, kidneys, and livers of male and female mice of various mouse strains and in female golden hamsters, using the enhanced model of amyloidogenesis, in which amyloid deposition in tissues, may be identified within 2 days and reach substantial amount within 4-6 days. The crush and smear technique, with an amyloid amount score range of 0-5, was used to semi-quantify the amount of amyloid, on different points in time. Results: Pronounced differences were observed in the amount of amyloid in spleens, as compared to livers and kidneys of amyloidotic Swiss mice, with an amyloid grade of 4.7 vs. 2.7 and 2.1 respectively. Amyloid was found in livers of only 65% of mice with amyloidotic spleens, and was limited to animals with splenic amyloid deposits graded higher than 3.5. Similar differences between spleens and livers were observed in other mouse strains (ICR, C57Bl, C3H and AKR), and in golden hamsters. Long term experiments, lasting up to 6 months, revealed that the rate of liver amyloidogenesis is much slower than that of the spleen. Female Swiss mice gave comparable results but with somewhat lower amyloid grade in female organs. Conclusions: In this study, tissue specific differences in the amount and rate of amyloid A deposition were observed in various strains of mice and in golden hamsters. The preferential organs of amyloid deposition appear to be different in man as compared to animal models of amyloidosis. Factors account for these tissue and strain differences may be important in the amyloidogenic pathway, and are yet to be determined.
(abstract 47)

**Attempts at suppression of amyloidogenesis in a mouse model by a variety of non-steroidal and other anti-inflammatory agents**


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Background: Inflammation is the underlying force driving AA amyloidogenesis. Experience with colchicine, suggests that its anti-inflammatory effect is instrumental in the prevention, deceleration and reversal of amyloidosis in familial Mediterranean fever. Similar role has been assigned to anti-TNF medications in rheumatoid arthritis. Surprisingly, commonly used anti-inflammatory medications are not routinely being employed in the treatment of amyloidosis, with only scarce evidence based data to explain this avoidance. Objective: To determine the efficacy of anti-inflammatory drugs of different modes of action at suppressing AA amyloidosis in a mouse model.

Method: AA amyloidosis was induced in Swiss male mice by a single intravenous injection of amyloid enhancing factor, followed by 3 successive daily injections of AgNO3. Suppression of amyloid formation was studied, using a number of anti-inflammatory preparations, given during amyloidogenesis, including several non-steroidal anti-inflammatory agents (NSAIDs), such as diclofenac, indomethacin and diflunisal, a variety of other anti-inflammatory agents such as the TNF-alpha inhibitor, etanercept, interferon-alpha and leflunomide, and different chemotherapeutic agents, including methotrexate, azathioprine and cyclophosphamide. The degree of splenic amyloid deposition was determined on day 4 to 6 using the crush and smear technique and a 5 grade scale. Colchicine with 80% inhibition of amyloidogenesis in our mouse model was used as a positive control. Results: Most drugs tested had borderline (10%-30%) or not at all amyloid suppressive potential. Only cyclophosphamide stood out as an effective anti-amyloid preparation with an inhibitory activity reaching 88% (P=0.0002) Conclusion: The routine clinical anti-inflammatory medications studied here, including the anti TNF preparation etanercept, and the chemotherapeutic agent azathioprine had no significant effect on AA amyloidogenesis. The only exception was cyclophosphamide, which join the amyloid retarding agents colchicine and adrenocorticosteroids. A controlled trial with cyclophosphamide for the treatment of AA amyloidosis is warranted

(abstract 49)

**Ambient temperature and amyloidogenesis in mice.**


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Background: Geographic differences in the distribution of amyloidosis have been noted, with higher rates of this inflammation-related complication in northern countries on the one hand and in Africa on the other hand. These deviations from the worldwide rate of AA amyloidosis were attributed to genetic backgrounds in Scandinavian patients and to increased exposure to infections in Africa. A recent study, which found the country of living as a major risk factor for amyloidosis, related the country effect to the level of medical service available in this countries. The role of ambient temperature, as another possible environmental factor for the development of amyloidosis has not been investigated previously. Aim: The present study is aimed to examine the influence of climate conditions and acclimatization to heat on the rate and amount of amyloid deposition in the mouse
spleens. Methods: The enhanced mouse model of amyloid induction, by which amyloid is generated within 3-6 days, following intravenous administration of amyloid enhancing factor on day one and daily injections of AgNO3 for 3 days, was applied to 3 groups of Swiss mice, exposed to 3 different environmental conditions, room temperature (19 to 24°C, served as control mice), cool environment (12 to 14°C), and warm temperature (32 to 34°C). The effect of acclimatization to heat was investigated as well, by exposing mice continuously to 32 – 34°C during the month preceding the amyloid induction experiment (group 4). The crush and smear technique was used for evaluation of the amount of splenic amyloid at the end of the experiment. Results: The mean amyloid load was evaluated as 3.4, 3.6 and 3.9 in mice staying during the experiment in room temperature and in cold and warm environment respectively (Non significant differences). The mean amyloid load was also comparable for mice staying in room temperature and mice with lengthy exposure to hot environment (acclimatization, 2.9 in both). Conclusions: Ambient temperature spanning from 12 to 34°C has no effect on amyloidogenesis in the enhanced mouse model. Other environmental factors should be explored to explain local deviations from the expected prevalence of AA amyloidosis

(abstract 64)

Is AA amyloidosis always secondary?

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Background: AA (Inflammatory) amyloidosis is a late complication of various inflammatory disorders. However, for a little number of cases, no underlying inflammatory disease is found after many investigations and a long follow-up. Patients and methods: all consecutive AA amyloidosis cases for which genetic analyses for hereditary fevers were done between 1997 and 2006. These analyses were suggested either because there were symptoms and a familial history compatible with this diagnosis, or because there was no obvious cause for AA amyloidosis. Each sample was accompanied by a clinical information form; however many of them were incomplete. Then, all medical doctors in charge of these patients (disseminated all over the country) were contacted to confirm the nature of the amyloid deposits, to precise the medical history, the associated diseases and the evolution of their patients, and to discuss the final diagnosis if no mutation for hereditary fevers was found. Results: 83 AA amyloidosis cases were analysed. No information was available in 17 cases. One case was localized in the bladder. Hereditary fever was confirmed by genetic analysis in 40 cases and suggested in 7 other cases (compatible clinical feature and heterozygous mutation of MEFV gene). There was an obvious family history of recurrent inflammatory syndrome and amyloidosis for 2 children but no known mutations for hereditary fever was found. For the 18 patients who had no mutations for hereditary fever, the causal disease for amyloidosis was: Crohn’s disease (7 cases), tuberculosis (4 cases), spondylarthropathy (3 cases), rheumatoid arthritis (1 case), familial cyclic neutropenia (1 case), Enterobacteriaceae recurrent infections in a polycystic liver and kidney disease (1 case), colonic adenocarcinoma (1 case). In 5 cases, no cause was found. These latter shared common characteristics: amyloidosis was revealed by a nephrotic syndrome, there was no familial history. There were large differences in the ages at diagnosis (13-70 years old), symptoms and evolution (stable state under dialysis for one patient, severe evolution of multifocal amyloidosis for one patient, death for 3 patients). Conclusion: This study illustrates that amyloidosis can be the first manifestation of inflammatory diseases for which amyloidosis is usually a late complication; long follow-up and clinical manifestations must be monitored carefully to come up to the diagnosis. Then, this study shows that AA amyloidosis can be independent from
any underlying inflammatory disease. We suggest that “primary” AA amyloidosis exists and should motivate to find new genetic and environmental factors involved in the occurrence of AA amyloidosis.

(abstract 184)

**The analysis of Interleukin-1 Receptor Antagonist and Interleukin-1β gene polymorphisms in Turkish FMF patients: Do they predispose to secondary amyloidosis?**


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Purpose: Amyloid development in familial Mediterranean fever (FMF) patients is associated with acute phase response and the acute phase reactant Serum Amyloid A which is induced by IL-1β. Its concentration can increase to more than 1000 fold during inflammation. In view of the inflammatory nature of FMF disease we have investigated whether IL-1β and IL-1 Receptor Antagonist gene polymorphisms may be involved in amyloid development in FMF patients.

Materials and Methods: 99 FMF patients without amyloidosis; 54 FMF patients with amyloidosis and 60 healthy controls samples were genotyped for IL-1β-511 (C/T) and IL-1β+3953 (C/T) polymorphisms using PCR-RFLP and for IL-1Ra VNTR polymorphism using PCR. Results: The allele and genotype frequencies of IL-1β-511 (C/T), IL-1β+3953 (C/T) and IL-1Ra VNTR polymorphisms in FMF patients with and without amyloidosis were all compared with those in controls. There were no significant differences between FMF patients with and without amyloidosis and healthy control samples for these polymorphisms (all P values are > 0.05). Also, these polymorphisms were not associated with M694V mutation in FMF patients with and without amyloidosis. Conclusion: IL-1β-511 (C/T), IL-1β+3953 (C/T) and IL-1Ra VNTR polymorphisms are not associated with the development of amyloid in FMF patients.

(abstract 44)

**Behçet Disease**

(abstract 44)

**Behçet’s disease with mucocutaneous lesions, ocular and central nervous system involvement: long-term efficacy of infliximab with extended administration intervals.**

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Behçet’s disease is a chronic relapsing, multisystemic, inflammatory disorder classified among the vasculitides. The disease is characterized by recurrent mucocutaneous lesions (the only symptoms in mild cases), ocular (50-70%), articular (50%), vascular (25%), gastrointestinal (5-10%) and central nervous system involvement (5-10%). The aetiology of the disease is unknown, however it is thought that tumor necrosis factor (TNF) play a central pathogenetic role in the inflammatory process, with important therapeutic implications. We present here the case of a 30 year old man who was referred to us in February 2004 when admitted to the Ophthalmology Department in our hospital for an episode of low visual acuity in the left eye caused by posterior uveitis. A medical history showed recurrent aphthous ulcers in the oral cavity, present since adolescence but more
frequent since 2002, an episode of phlebitis in the right upper limb at the age of 18, lower limb athralagias which had been present for about a year, along with subcutaneous painful erythema nodosum and slight fever. The latter skin lesions usually appeared every two months and resolved in 1-2 days. The patient had also experienced occasional episodes of low visual acuity which had not been investigated beforehand. Laboratory tests showed slight leuokopenia (WBC about 4000/μL) and hypogammaglobulinemia; characterization for HLA B51 proved positive, inflammatory process indices were within normal limits. Behçet’s disease was diagnosed based on the afore mentioned clinical picture. Ocular fluorangiography indicated widespread perivascular hyperfluorescence caused by a vasculitic process; fundoscopy highlighted a flame hemorrhage of the upper sector to the left of the eye and vitreous thickening. An MRI brain scan detected gliosis thus confirming suspicion of disease. Initially, the patient was treated with three IV steroid boli (metilprednisolone 1 g) and later by oral steroids (tapering doses of prednisone 1 mg/Kg), azatioprina (150 mg/die) and steroid injections in the retrobulbar site. Methotrexate (15 mg i.m. a week) was added to therapy after 3 months owing to poor response, above all in ocular involvement. Due to the persistent activity of the disease, (two re-exacerbations of posterior uveitis in few months), after adequate tuberculosis screening and exclusion of other infectious processes, cardiovascular disease, neoplasias and /or demyelinating diseases, the patient was administered therapy consisting of infliximab 3 mg/Kg at time 0, 2 weeks, 6 weeks and then every 8 weeks. After the first administration in January 2006, the patient observed immediate benefits to both ocular and mucocutaneous symptoms. Therapy effectiveness was confirmed by the fundus exam and fluoroangiography performed two weeks later. Methotrexate therapy was maintained in combination with antiTNFα therapy. Tapering doses of prednisone were slowly tapered to a minimum maintenance dose. Brain MRI performed 10 months after initiation of treatment with infliximab was unchanged. From November 2007, disease stability and a prolonged ocular and mucocutaneous symptom free period allowed us to extend administration intervals to 9 weeks and then to 10, aiming at a further extension to 11 weeks if clinical conditions remain stable. We have found in the scientific literature that the published evidence on the use of anti-TNF agents in Behçet’s disease consists mainly of reports on the open use of infliximab, principally evaluated as an add-on therapy. An expert panel meeting was held in May 2006, in order to review the currently available treatment options for Behçet’s disease and the medical needs for those patients with severe disease, and to develop a consensus on the positioning of anti-TNF agents. Until results from adequately powered, randomized controlled clinical trials are available, anti-TNF agents are recommended to be used with caution only for selected patients with severe disease, such patients with two or more relapses of posterior uveitis per year (as in the case presented), low visual acuity due to chronic cystoid macular oedema, active central nervous system disease, intestinal inflammation or arthritic and mucocutaneous manifestations that reduce significantly the quality of life. In most cases of ocular involvement reported in literature infliximab is administered at a dose of 5 mg/Kg, while a good constant response with only 3 mg/Kg has currently been achieved in the case reported. This has recently allowed us to extend the administration intervals in an almost symptom - free patient to 24 months from initiation of therapy.

(abstract 45)

PED-BD: An international cohort study for pediatric Behçet’s disease

isabelle k. (1)*, tu-anh t. (1), khaoussou s. (1), alexia l. (1), scientific committee P. (1)

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auto-inflammatory diseases, Paris-Le Kremlin-Bicêtre, France; 7: Ankara, Turkey; 8: Montpellier, France; 9: Paris-Pitié Salpêtrière, France. Rationale BD is exceptionally observed before the age of 16 years and raises diagnosis problems because there is no specific biologic marker. Sets of clinical criteria have been proposed for adult patients only. They lack of specificity in children where the disease is often uncompleted or atypical. Aim of the study is to set-up an international cohort of patients selected on homogenous criteria established by a committee of experts, in order to help further epidemiological studies. The cohort is aimed also at supporting genetic studies. Patients and methods Centers from all over Europe, specializing in pediatric BD are expected to collaborate under the auspices of the PReS (Pediatric Rheumatology European Society) and the ISBD (international society for Behçet’s disease), to document their patients into a single database that will be available online. The scientific committee, during the last PReS meeting, has been established a list of calling signs (minimal requirement), on consensus basis, in order to define the criteria for entering the study. The participants have reviewed all items of patient charts in details. Long-term documentation is requested. Data will be periodically examined by the international scientific committee of experts and the system will recall the participants each year, automatically, to update the patient data. The study has received an ethical committee agreement. Informed consent will be obtained for anonymous collection of data and DNAs (according to the legislation of each participating country). Criteria of judgment Patients charts will be reviewed each year by the expert committee in order to classify them as definite BD (consensus), probable BD (majority of votes), or not BD. Statistics Univariate and multivariate analyses will compare each group of patients and will allow the calculation by symptoms of risks for developing BD. Financial support source is the Assistance Publique Hôpitaux de Paris, PHRC call 2007 Total 4-y grant 255 000 € Agenda A two-month period pilot study involving members of the scientific committee is ongoing from January 2008. First results will be presented during the FMFSAID meeting Perspectives This research designed to define tools for early diagnosis of BD in children is mandatory for further epidemiological studies on this topic. In the future, the prospective registration of new cases will permit better understanding of its natural history, its outcome (morbidity, mortality) and its prognosis thanks to long term follow-up and to identify risk factors. Its specific focus on pediatric cases will favor the identification of genetic factors that will be useful for earlier diagnosis of this severe and complex disease.

Blau's syndrome e other granulomatous diseases

(abstract 167)

Chitotriosidase Activity in juvenile sarcoidosis

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Background Sarcoidosis is an inflammatory disorder of unknown aetiology identifiable by the formation of confluent noncaseating granulomas. It is characterized by lymphocyte and macrophage activation and migration into involved organs. Chitotriosidase belongs to the chitinase protein family and is secreted by activated macrophages. The chitinases are able to catalyze the hydrolysis of chitin or chitin-like substrates such as 4- methylumbelliferyl chitotrioside. Methods Chitotriosidase activity was determined using the substrate 4-methylumbelliferyl-DNN’N”-triacetylchitotriosiose (MUbGlc-Nac, SIGMA Chemical Co). The substrate and serum was incubated with the serum in a citrate/phosphate buffer. The reaction was stopped by adding glycina buffer. The fluorescence of 4-methylumbelliferone was evaluated by fluorimeter at excitation 365 nm and emission 430 nm Result We report about chitotriosidase measurements in patients with juvenile sarcoidosis. They presented a serum chitotriosidase level up to 1658 nmol/h/ml at disease
onset before therapy. Erythrocyte sedimentation rate (ESR) and angiotensine converting enzyme was elevated. Under medication clinical activity improved, ESR and ACE were normalized. The chitotriosidase levels were below 700 nmol/h/ml. The chitotriosidase level in normal healthy donors was < 500 nmol/h/ml. Conclusion. Serum chitotriosidase levels could be a marker for disease activity in sarcoidosis.

(abstract 26)

An case of early-onset sarcoidosis who showed spontaneous regression of his clinical manifestations but inherited skin eruption to his baby: what should we call this systemic inflammatory granulomatosis


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Early-onset sarcoidosis (EOS, MIM #609464) usually appears before 4 years of age and has a distinct triad of skin, joint and eye disorders that is chronically progressive and in many cases result in severe complications, such as blindness and joint destruction. Here we reported a case of EOS who showed spontaneous regression of his clinical manifestations but inherited the disease-associated mutation in NOD2 and the same eruption to his baby boy. A 13-year-old boy with lichenoid papules on the trunk and extremities with 8 years history visited our clinic in 1986. After topical corticosteroids treatment, the skin eruption had temporarily disappeared, but always recurred. He had accompanied postsynechia iridis of both eyes and received trabeculotomy due to secondary glaucoma. Skin biopsy performed at the age of 15 showed epithelial granulomas in the dermis, which led us to the diagnosis of EOS. When he became 20 years old, his eruption showed spontaneous regression without any apparent incentive episodes and at that time his unaided visual acuity kept 0.7 in both eyes. During his clinical course, he had never experienced pulmonary involvement and joint dysfunctions. This rare sporadic granulomatosis, as well as autosomal-dominant disorder with same clinical manifestations termed as Blau syndrome (BS, MIM #186580), has been revealed to be associated with NOD2 mutation. In 2004 after providing the opportunities for the consultation of genetic disorders and obtaining the informed consent, we performed his genetic analysis and found a heterozygous R334W mutation in NOD2. In 2007 he got his first son who was born by normal delivery following full-term uncomplicated gestation. When his baby became 8-month-old, asymptomatic papules were appeared on the trunk and extremities that were quite similar with his father’s eruption. Two months before this episode the baby received BCG vaccination in his left arm, where non-pruritic solid papules were accompanied. We have not performed skin biopsy yet. Laboratory findings were all within normal limits and physical examination revealed no involvement of eye and joints right now. His genetic analysis revealed the same heterozygous R334W mutation. EOS/BS-associated NOD2 mutations can induce spontaneous activation of NF-κB. However, much about the pathophysiology of EOS/BS remains unclear. We do not know why and how constitutive NF-κB activation results in granuloma formation predominantly at the eyes, skin and joints. By the analysis with our recruited 20 cases with NOD2 mutation in Japan, the onset and clinical severity in EOS/BS is not always associated with NOD2 mutant-derived NF-κB activity. In contrast, there are remarkable differences on the onset and the severity of the each symptom even among the patients with same R334W mutation in our study. The clinical manifestations in a member of BS with same genetic mutation of NOD2 are also reported to be different between each patient. For example, in a single family of 11 individuals from 4 generations that Blau originally reported in 1985, 10 had arthritis, of whom 2 had skin, eye and joint involvement, and one had skin and joint disease. One patient had iritis only. In addition, our
present case with spontaneous regression of his clinical manifestations may also indicate that
clinical symptoms as EOS/BS are led not only by genetic NOD2 mutation but also required for
other exogenous factors, even though we unfortunately cannot identify the cue for provoking his
regression in this case. On the other hand, when a sporadic EOS case gets baby who shows the
same clinical symptoms associated with inherited NOD2 mutation as our present case, what should
we call this systemic inflammatory granulomatosis associated with NOD2 mutation?

(abstract 141)

**NOD2 mutation-associated Pediatric Granulomatous Arthritis (PGA): An expanding
phenotype.**


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From the description of Early Onset Sarcoidosis (1966) and Blau Syndrome (1985) it has become
clear that both conditions represent the sporadic and familial form of the same disease. Pediatric
Granulomatous Arthritis (PGA), the newly coined name for the disease is characterized by a triad of
polyarthritis, pan-uveitis and rash and is associated in 50-100% with mutations within or near the
NACHT domain of NOD2. The presence of epitheloid granulomas (EG) in synovium or dermis is
the pathologic hallmark. Until recently large vessel vasculopathy and cranial nerve involvement
were the only clinical features reported in non-terminal stages outside the classical triad. Through
the International Registry and the genotyping we identified a number of previously unrecognized
manifestations. Reporting those manifestations is the purpose of this communication. Results: From
its inception in 2005, 102 individuals have been entered and tested. Of them 31 (15 from 5 familial
pedigrees and 16 sporadic) with mutation have been identified and are the basis of this report. In
this series, the largest to date, 16 have substitution R334W, 12 substitution R334Q, two E383K and
one W490L. There are 12 females and 19 males. Average age of onset is 27 mos. (3-168) and at
recruitment 4-49 years. All have the disease classical triad at presentation with skin rash being the
most common initial symptom. Two had no EG on biopsy (1synovium, 1 skin). None had ANA or
RF. We observed no cranial nerve involvement or large vessel vasculopathy. New phenotypic
findings: Erythema nodosum-2 cases, granulomatous lymphadenopathy (1), sialoadenitis (1),
hepatic granuloma (1), splenic involvement (1), fever (1), interstitial lung disease (1), moderate
(treatment requiring) arterial hypertension not associated with renal artery disease (3), pericarditis
(1), glomerulonephritis with EGs (1), pulmonary embolism (a 38 year-old woman with no risk
factors), palpable purpura (2-documentted leukocytoclastic vasculitis in one). Conclusion: These
manifestations do not appear to be related to any particular mutation, disease duration or with being
familial or sporadic. PGA is a multisystemic disease with increasing overlapping symptoms with
classical sarcoidosis. Additional phenotype-modulating genetic factors may be involved.

(abstract 164)

**Novel Clinical Spectrum of NOD2 Mutations in Inflammatory Disease**

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BACKGROUND NOD2 encodes an intracellular pathogen recognition receptor and represents a
major susceptibility gene for Crohn’s disease. We describe 3 cases of granulomatous inflammatory disease expanding on the previously described clinical spectrum of NOD2 associated disease. In addition one of these cases had periodic fever and one a mutation in mevalonate kinase (MVK).

CASE 1 A 19 year old woman presented with swollen lower lip and cheeks associated with facial nerve entrapment. Histology revealed subepithelial infiltrate with granulomas within corial papillae compatible with orofacial granulomatosis. Genetic screening revealed NOD2 R702W and MVK V377I mutations. Serum IgD and urinary mevalonic acid normal. Infliximab followed by etanercept produced transient improvement but sirolimus produced dramatic and sustained benefit. CASE 2 A 37 year old woman developed fevers, jaundice and watery diarrhoea. Past history included Common Variable Immunodeficiency Disorder diagnosed aged 33 years and autoimmunity from childhood (diabetes mellitus, hypothyroidism, idiopathic thrombocytic purpura and haemolytic anaemia). Cholecystectomy suggested polyarteritis nodosa of the cystic artery with diagnosis confirmed by coeliac axis angiography. Liver biopsy demonstrated granulomatous hepatitis. No mutations detected in TNFR1, MVK or MEVF but NOD2 R702W positive. CASE 3 A 40 year old man presented with a 10 year history of recurrent fevers associated with joint pains and lethargy on a background of unexplained pericarditis. Acute phase response during fever attack was CRP 240mg/l and ESR 79mm/hour. CT scan showed cervical and para-aortic lymphadenopathy and hepatosplomegalgy. Lymph node biopsy revealed granulomas and fibrosis. No mutations detected in TNFR1, MVK or MEVF but NOD2 R702W positive. Good clinical response achieved with methylprednisolone followed by methotrexate. DISCUSSION NOD2 mutations occur in healthy individuals but the described role of NOD2 mutations in potentiating both NF-KB activity and IL-1B processing [1] suggests a plausible pathogenic role of these mutations in both the granulomatous and febrile manifestations of these cases. In conclusion, complex inflammatory phenotypes may reflect polygenic defects in genes of innate immunity including NOD2 and MVK (case 1) in addition to other as yet unidentified genes. [1] Maeda S, Li-Chung H et al; Science 2005; 307: 734-738

(abstract 193)

INVESTIGATION OF NOD2 POLYMORPHISMS IN COMMON VARIABLE IMMUNODEFICIENCY (CVID) AND ITS GRANULOMATOUS AND ENTEROPATHIC MANIFESTATIONS


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BACKGROUND CVID is a primary immunodeficiency with heterogeneous genotype and phenotype. It is defined by hypogammaglobulinaemia and poor vaccine responses in the absence of secondary causes. Clinical manifestations include recurrent infections and immune dysregulation eg: granulomas, enteropathy, lymphoproliferation and autoimmunity. Genetic defects associated with CVID to date account for a minority of cases. Aberrant NF-κB signalling pathways have been implicated in immunodeficiency in addition to the role of NOD2 and NF-κB in Crohn’s and other granulomatous diseases. We therefore hypothesized that NOD2 polymorphisms may contribute to CVID, or affect its disease expression e.g. presence of granulomas or enteropathy. Approximately 40% of Crohn’s disease patients carry at least one of the 3 polymorphisms (G908R, R702W and L1007f) compared to 7.8% of healthy controls. SUBJECTS A UK national cohort of 118 CVID patients fulfilling European Society for Immunodeficiencies’ criteria were studied. Data was collected on presence of granulomas and histologically confirmed enteropathy. Controls reflected...
938 healthy UK individuals from published studies. METHODS Restriction endonuclease and SSP PCR methods were used to examine for G908R, L1007f and R702W NOD2 polymorphisms from peripheral blood samples. RESULTS Allele frequencies of the 3 polymorphisms were not significantly increased in CVID patients compared to controls although there was a trend towards increased G908R polymorphisms in CVID patients (p=0.108). Subgroup analysis revealed 14% of the CVID cases studied were complicated by granulomas and 8% by enteropathy. The G908R polymorphism was increased in the enteropathic subgroup compared to controls (p=0.048) but no significant differences were found in polymorphisms in the granulomatous subgroup compared to controls. CONCLUSION NOD2 provides a link between environmental factors eg: bacterial muramyl dipeptide and cellular activation with resultant inflammation and granuloma formation. Frequency of the three NOD2 polymorphisms studied was not however increased in this CVID cohort or it’s granulomatous subgroup compared to controls. An association of the G908R polymorphism in the enteropathy subgroup was suggested. but greater numbers in the subgroup are required to confirm this association. [a] Bacchelli C, Buckridge S et al. Clin Exp Immunol 2007; 149: 401-409

**Chronic Recurrent Multifocal Osteomyelitis**

(abstract 180)

**Neutrophil Dysfunction in a Family with a SAPHO-like Phenotype**

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Background: SAPHO (synovitis, acne, pustulosis, hyperostosis and osteitis) syndrome is a complex disorder characterized by chronic inflammation of the bone, skin and joints. The etiology is unknown but phenotypic characteristics suggest that it is a disorder of innate immune system. Most cases are sporadic. We describe a family with multiple affected family members that segregate a SAPHO-like phenotype (osteitis, severe acne, pustulosis) and report results of neutrophil functional studies and candidate gene analysis. Methods: The proband had recurrent multifocal osteitis, pustulosis and acne. She had multiple family members who also suffered with inflammatory dermatosis with or without osteitis. Written informed consent was obtained from each participating family member. A detailed family history was obtained and a pedigree was constructed. Medical records were reviewed, DNA was collected and several candidate genes were sequenced. Multiple neutrophil functional studies were performed on the proband. Results: The pedigree segregates chronic recurrent osteomyelitis and an inflammatory dermatosis in a pattern that suggests an autosomal dominant disorder. Nitroblue tetrazolium and myeloperoxidase assays were normal in the proband. Neutrophil chemotaxis to IL-8 and C5a and neutrophil migration assays revealed no differences between controls and affected. However, an abnormality in the luminol but not the isoluminol respiratory burst assays following stimulation with PMA was seen in neutrophils from the affected proband and her mother. Oxidant production is also reduced in the proband and her mother when neutrophils were treated with fMLP, PAF + fMLP or TNF. No mutations were detected in the coding regions and splice sites of the candidate genes PSTPIP1, PSTPIP2, LPIN2 or p40phox. Conclusions: This family segregates a disorder characterized by chronic inflammation of the skin and bone. The inheritance pattern suggests an autosomal dominant disorder. Functional differences in neutrophils exist between affected individuals and controls. The biological significance of this defect remains unknown. It will be important to determine if this is a generalizable finding in SAPHO syndrome and related disorders. Identification of the gene defect in this family may help shed light on an immunologic pathway that when dysregulated can cause osseous and cutaneous inflammation.
Association of chronic recurrent multifocal osteomyelitis and Crohn’s disease in children


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Chronic recurrent multifocal osteomyelitis (CRMO) is the most severe form of chronic nonbacterial osteomyelitis (CNO). In children and adolescents CNO predominantly affects the metaphyses of the long bones, but lesions can occur at any site in the skeleton. Other organs (the skin, eyes, gastrointestinal tract and lungs) can also be affected. Here we describe the occurrence of Crohn’s disease in four patients, previously diagnosed with CRMO. All patients (7 – 16 years, three boys, one girl) presented with multilocal bone pain, lasting several weeks to months. Magnetic resonance imaging showed multifocal bone inflammation. The diagnosis of non-bacterial osteomyelitis was confirmed by diagnostic biopsy, including extensive microbial analysis. At the time point of diagnosis two out of four patients already presented with diffuse abdominal pain, diarrhea, intermittent subfebrile temperatures and weight loss. The remaining two developed abdominal symptoms one and two years after the diagnosis of CRMO. Gastroduodenoscopy and colonoscopy has been performed in all patients and revealed considerable inflammation of the terminal ileum and colon. Crohn’s disease was confirmed histologically by the presence of mucosal ulcers, fistulas and granulomas. A multimodal anti-inflammatory therapy, using local and systemic steroids, local mesasalazine and systemic sulfasalazine, in addition to azathioprine, was initiated in all four patients. Chronic inflammatory bowel disease is a rare associated feature of chronic non-bacterial osteomyelitis. Therefore, patients with CRMO presenting with abdominal symptoms should be screened for the presence of gastrointestinal inflammation. Crohn’s disease and CRMO seem to be based on a common autoinflammatory process. Disturbed recognition/signalling by receptors of the innate immune system has been linked to Crohn’s disease, thus, a similar pathway leading to generalized autoinflammation might be if pathogenic relevance in CRMO.

Chronic Recurrent Multifocal Osteomyelitis (CRMO): four cases treated with aminobisphosphonate (pamidronate)


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CRMO is an autoinflammatory disease of children and young adults, characterized by the insidious onset of local pain and swelling in affected bone and fever. The bone lesions are radiologically characterized as multiple luciens surrounded by defined zones of patchy but dense sclerosis, cortical thickening from periosteal new bone formation, and increased bone size with different bones involved. Diagnosis is based upon laboratory tests (elevated levels of CRP/ESR), bone scintigraphy and MRI findings and is proved by open bone biopsy. Recently bisphosphonate therapy, and particularly i.v. pamidronate, has been proposed as treatment for patients both CRMO who do not
responder to NSAIDs therapy. We report 4 cases of children affected by CRMO treated with a therapeutic cycle of aminobisphosphonate (pamidronate). In all pts. the diagnosis of CRMO was confirmed by bone scintigraphy, MRI and open bone biopsy. Pt.1. 12-years-old age (disease onset 10 yrs) presented with pain and swelling of right ankle, hip, knee and breastbone; CRP 2.4 mg/dl, ESR 100 mm/h. CRMO of right distal tibial epiphysis, right peroneal malleulus, breastbone and right hip. Pt.2. 5-years-old age presented with post-traumatic back pain in the past month and pathologic fracture of three thoracic vertebrae; normal values of CRP and ESR. CRMO of right iliac wing and thoracic spine (T8-T9-T10). Pt.3. 8-years-old age presented lumbar pain and swelling in the past month associated with antalgic scoliosis; CRP < 0.5 mg/dl, ESR 40 mm/h. CRMO of left distal tibial epiphysis, thoracic spine (T7-T8), right iliac wing. Pt.4 17-years-old age (disease onset 11 yrs) presented with post-traumatic pain and swelling of right hip and heel; CRP 1.6 mg/dl, ESR 29 mm/h. CRMO of bilateral distal tibial epiphysis, left calcaneus and neck of right femur. All 4 pts. were treated with 3 infusions of pamidronate 0.5-1.3 mg/ kg/day in 250 ml of saline solution i.v. infusion at day 1,3,5 and then monthly. Severe adverse reaction was not found except for low-grade fever and lassitude on the day following administration. In all pts a remarkable improvement of painful symptoms was quickly achieved. MRI performed 1-3 months after treatment showed an important reduction of osteolytic, sclerotic and reactive bone lesions, and normalization of inflammatory values was observed. Our experience suggests that pamidronate maybe an efficacious alternative to conventional treatment in CRMO.

Damps, PAMPs and alarmins

(abstract 123)

DAMPs induce IL-1beta synthesis, processing and secretion: modulation by extracellular REDOX

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Immune responses are initiated and perpetuated by molecules derived from pathogen-associated (PAMP) or damage-associated (DAMP) molecular pattern molecules. DAMPs include normal cell constituents released by damaged or dying cells and components of the extracellular matrix released by the action of proteases at the site of tissue injury. Toll-like receptor (TLR) 4, among the many pattern recognition receptors expressed by mammalian cells, recognizes both DAMPs and PAMPs and, hence, is involved in the inflammatory response to both infectious and non-infectious agents. TLR activation triggers several signalling pathways leading to expression of inflammatory genes. Thus, an important question is whether DAMPs can promote directly the secretion of proinflammatory cytokines. Many DAMPs are nuclear or cytosolic proteins with defined intracellular function that, when released outside the cell following tissue injury, move from a reducing to an oxidizing milieu resulting in their functional denaturation. Therefore, the redox state of the microenvironment may modulate the activity of DAMPs. Here we show that in primary human monocytes different DAMPs, such as HMGB1 and the calcium-binding protein S100A12, are competent to induce synthesis and secretion of IL-1beta in an inflammasome-dependent manner. This effect is not due to LPS contamination of the recombinant protein, as it is seen even at very low protein concentration and is unaffected by the antibiotic polymyxin B, which blocks LPS through binding to its toxic component lipid A. Furthermore, the low molecular weight hyaluronic acid, which derives from matrix degradation, is also able to induce IL-1beta synthesis and secretion. Interestingly, the DAMP-induced IL-1beta secretion is further augmented by the addition of reducing agents, such as DTT, to the culture medium, indicating that a reduced extracellular milieu might improve or prolong the activity of DAMPs. However, this effect is likely due to redox
modifications of monocytes rather than of the recombinant proteins. Indeed, similar levels of IL-1beta are secreted when untreated DAMPs or DAMPs pre-reduced in vitro are used to stimulate monocytes, without any further reduction of the culture medium. Thus, endogenously released cell constituents can induce inflammation through the activation of IL-1beta synthesis, processing and secretion and the redox state of the extracellular microenvironment modulates the process.

(abstract 161)
The Damage Associated Molecular Pattern (DAMP) Molecules and TLR-4 ligands MRP8/14 in the pathogenesis of Familial Mediterranean Fever (FMF)
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Background: The pro-inflammatory Damage Associated Molecular Pattern (DAMP) molecules Myeloid-Related Protein (MRP)8 and 14 have been recently identified as ligands and activators of toll like receptor (TLR)-4. Familial Mediterranean Fever (FMF) is an auto-inflammatory syndrome associated with activation of phagocytic cells and hypersecretion of the proinflammatory cytokine IL-1β, but exact pathogenic mechanisms are still elusive. Release of IL-1 and MRP8/14 follows an alternative route of secretion. Objectives: To evaluate MRP8/14 serum levels in FMF patients during high inflammatory episodes and during successful therapy. To study expression and secretory mechanisms of MRP8/14 in FMF. Methods: 70 genetically proven FMF patients were followed up longitudinally over a period of 18 months. Serum concentration of MRP8/14 determined by ELISA and additionally ESR, CRP and SAA as classical inflammation markers were analysed before starting of therapy and during colchicine treatment. As control groups we measured 17 Neonatal-Onset Multisystem Inflammatory Disease (NOMID), and 18 Muckle Wells Syndrome (MWS) patients. Gene expression of MRP8/14 was analysed in PBMCs of FMF patients via RT-PCR compared to healthy controls. Results: The mean serum levels of MRP8/14 in inflammatory episodes in FMF (343.210±202.210 ng/ml; n=17) were significantly higher than in NOMID (2.830±580 ng/ml; p<0.001), or in MWS (3.205±585 ng/ml; p<0.001). FMF patients treated with colchicine and not exhibiting any attacks during the study period (5480±1900 ng/ml; n=28) had significantly lower MRP8/14 levels than patients treated with colchicine exhibiting complaints typical for FMF (34.700±14.580 ng/ml; p<0.001; n=20), and also than homozygous patients never experiencing any clinical signs and not being treated with colchicine (22.310±10.110 ng/ml; p<0.05 n=5). Gene expression levels of MRP8/14 in patients with active FMF during inflammatory attack were 200 times higher than in healthy controls. Conclusion: MRP8/14 as a marker of phagocyte activation is highly overexpressed and secreted in patients with FMF. In comparison to other IL-1 driven diseases with consistently lower serum levels these new TLR-4 ligands might represent an independent inflammatory mechanism. Measurement of MRP8/14-levels in FMF might be a valuable tool to reflect disease activity and response to anti-inflammatory therapy, and might even be a highly sensitive marker for subclinical inflammatory activity.

Familial Mediterranean Fever
(abstract 121)

Novel insights into the inheritance and diagnosis of familial Mediterranean fever (FMF)
Since the genetic testing of MEFV began, it has been observed that a substantial number of FMF patients possess only one demonstrable mutation, and recent reports have also raised the question of possible dominant inheritance. These single-variant patients often have a typical disease history and respond well to colchicine. One explanation for this observation may be a lack of sensitivity in screening techniques. We report an extensive search for a second MEFV mutation in 41 patients clinically diagnosed with FMF and carrying only one structural MEFV mutation. Utilizing standard capillary electrophoresis sequencing, this cohort of patients was screened for a second disease-associated mutation in 10 exons of MEFV and a second mutation was not identified. A subset of 10 patients was then sequenced for the entire 15kb MEFV genomic region using a hybridization-based chip technology (Callida Genomics, CA). We identified 44 SNPs, including 22 novel SNPs, predominantly in introns 1, 2, 3, 4, and 6. Each patient was heterozygous for at least one of many SNPs in the MEFV genomic region, arguing against the presence of large genomic deletions. Haplotype analysis of patients with positive family history did not identify the common haplotype that should be associated with the transmission of the second FMF allele. Allelic expression using total PBMC-RNA, showed that both MEFV transcripts were expressed. qRT-PCR was performed to compare the expression levels of the group of single-variant patients with patients possessing two FMF-associated mutations and we did not observe a significant difference between single and double variant patient groups. We considered the possibility of a digenic model of inheritance by examining candidate genes encoding proteins known to interact with pyrin. Five proteins were chosen for genetic analysis in 10 patients: ASC, SIVA, PSTPIP1, POP1, and POP2. Two novel variants were identified in ASC and SIVA, but the SIVA substitution was established to be a polymorphism in a panel of Ashkenazi Jewish controls. The ASC variant had a frequency of 0.004 in a panel of Caucasian and Ashkenazi Jewish controls and awaits further characterization. Our data underscore the existence of a significant subset of FMF patients who are carriers for only one MEFV mutation. Thus, FMF may not be a simple monogenic inflammatory disease and FMF phenotype may occur in patients with only one MEFV mutation in the presence of other permissive alleles.

(abstract 144)

Can Toll-like receptor 2 polymorphism affect the phenotype of heterozygous?


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FMF is the most common of the monogenic autoinflammatory diseases caused by mutations in the MEFV gene. Although FMF is an autosomal recessive disease patients with one mutation do sometimes display the phenotype. Unidentified MEFV mutations or other genetic factors are thought to be involved. Toll like receptor 2 plays a major role in innate immune activation through recognition of microbes. The aim of this study was to search whether Arg753Gln TLR 2 polymorphism had an impact on the development of the clinical manifestations of FMF in the children heterozygous for MEFV mutations. We studied 24 children diagnosed clinically as FMF with mutation in one of their alleles. 116 healthy controls were studied as the control group. The TLR 2 Arg753Gln polymorphisms were analyzed with PCR-RFLP based method. There was a significant difference between the frequencies of TLR-2 Arg753Gln polymorphism in healthy controls (6%) and heterozygotes (25%) (p=0.01). We suggest that this polymorphism may be one of
the factors affecting the phenotypic appearance in heterozygote patients in areas where there is a high infection rate. These results need to be confirmed after these patients are tested for mutations in other autoinflammatory genes.

(abstract 146)

**Differences In The Severity Of The Phenotype Of Children and Adolescents With Familial Mediterranean Fever Residing In Turkey and Germany**


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Familial Mediterranean Fever (FMF) is worldwide the most common autoinflammatory disease. It has been long known that environmental factors affect the phenotype since patients in the United States are not expected to develop the complication of secondary amyloidosis. To substantiate this hypothesis we compared the disease-severity in Turkish FMF patients living in Turkey and Germany, based on a modified score for children. A total of 53 Turkish children living in Turkey were compared to 45 Turkish children born and raised in Germany. All were under the age of 18 years, mean age among the group from Turkey and Germany was 42.2 (range 2-120 months) and 44.29 (range 3-178 months) months, respectively. No sex difference was present between the two groups. M694V was the leading mutation in both groups. There was no significant difference between the last visit mean CRP and ESR levels of the group from Turkey (mean CRP 0.83 mg/dl, mean ESR 16.9 mm/hr) and Germany (mean CRP 0.5 mg/dl, and mean ESR 16.2 mm/hr). The score developed by Livneh et al was modified by the integration of the recommended age-related doses, previously published by us. Additionally, disease severity was determined by the use of the scoring system developed by Pras et al. There was no correlation between the disease severity defined by the different scoring systems and the acute phase reactants. According to the modified Livneh score, 78.2 % of patients from the group living in Turkey had a severe course compared to 34.1% from the group living in Germany. Pras scores were also higher in the patients born and grown up in Turkey (34.5%) compared to patients living in Germany (15.4%). The difference between the two groups for both scoring systems were statistically significant (p< 0.05 for both). We suggest that environmental factors may affect the severity of FMF even if they were coming from the same ancestors.

(abstract 168)

**Incidence of MEFV exon 10 and exon 2 polymorphisms in multiple Asian, European and African countries and a novel test for geographically restricted positive selection pressure**


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To address the paucity of data on the incidence of mutations in exon 10 of MEFV and the E148Q exon 2 polymorphism in different regional populations, as well as the geographic distribution of these polymorphisms, we sequenced exon 10 and genotyped the E148Q polymorphism in 26 groups
from 21 countries in Europe, North America, Asia and Africa (n = 3,237). The dataset comprised samples from Armenia, the Armenian Diaspora (U. K. and U.S.A.), Azerbaijan, Cameroon, Ethiopia (five ethnic groups: Afar, Annuak, Amhara, Maale and Oromo), China, Georgia, Greece, Iran, Italy, Malawi, Mongolia, Morocco (Berber), Mozambique, Papua New Guinea, Sudan, Sweden (Saami), Syria, Turkey, Ukraine and United Kingdom. We report that the exon 10 mutation resulting in the amino acid change A744S associated with Familial Mediterranean Fever was observed in four of the five Ethiopian groups and was greater than 6% in the Maale. We then developed a permutated odds ratio test that compared the frequencies of synonymous and non synonymous mutations with the frequencies of un-mutated sites to detect evidence of positive selection acting on exon 10 in the Armenian region but not in other geographic areas. The results of the geographic survey and test for selection are presented.

(abstract 199)

Dissecting Inflammatory and Chemotactic Pathways in Familial Mediterranean Fever


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Background: Familial Mediterranean fever (FMF) is a heritable autoinflammatory disease characterized by substantial neutrophil influx at sites of serosal and synovial involvement. Inflammatory attacks are usually prevented by prophylaxis with colchicine, a microtubule inhibitor. Pyrin, the FMF protein, colocalizes with microtubules and may impact leukocyte cytoskeletal functions such as adhesion and migration. We therefore sought to further investigate leukocyte migration in FMF. Methods: Peripheral blood samples were obtained from 25 treated patients, 10 untreated patients, and 16 controls. Subsets of patients were studied in several assays. Chemokines produced from cultured supernatants and from serum samples were evaluated with the Luminex immunoassay. Chemotaxis assays included a transwell system and a live imaging protocol with granulocytes stimulated to various chemoattractants, including MIP-1 alpha, IL-8 and fMLP. Analysis of gene expression sequences with the Affymetrix system was utilized in 8-paired patient samples who underwent colchicine withdrawal. Results: Microarray analysis revealed a “chemotactic signature” induced by colchicine withdrawal with upregulation of major chemotactic genes including CRK, BCL6, ADAMTS 10, and downregulation of genes involving chemokine receptors and cell adhesion molecules such as CCR 7 and CXCR3. At 72 hours off colchicine, monocytes from FMF patients stimulated with LPS produced more TNF-alpha and IL-12p70. Moreover, neutrophil activation was increased, as seen with the downregulation of the L-selectin marker, CD 62L. Patients off colchicine demonstrated significant hyperresponsive cell movement compared with treated patients and controls, although such movement was found to be random (chemokinetic) and less directed (chemotactic) when visualized. FMF patients on colchicine showed elevated serum levels of the chemokines, MIP-1 alpha and beta, as compared to controls. Conclusion: A cascade of intracellular events involving chemokine signaling can impact leukocyte migration in FMF. Current studies are focused on determining the extent to which these effects represent the underlying pathophysiology of FMF, independent of colchicine, by studying colchicine naïve patients.

(abstract 135)

Validation of a diagnostic score for molecular analysis of hereditary autoinflammatory...
Objective: Among children with periodic fever of unknown origin, only 20-25% are affected by one of the known hereditary autoinflammatory diseases, namely familial Mediterranean fever (FMF), TNF receptor associated periodic syndrome (TRAPS), and mevalonate kinase deficiency (MKD). Aim of the study was to validate a set of clinical parameters able to predict gene mutations in hereditary autoinflammatory diseases associated to periodic fever. Patients & Methods: 234 consecutive patients with a clinical history of periodic fever were screened for mutations of MVK, TNFRSF1A and MEFV genes and detailed clinical information was collected. A Diagnostic score was formulated on the basis of a univariate and multivariate analysis in genetically positive and negative patients (training set). The Diagnostic score was validated using an independent set of 76 patients (validation set). Results: Age at onset (OR=0.94, p=0.003), positive familiar history (OR=4.1, p=0.039), thoracic (OR=4.6, p=0.05) and abdominal pain (OR=33.1, p< 0.001), diarrhea (OR=3.3, p=0.028) and oral aphthosis (OR=0.2, p=0.007) were the variables independently correlated to the positivity at the genetic testing. These variables were combined in a linear score whose ability to predict the risk of positive results at the genetic testing was validated on an independent dataset. The Diagnostic score in the validation set revealed high sensitivity (82%) and specificity (72%) in discriminating positive and negative patients. A regression tree analysis was able to provide, for patients with a high risk to be positive at the genetic test, the most reasonable order of the genes to be screened. Conclusions: The proposed approach in patients with periodic fever will increase the risk in obtaining a positive results in genetic testing with good specificity and sensitivity. Our results further suggest the order of the genes to be screened. To the work has also contributed the Italian group of Autoinflammatory diseases consisting of A. Meini (University of Brescia), F. Zulian (University of Padua), L. Obici (University of Pavia), L. Breda (University of Chieti), S. Martino (University of Turin) and A. Tommasini (University of Trieste).

The MEFV Gene 3'-UTR Alu Repeat Polymorphisms in Patients with Familial Mediterranean Fever


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Objective. The MEFV gene mutations can be detected in the majority of FMF patients, but there is an important proportion of patients with FMF phenotype who carry a single or no coding region
mutation. This study aimed to investigate the promoter region and 3’-UTR polymorphisms of the MEFV gene in a group of FMF patients with no coding region mutations to identify variations with a possible role in the regulation of MEFV expression. Methods. The study group consisted of 289 patients with FMF and 103 ethnically-matched healthy individuals of Turkish origin. All individuals were first genotyped for five most commonly observed mutations (M694V, M680I, V726A, E148Q and M694I). Then, the coding regions of the MEFV gene in patients carrying none of the 5 mutations were amplified and screened by using single-stranded conformation polymorphism and DNA sequencing. After the exclusion of patients with mutations in exons, the promoter and 3’-UTR regions of the MEFV gene were investigated in the remainders. For the haplotype analysis, all study group genoyped for two of the 3’-UTR single nucleotide polymorphisms (SNP). Results. Genotyping for five mutations revealed 186 patients (64.4%) with two mutations, 61 patients (21.1%) with one mutation, and 42 patients (14.5%) with no mutation. The carrier rate for healthy controls was found to be 10%. After the screening of the all 10 exons in patients with none of the 5 mutations, we identified 36 patients (12.5%) as having no coding region mutations. Analysis of the 3’-UTR region showed two Alu repeats (AluSx and AluSq), which were located in the 3’-UTR of the reference mRNA sequence. Sequencing of the 3’-UTR of the MEFV gene showed several SNPs, which were clustered in 2 haplotypes. With the genotyping of all study group for two of the 3’-UTR SNPs (rs2741918 and rs450021), we observed a significant increase in the frequency of heterozygotes for 3’-UTR haplotypes in FMF patients with no coding region polymorphisms compared to healthy controls (75% versus 48.5%, P = 0.006, OR = 3.2, 95% CI 1.4-7.4). Conclusion. This study showed a group of 3’-UTR polymorphisms in the MEFV gene, which are clustered in two haplotypes, and a genetic association was observed between the 3’-UTR polymorphisms and FMF patients with no coding region mutations. These findings may suggest a role for 3’-UTR sequences in the regulation of the MEFV gene expression.

(abstract 147)

S-100A12 as a sensitive marker for the detection of inflammation in children and adolescents with Familial Mediterranean Fever

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S100A12 is a member of the Damage-Associated Molecular Pattern molecules (DAMP). It is expressed by activated granulocytes and exhibits its proinflammatory capacity by binding to RAGE, a receptor found on endothelium and various cells of the immune system. Its role as marker for inflammation in Familial Mediterranean Fever (FMF) is unknown. In a cross-sectional study 51 children and adolescents with the clinical and/or genetical diagnosis of FMF were followed up over a period of 18 month (mean age 10.5 yrs [3.2 – 20.4]; 19 females, 32 males, total number of visits 192). Patients presented to the pediatric outpatient clinic of the Charité, Berlin. Patients were categorized into 4 groups: (1) pats. treated with colchicine not exhibiting any attacks (n=28); (2) pats. treated with colchicine exhibiting attacks at some time during the 18 months (n=20); (3) pats. with the genetical diagnosis of FMF never having any clinical signs and not being treated (n=5) and (4) pats. newly diagnosed having FMF (n=7). During the clinical visits CrP, ESR and serum amyloid A protein (SAA) were determined. Concentrations of S100A12 were analyzed using an ELISA system established in our laboratory. In active FMF S100A12 is highly increased, in some cases concentrations were > 25000 ng/ml (upper limit of healthy controls 120 ng/ml). Mean concentration in group 1 was 490 ng/ml ± 80, which was significantly lower as compared to all other groups (vs. group 1 p< 0.001; group 2 p< 0.001; group 3 p< 0.011). In group 2 mean
concentration was 5380 ng/ml ± 1200 and in group 3 2540 ng/ml ± 1210 (no statistical difference). In group 4, mean concentration was 30000 ng/ml ± 18260, thus being statistically increased compared to the other groups (vs. group 1 p< 0.001; group 2 p=0.06; group 3 p=0.06). In general, S100A12 correlated with CrP (r=0.47, p< 0.001) and ESR (r=0.29; p< 0.01). But when comparing group 1 to group 3, S100A12 was the only increased inflammation marker (vs. group 1 p< 0.011). High S100A12 concentration correlated with the occurrence of a homogygous M694V mutation. In conclusion, S100A12 is a sensitive surrogate marker for disease and inflammation monitoring in FMF. In asymptomatic subjects with two mutations within the MEFV gene it is more sensitive in the detection of subclinical inflammation compared to the standard inflammation markers. In untreated patients, high concentrations of S100A12 can be helpful to differentiate FMF from other febrile conditions.

(abstract 170)

**ROLE OF SMALL INTESTINAL BACTERIAL OVERGROWTH IN COLCHICINE NON-RESPONDERS**

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Familial Mediterranean Fever is an autosomal recessive disease due to a genetic unbalance of innate immune response. The periodic attacks could derive from interplay of genetic and environmental factors. It was described that some infections as Helicobacter pylori can increase the severity and frequency of Familial Mediterranean Fever attacks. Small intestinal bacterial overgrowth could be associated with a releasing of antigens or metabolic productions. We hypothesized this condition could trigger Familial Mediterranean Fever attacks and consequently could lead to unresponsiveness to colchicine. Twenty Familial Mediterranean Fever non-responders, with a small intestinal bacterial overgrowth diagnosed by a H2 glucose breath test, were enrolled in our study. We evaluated laboratory and clinical features before and after three months from the eradication. A questionnaire regarding subjective severity of disease was administered. We demonstrated that the eradication of the small intestinal bacterial overgrowth leads to a significant improvement both of laboratory and clinical features. The production of bacterial antigens could induce Familial Mediterranean Fever attacks and consequently could simulate a colchicine unresponsiveness. We suggest that patients with unresponsiveness to colchicine treatment should be investigated for small bowel bacterial overgrowth by a fair and simple test as H2 glucose breath.

(abstract 222)

**TRENDS IN COLCHICINE TREATMENT IN FAMILIAL MEDITERRANEAN FEVER (FMF)**

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35 yrs after the colchicine (Co) introduction in FMF treatment, many issues, such as co-resistance (CoRs) or intolerance (CoI), still remain unresolved. To evaluate the current trends, we sent a
questionnaire to clinical FMF centers worldwide. We received 24 completed questionnaires from 8 countries, covering a total of 4563 treated patients (pts), 1230 < 10 y.o. and 2473 ≥ 20 y.o. Interviewed physicians have had experience with Co for 15.5 yrs on average. 83% of them perform a diagnostic test with Co (43% regularly). In adults, 71% of them use 1 mg/d initial dosage, 86% 1-1.5 mg/d maximum dosage, 57% 2 mg/d as the highest tolerated dosage. In children < 5 yrs of age, 93% of them use ≤ 0.5 mg/d initial dosage, 80% ≤ 1 mg/d maximum dosage and only 33% reached 1.5-2 mg/d; in children ≥ 5 yrs of age, 60% of them use ≤ 0.5 mg/d initial dosage, 93% ≤ 1 mg/d maximum dosage, 47% reached 2.5 mg/d. All the physicians monitor Co dose on the disappearance of attacks, 64% also on reduced acute phase reactants (APR). 76% think that protracted arthritis is not linked to Co, 53% leg pain. 73% perform a gradual increase of Co dose; 65% went over 2 mg/d mainly in adults. 95% of them use ≥ 2 mg/d in Co resistant, 75% use 1-1.5 mg/d in intolerants. 75% of them don’t increase Co before menses but 83% increase it in proteinuric pts. 85% keep the same dose in pregnancy; adverse effects with statins and macrolides were observed. Up to 33% of pts reduced and 1-10% stopped Co because of side effects (gastrointestinal 90%). 50% of the physicians suggest to test response and tolerance every 6 months: all ask for urinalysis; 87% WBC, 74% ESR and 69% liver tests and CRP, 39% muscle enzymes. Few cases of allergy were reported. 45.5% suggest lactose withdrawal. 83% consider diarrhea as CoI, 56% myopathy, 48% neuropathy. 91% define a CoRs on the basis of observation time, 83% on the basis of attack frequency, 56% of Co dose, 48% of abnormal APR during attack-free period. In terms of the criteria for CoRs, 94% of physicians refer to attacks, 55% to persistent high APR, only 16% to onset or worsening of renal disease and 11% to Co dose. 84% suggest diarrhea as sign of CoI, 35% neuropathy, myopathy, leucopenia, only 16% refer to Co dose. Only 39% tried an alternative treatment (anti-TNF-a, IFN or thalidomide). This study shows a large variance in Co use for FMF worldwide and confirms the need of standardized definitions of Co resistance, intolerance and guidelines.

Clinical Disease among FMF Heterozygotes

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Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by recurrent attacks of fever and serositis. Traditionally FMF is considered an autosomal recessive disorder. In most patients who had undergone genetic analysis two mutations in the disease gene (MEFV) were demonstrated, but in 15-25% only one mutation was found. Most of these patients were checked for only a limited number of specific mutations and were thought to carry less common MEFV mutations on the second allele. However, a number of studies failed to demonstrate such mutations even when MEFV was fully sequenced. We performed a comprehensive genetic workup of 22 patients that were previously found to carry one mutation, all of whom were diagnosed as suffering from definite FMF according to the Tel Hashomer criteria. The analysis included full sequencing of the cDNA, multiplex ligation-dependent probe amplification (MLPA), and haplotype analysis in patients who
had siblings that also suffered from FMF. In two of the 22 patients a second mutation was found. However, in 20 of the patients we could not identify an additional mutation, a large genomic deletion or duplication on the second allele. Analysis of SNP's along the cDNA ruled out lack of expression of one of the alleles. In two of the three families where more than one sibling was diagnosed with FMF we could show that the affected siblings inherited a different MEFV allele from the parent without the MEFV mutation. In the 20 patients with one mutation the most prominent features were fever and abdominal pain. Arthritis and ELE were rare and amyloidosis was not found in any of them. The heterozygous patients tend to have a relatively mild disease but can not be distinguished on a clinical basis from homozygotes. The findings presented above are highly consistent with the existence of a clinical phenotype among some FMF heterozygotes and could explain vertical transmission in some families. Although rare reports on disease occurring in the context of one mutation have been previously published, our data suggests that the extent of this phenomenon may be much more common than previously thought.

(abstract 124)

**Altered Development and Function of CD11c+ Cells in Pyrin-Null Mice**


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The familial Mediterranean fever (FMF) protein, pyrin, is encoded by the MEFV gene and expressed in myeloid/monocytic cells upon lipopolysaccharide (LPS) stimulation. Mutated pyrin causes FMF, a recessively inherited autoinflammatory syndrome, presenting in humans with recurrent episodes of fever, polyserositis and subsequent development of amyloidosis. Although pyrin is known to modulate inflammation and apoptosis, its overall function has not been completely elucidated. To better understand the physiologic role of pyrin in vivo, we have successfully generated pyrin-null mice (pyrin -/-) by disrupting exons 1 and 2 of murine Mefv. These animals were produced at the expected Mendelian frequency, developed normally, and reproduced as well as wild-type (WT) littermates. Resident peritoneal cells from both pyrin -/- and WT mice were harvested, adherent cells were treated with LPS ex vivo, and the mRNA was compared by microarray analysis. A large functional group of differentially expressed genes was found to be involved in inflammation and the immune response. Of these genes, CD11c – the prototypic marker of murine dendritic cells (DCs) – was downregulated in pyrin -/- mice relative to WT animals, which was confirmed by quantitative real-time PCR. Flow cytometric analysis of peritoneal cells and splenic leukocytes revealed a 20% and 15%, respectively, reduction of the percentage of CD11c+ cells in pyrin -/- compared to WT mice. Following the ex vivo generation of DCs from the bone marrow, a similar difference of CD11c+ cells between pyrin -/- and WT mice was found, which was mainly caused by a reduced percentage of CD11c+ precursor cells in pyrin -/- relative to WT animals. Severe abdominal symptoms, as peritonitis and diarrhea, often accompany the febrile episodes of FMF. This prompted us to further analyse the intestinal CD11c+ cell distribution and function in pyrin -/- and WT mice. Whereas the percentages of CD11c+ cells were comparable between pyrin-/- and WT animals, intestinal CD11c+ lamina propria cells of pyrin -/- mice appeared to show an aberrant pattern of cytokine expression, which was not seen for splenic CD11c+ cells of these mice. Based on these results, we hypothesize that pyrin is involved in the development of dendritic cells from their myeloid precursors and may exert a tissue-specific
functional deficit potentially leading to a dysregulated intestinal immune homeostasis.

(abstract 176)

RNA interference of MEFV in THP.1 cells reveals a role for endogenous pyrin in Toll-like receptor signaling (TLR) that is mediated by the transcription factor IRF2

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The gene causing familial Mediterranean fever (FMF), MEFV, encodes a protein, pyrin that is expressed at high levels in granulocytes, monocytes, dendritic cells and in some human myeloid leukemia cell lines, such as THP.1. Although pyrin appears to play a role in the regulation of IL-1? and NF?B activation, its function has not been completely defined. To provide molecular insight into pyrin function, RNAi technique was employed to compare gene expression profiles between THP.1 cells expressing endogenous pyrin (SC) and cells in which the gene had been knocked down (siMEFV). Using Affymetrix cDNA microarray analysis to identify potential novel pyrin-dependent pathways, we identified over 300 genes differentially expressed in siMEFV treated cells compared to control. Based on Gene Ontology Classification, subsets of down regulated genes (CD36, CD14, MD2, TIRAP and MyD88) were found to be involved in Toll-like receptor (TLR) signaling. Western blot analysis confirmed reduction of protein for CD36 and MyD88. Flow cytometry analysis of CD14 demonstrated a 2-fold reduction in siMEFV treated cells compared to SC. It has been previously shown that CD36 functions as a co-receptor involved in the recognition of LTA via the TLR2/6 pathway. Functional analysis for CD36 showed inhibition of TNF? production in siMEFV treated cells after LTA stimulation. Consistent with the down regulation of the genes identified, stimulation of TLR agonists revealed a reduction in TLR2/1, TLR2/6 and TLR4 signaling. NF?B activation with E. coli LPS was also suppressed. Phagocytosis assays demonstrated a reduction in the ability of siMEFV treated cells to internalize E. coli bioparticles. Interferon regulatory factor 2 (IRF2) was found to be down regulated in siMEFV treated cells. To investigate the possibility of IRF2 as a common regulator of the TLR genes investigated, promoter analysis was used. We found that downregulated TLR genes showed enrichment of binding sites for the interferon regulator factor family (IRFF). Expression profile screening of IRFF members revealed IRF2 as the most significantly changed IRFF. IRF2 was validated by qRT-PCR and Western blot. FMF patients on and off colchicine had reduced IRF2 mRNA relative to controls, indicating that mutations in MEFV may in some cases act like knockdown in THP.1 cells. These data suggest that, directly or indirectly, endogenous pyrin facilitates signaling in part by its effect on IRF2.

(abstract 87)

A NEW SET OF CRITERIA FOR THE DIAGNOSIS OF FAMILIAL MEDITERRANEAN FEVER IN CHILDHOOD


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Objective. Although the gene of familial Mediterranean fever (FMF) was identified a decade ago the diagnosis is still based on clinical criteria. Existing criteria have been developed mainly for adult patients. However, as the episodes of FMF typically appear in childhood, these criteria need validation in children. The purpose of the present study is to establish a new set of criteria for use in childhood. Methods. The study group consisted of recently diagnosed FMF patients who had mutations at both alleles and who were initially examined in one of the four main centers for pediatric nephrology and rheumatology. 170 consecutive FMF patients (88 males, 82 females) between August 2007 and January 2008 were interviewed by one of the experienced physicians about the presence of 35 features and manifestations of FMF at the time of diagnosis. Controls were consecutive patients without FMF (n: 83) who had episodes of fever and clinical features mimicking that of FMF. The diagnostic performance of the candidate features was assessed by multiple logistic regression analysis. Outcome variable (FMF or Controls) was cross-classified with a predicted group variable whose values were derived from the estimated logistic probabilities. To obtain the derived predicated variable, a cut-point (0.50) was defined and compared each estimated logistic probability to 0.50. If the estimated probability of a patient exceeds the 0.50 then the patient was considered as a FMF, otherwise the patient was considered as a control. Results. The multiple logistic regression analysis showed that 6 of the 85 candidate criteria discriminate FMF from controls with a sensitivity of 92% and specificity of 94%. These 6 criteria were; fever (over 38°C, 6-72 hours of duration), abdominal pain (6-72 hours of duration), chest pain (6-72 hours of duration, unilateral), arthritis (6-72 hours of duration, oligoarthritis), exertional leg pain and family history of FMF. The presence of two or more of these six criteria diagnosed FMF with a sensitivity of 91% and a specificity of 88%. An equation for probability was also developed for calculating the likelihood of FMF that may guide clinicians to seek genetic analysis. Conclusion. The proposed diagnostic criteria were found highly sensitive and specific and may be used to diagnose FMF and to distinguish it from other periodic fever diseases in childhood.

(abstract 53)

EXPERIENCES OF HEALTH CARE FOR CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER IN ARMENIA

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Familial Mediterranean Fever (FMF) is a serious medical and social problem for Armenia. It’s determined by the ethnic origin of disease, high gene frequency (up to ~30%). As most cases of disease start at early age, early diagnosis and proper management of cases of FMF in childhood is crucial for prevention of life-threatening complications of diseases. In the latest history, Armenian pediatricians started observations of FMF in Armenian children in early 60s (Astvatstaryan V et al, 1962). Epidemiological observations done in 90s showed that FMF is spread unequally among children’s population of different regions of the country and the prevalence rate varied from 1:700 to 1:5000. The rate some correlated with some geographic characteristics such as attitude over sea level, annual duration of sun exposure and wind’s average annual speed. Also the prevalence rate is relatively higher in the rural areas, especially among the descendants of the people, migrated from some particular regions of the Western part of the Armenian plateau. Amyloidosis was common:
before introduction of regular therapy by colchicine the frequency of amyloidosis among children with FMF (until age of 15) was as high as 16.2%. Over last years, especially last decade, the number of children with FMF in Armenia has been dramatically and constantly increasing. For instance, comparison of number of cases registered by the health care facilities showed that during past 12 years the number of pediatric FMF patients increased in some regions two times. Moreover, FMF becomes “younger”: currently average age of FMF manifestation is 3 years and the number of cases of FMF, diagnosed among children up to 3 years- old increased from 1.5% up to 8.5%. Also we register more often severe and atypical course of disease and cases of prolonged arthritis, protracted febrile myalgia, skin lesions as well as family cases. Taking into consideration increasing prevalence of FMF, high incidence of amyloidosis, need for early detection of disease in children, the problem of FMF, its treatment and follow up care were recognized as one of priorities of the Armenian health care system. Generally, most health services for children with FMF are under state financing. The National Pediatric FMF Centre has been established at the Institute of Child and Adolescent Health; the Centre provides not only diagnosis, but also long-term follow up care for sick children up to age of 18 years. In 2003 the program on “Early diagnosis and treatment of FMF in children in Armenia” has been initiated; the “Howard Karagheusian” Commemorative Corporation (which is a recognized benevolent organization of the Armenian Diaspora) supported the program. The program currently is being implemented in close collaboration with the Ministry of Health of the Republic of Armenia, the Yerevan State Medical University and the Centre of Medical Genetics and Primary Health Care. The main goal of programs is improving overall management of the patients and prevents development of the complications. For that a number of interventions were started, including educational, clinical, research activities. Educational activities include developing and publishing the guidelines for doctors and nurses, publishing of leaflets for parents, emission of educational programs by mass media. Over last years the Centre's staff regularly visited provinces of the country and provided the screening of patients, suspicious for FMF. Considering high prevalence of FMF in some regions, the regional branches of the FMF centre have been established in large cities. The program resulted in increasing referrals and a number of patients with FMF on regular follow up care: since implementation of the program the total number increased from 500 to 1500. The annual number of newly diagnosed pediatric cases reached about 300 in 2007. Last 5 years no new case of amyloid complications has been registered among patients managed by the Centre. Most patients (87%) are citizens of Armenia, while 13% are Armenians from different countries of former USSR (Russia, Georgia, Ukraine etc). Since 2003 thanks to support of the “Howard Karagheusian” Commemorative Corporation, patients of the Centre are provided by colchicine free of charge. The Centre is planning to expand regional activities, improving access to care and mechanisms for colchicine supply, continuing educational programs. Also some studies are going-on or planned, including epidemiological aspects, atypical forms of FMF, mechanisms of development of amyloidosis and peculiarities of dialysis and kidney transplantation in case of FMF.

(abstract 54)

COMPARATIVE EVALUATION OF THE EFFECT OF IMMUNOGUARD®, KANJANG® AND COLCHICINE ON NITRIC OXIDE LEVEL IN BLOOD PLASMA OF CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER IN DEPENDENCE OF MEFV GENE MUTATIONS

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Recently a new non-toxic anti-inflammatory herbal preparation known as ImmunoGuard® has been shown to relieve inflammatory attacks in Familial Mediterranean Fever (FMF). The main curative effect of ImmunoGuard® relates to its active principle, i.e. the andrographolide. Andrographolide is a well-known anti-inflammatory, analgesic and immunostimulatory agent presents in extracts of Andrographis paniculata. It is used, particularly, in adaptogene KanJang® for prevention and treatment of common cold in Scandinavia. KanJang® contains relatively higher concentration of the andrographolide. Unlike conventional NSAIDs, the mechanism of action of andrographolide is probably associated with the inhibition of the PAF-mediated inflammatory response, the inhibition of expression of an important mediator of inflammation of nitric oxide (NO) synthesis in macrophages, and the modulation of production of cytokines (IFN- and TNF-a). Recently we have evaluated the effect of adaptogen ImmunoGuard® to the level of NO in blood plasma of the children with FMF. There was found a relationship between beneficial effect of ImmunoGuard®, reducing severity of inflammatory attacks in FMF patients and their NO blood level. It can be speculated that decrease of the blood NO level can provoke an inflammatory attack in FMF patients. Considering the anti-inflammatory efficacy of andrographolide we conducted the comparative evaluation of the effect of ImmunoGuard®, KanJang® and Colchicin to NO level in blood plasma of children with FMF depending on MEFV gene mutations spectrum. NO was measured (as an accumulation of plasma NO3-/NO2 by capillary electrophoresis) in total 193 FMF patients (mean age 7.5) blood plasma; 46 patients were treated with ImmunoGuard®, KanJang®, 36 patients received placebo, 111 children were permanently treated with Colchicine, and 22 healthy individuals were taken as a control group. The level of NO in the blood plasma was investigated in several groups of FMF patients with different MEFV gene mutations (genotypes) before and after 3, 6, 9, 12 months of the treatment during the attacks and in attack free periods of the disease. The clinical trial with ImmunoGuard®, KanJang® for the 1st group of patients with most common and pathogenic (hard) mutations (M694V/M694V; M694V/M680I; M694V/V726A; M680I/M680I) was of shorter term and lasted 6 months. Afterwards the colchicinotherapy was started. The 2nd group of the FMF patients with the carriers of some heterozygous (M680I/N; M694V/N; V726A/N) mutations as well as in (the 3rd group) some compound-heterozygotes (M694V/R761H; M694V/E148Q; M680I/R761H; V726A/M680I; V726A/R761H) were treated with ImmunoGuard®, KanJang® for 12 months. Colchicinotherapy has shown the NO blood level close to that of healthy subjects just after 3 months (p < 0.05) treatment in all types of MEFV gene mutations and this normal level persisted during the whole period of clinical trial (one year). The blood plasma level of NO in all three groups of FMF patients with different MEFV gene mutations after treatment with ImmunoGuard®, KanJang® was almost the same. NO blood plasma level in FMF inactive patients has been significantly increased (p=0.0025) after 3 months treatment in comparison with patients treated with colchicine and control healthy children. The severity and duration of the FMF attacks decreased. However, the patients with hard mutations (the 1st group) after treatment with ImmunoGuard® as well as KanJang® have shown more expressed and persisted elevation of the NO blood levels during all 6 months of the trial. Elevation of the NO blood plasma levels in the rest two groups of FMF patients was less expressed and in further tended to decrease rapidly and equal to that of patients subjected to colchicinotherapy and healthy persons. These data confirm that new herbal drug-adaptogenes ImmunoGuard® and KanJang® can be used as an additional safe and effective medicine for the management and treatment of some FMF patients, especially in cases with mild and moderate course of the disease (rare, short-term attacks with chest pain predominance, etc.) with some spectrum of MEFV gene mutations (heterozygous and some compound-heterozygous genotype).

(abstract 35)

Familial Mediterranean fever affects the composition of intestinal microbiota.
Familial Mediterranean fever (FMF, MIM249100) is a recessively inherited autoinflammatory disorder characterized by recurrent self-resolving attacks of fever and polyserositis, with chronic subclinical inflammation during the asymptomatic remission periods. Mutations responsible for the disease were located in the MEFV gene, which encodes pyrin/marenostrin, the protein involved in regulation of the innate immunity. In our study we investigated how this disease may affect the composition of intestinal microbiota. A total of 19 FMF patients with clinically confirmed disease and eight healthy individuals were enrolled in this study. All study participants were genotyped for mutations in the MEFV gene. The composition of commensal intestinal bacteria was determined by two independent techniques, sequence analysis of 16S rDNA clone libraries and FISH analysis using 12 hybridization probes covering almost the entire human gut bacterial diversity. These analyses demonstrated that the disease causes significant changes in bacterial community structure characterized by major shifts in bacterial populations within the Bacteroidetes, Firmicutes, and Proteobacteria phyla in remission and acute states of disease. In remission, bacterial diversity values were higher than in control but the bacterial composition was deviant from the norm, with the significant increase in proportion of Enterobacteriaceae, Acidaminococcaceae, Ruminococcus and Megasphaera and significant reduction of Roseburia. Attack periods were characterized by depletion of total numbers of bacteria and loss of bacterial diversity. The proportion of Prevotellaceae, Dialister and Prevotella was significantly lower while the Porphyromonadaceae, Phascolarctobacterium, Faecalibacterium, Roseburia, Acidaminococcaceae, and Parabacteroides were significantly increased during attacks. Interestingly, there were also differences due to the disease state (remission or attack) and the affected bacterial groups were Acidaminococcaceae, Porphyromonadaceae, Megasphaera, Dialister, Faecalibacterium, and Parabacteroides. Discriminant function analyses of clone libraries and FISH data revealed three distinct clusters in bacterial distribution that are highly specific, well separated, and distinct for healthy controls and for the remission and attack phases of FMF. This is the first report, which reveals that the autoinflammatory disease state such as FMF affects bacterial diversity in the gut and results in specific restructuring of its composition. It is not clear what are the mechanisms involved in selection of certain groups of bacteria in FMF. In attack this may be explained by the innate immunity activation resulting in acute inflammation, with elevated body temperature due to fever as well as by PMN infiltration, which causes local oxidative stress, harmful for the mostly anaerobic bacteria in the gut. Still, the microbiota composition does not return to the normal state in asymptomatic FMF patients suggesting continued selection in remission, possibly through the adaptive immunity, which is activated in acute inflammation period and primed against certain groups of commensal bacteria in the gut.

(abstract 36)

Cytokine network alterations in sera of Armenian patients with Familial Mediterranean fever


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Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease, predominantly
familial Mediterranean fever (FMF), one of the most common autoinflammatory syndromes, is caused by recessive mutations in the MEFV gene, which encodes pyrin/marenostrin. FMF is characterized by acute, self-limiting episodes of fever and serositis with massive influx of polymorphonuclear neutrophils into the affected sites. Despite the well-defined genetics of the disease, the mechanisms that trigger periodic acute inflammation attacks and contribute to chronic subclinical inflammation in remission periods of FMF remain largely unknown. In our study, we investigated the possibility that intestinal commensal bacteria and their ligands may be responsible for the disease phenotype in a genetically susceptible host. The normal immune system maintains the low-level inflammation in the gut and this inflammation is tightly regulated. But in autoinflammatory disorders such as FMF, the immune system may over-react and mount inadequate

Specific immune responses to commensal microbiota in FMF


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responses against commensal bacteria. Thus, we investigated the systemic immune responses to commensal bacteria (bacterial antigens) in FMF in comparison with healthy subjects. For this, we isolated a number of bacteria from fecal specimens of four FMF patients. Taxonomic affiliation of bacteria was confirmed by sequence analysis of 16S rRNA genes. The Bacteroides species were the most frequently isolated bacteria, followed by Escherichia coli. Other frequently isolated bacteria were from the genera Parabacteroides, Enterococcus, and Lactobacillus. In total, the lysates of 16 different bacterial strains belonging to five bacterial genera were subjected to immunological analyses. The levels of specific IgG and IgA in the sera of 13 FMF patients and 11 healthy controls against the antigens of commensal bacteria such as Bacteroides, Parabacteroides, Lactobacillus, Enterococcus, and Escherichia coli were measured by ELISA and analyzed further by Western blot. Significantly increased titers of IgG against the antigens of P. distasonis, E. coli and B. ovatus were observed in the sera of FMF patients in comparison with controls, but there were no significant differences in IgA titers. Western blot analysis demonstrated the presence of IgG antibodies directed against the multiple protein antigens of commensal bacteria. In respect to colchicine treatment, no significant differences in IgG titers in the sera of colchicine-treated or untreated FMF patients were observed. On the contrary, IgA titers tended to be statistically higher in the sera of colchicine-free FMF patients than in the colchicine-treated group. Thus, FMF is characterized by the overly aggressive adaptive responses against the commensal microbiota, which is usually well tolerated by the normal immune system. It remains to be seen, however, whether this is the result of excessive translocation of bacterial antigens through the epithelial barrier or the consequence of heightened sensitivity to the normal bacterial antigens in this disease.

(abstract 59)

Impaired resolution of inflammation during Familial Mediterranean fever is associated with heightened endotoxin susceptibility of monocytes and neutrophils

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1) Samvel Avetisyan; 2) Gagik Hakobyan; 3) Impaired resolution of inflammation during Familial Mediterranean fever is associated with heightened endotoxin susceptibility of monocytes and neutrophils Tigran K. Davtyan1, Samvel A. Avetisyan2, Vachagan A. Harutyunyan2, Gagik S. Hakobyan3 1Laboratory of Immunology and Virology, "Armenicum" Research Centre, CJSC Armenicum, 37 Nalbandyan str., Yerevan 0001, Republic of Armenia 2Department of Pathophysiology; Yerevan State Medical University, Yerevan State Medical University, Koryun 2 St., Yerevan 0025, Republic of Armenia 3Department of Internal Medicine; Yerevan State Medical University, Yerevan State Medical University, Koryun 2 St., Yerevan 0025, Republic of Armenia Familial Mediterranean fever (FMF) is a relapsing autoinflammatory disorder, caused by various mutations in the gene MEFV, which encodes a protein called pyrin, expressed in neutrophils and activated monocytes. We have shown, that induction of monocyte endotoxin homo- and cross-tolerance takes place in FMF patients during attack, whereas monocytes from patients in attack-free period failed to induce LPS tolerance and exhibited heightened sensitivity to bacterial endotoxin. Induction of anti-inflammatory cytokine synthesis polarization and enhancement of LPS-induced monocyte apoptosis was observed in FMF patients during attack, whereas monocytes from patients in remission period exhibited pro-inflammatory cytokine polarization and resistance to repeated LPS-induced apoptosis. Colchicine induced anti-inflammatory cytokine synthesis and caused down-modulation of monocyte apoptosis, whereas cytokines did not alter LPS-induced monocyte apoptosis. We demonstrated that impaired LPS tolerance induction in attack-free FMF patients correlates with different time course pattern of LPS-induced changes of CD14 and CD11b co-receptor monocytic surface expression, which is characterized by delayed turnover of CD14 or increased surface retention of CD11b receptors on
monocytes during stimulation with LPS. In addition, enhancement of LPS-induced apoptosis of neutrophils is observed in FMF patients, which is furthermore confirmed by the facts that neutrophils from previously unexposed to Salmonella enteritidis FMF patients exhibited heightened susceptibility to the LPS of this pathogen similar to that of Salmonella enteritidis infected patients. In conclusion self-limited nature of attacks during FMF may represent periods of inflammation resolution compensatory to continued sub-clinical inflammation during the remission.

(abstract 5)

**Peculiarities of the course of familial Mediterranean fever in patients survived after diffuse purulent peritonitis**

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Familial Mediterranean fever (FMF) is a periodic febrile disorder, characterised by fever and serositis. The abdominal attacks of FMF yield a pattern of an acute abdomen. On this background the acute appendicitis, provoking attack of FMF, can not be diagnosed beforehand and can result in development of diffuse purulent peritonitis (DPP). Among about 5000 observed by us patients we found 33 such cases. Among them 19 patients were men (57,6 +/- 8,60 vs 59,8 % in general contingent of FMF patients), 14 – women (42,4 +/- 8,60 vs 40,3 %, NS). With mixed form of FMF suffered 21 of them (63,6 +/- 8,37 vs 56,6 %), abdominal – 9 (27,3 +/- 7,75 vs 34,9 %), thoracic – 3 (9,1 +/- 5,00 vs 8,5 %). The purpose of the present research was comparison of the course of FMF in these patients and in patients with FMF of the general contingent. The mean age of manifestation of FMF attacks in investigated contingent of patients was 13.1 years (1 monthly age up to 38 years old). DPP and followed laparotomy were in average age 20.6 years old (range 2-67). In patients survived DPP, only in young age the higher probability of often attacks (100 +/-4,65 vs 40,0 +/-0,83 %, within the second decade of life) and the expressed articular syndrome (79,0 +/-9,35 vs 36,4 +/-0,81 %, during the second, and 100 +/-7,98 vs 35,5 +/-0,96 % - within the third decade of life), than in the general population of patients with FMF is observed. It is possible, that DPP promotes complications of the autoimmune disorders (few observations) and adhesive desease (12,1 +/-5,68 % vs 2,88 +/-0,249 %), but does not influence on the frequency of complication with amyloidosis (5,3 +/-5,12 vs 6,67 +/-0,42 %, within the second decade of life). Thus at the patients with FMF who has survived after DPP, only at young age attacks of FMF are observed more often than in the general contingent of patients, and the articular syndrome happens more expressed. It is possible, that DPP promotes also to complications of the autoimmune nature, and also to adhesive processes in an abdominal cavity, but does not influence on the frequency of amyloidosis. The described events should guard also doctors about an opportunity of development of an acute appendicitis on a background of attack of FMF, and despite of rather favorable course of peritonitis at FMF patients, it is desirable to observe an attack of FMF in a hospital and at the patient without previuos performed appendectomy in case of the slightest deflection of FMF attack from the previus observed stereotype attacks - to operate patients.

(abstract 6)

**Probability of renal amyloidosis in patients with familial Mediterranean fever in dependence on dynamics of attacks frequency and articular syndrome during first decades of life**

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There is collected information about 1894 patients - inhabitants of Yerevan, suffering from classical (serositical) forms of familial Mediterranean fever (FMF). In inhabitants of Yerevan the probability of amyloidosis is influenced basically with course of attacks in the first decade of life even if after often attacks in the first decade of life there comes the season of infrequent attacks in the second decade (probability of survival without amyloidosis up to 20-years age – 72.2 %). However rate of FMF attacks in the first decade of life has much smaller value from the point of view of probability of amyloidosis, than age of the beginning of FMF attacks. In inhabitants of Yerevan the probability of amyloidosis is influenced strongly with course of articular syndrome in the first decade of life even if after the expressed articular syndrome in the first decade of life there comes the season of not expressed articular syndrome in the second decade (probability of survival without amyloidosis up to 20-years age – 86.05 %). The expressiveness of articular syndrome in the second decade of life of FMF patients, at inexpressiveness of last in the first decade, also apparently influences on probability of survival without amyloidosis (89.11 %). Researches at a 40-years boundary have shown that course of articular syndrome in the first decade of life has crucial importance by way of probability of amyloidosis development. Even presence of articular syndrome from the second till the fourth decades of life in an event of that absence in the first decade of life, is the much more favorable course of FMF (probability of survival without amyloidosis up to 40-years age – 66.67 %). Besides improvement of articular syndrome after 3 decades of the expressed articular syndrome reduces chances of survival without amyloidosis (54.55 %). It is interesting, that chances of a similar surviving are higher, if after not expressed articular syndrome in the first decade decades of the expressed articular syndrome follow (100 %), than when during all 4 decades of life in FMF patients it was not observed the expressed articular symptomatology (91.46 %). In the prognostic plan the presence of often attacks of FMF in the first decade of life has the greater value, than an expressiveness of articular syndrome for the same period (in an event of often attacks and the expressed articular syndrome – 82.51 %, without the expressed articular syndrome – 88.21 %). However, at absence of often FMF attacks in the first decade of life the expressiveness of articular syndrome for the same period also has significant prognostic value by way of probability of complication of FMF with amyloidosis (93.96 vs 98.12 %).

(abstract 21)

The frequency of development of amyloidosis in patients with familial Mediterranean fever in cases of different surgical pathologies

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Background: Familial Mediterranean Fever (FMF) is an inherited auto-inflammatory disease, characterised by recurrent and self-limiting episodes of fever and serositis, in particular peritonitis that simulates perfectly an acute abdomen. Increased acute phase reactants and white blood cells count contribute to confound the diagnosis in the patients with acute abdomen due to FMF. So FMF patients often report very long clinical histories including even some surgical operations. Some FMF patients develop generalized amyloidosis, which can be fatal. Colchicine therapy modifies the natural history of the disease by decreasing the attack frequency and preventing amyloid deposition. In the scientific literature we have not found if the surgical operations influence on the probability of amyloidosis in FMF patients. The purpose of the study was to investigate the frequency of development of amyloidosis during definite decades of life in patients with FMF in cases of different surgical pathologies. Methods: According to data of the journal of colchicine distribution of the Republican Scientific-practical Center on FMF, of the journals of line of hospitals of Yerevan, to data, given by the district therapeutics of the polyclinics, and to data obtained from
another sources, it is composed the register of FMF patients and patients suffered with FMF-like diseases, which on 25.12.2000 included 10079 patients. For period 1977 - 2000 years catamnestic data of 4488 patients are obtained. With classical (serositical) forms of FMF suffered 4167 patients, among these 2490 were of masculine gender (59.8%), 1677 - feminine (40.3%). With mixt form of FMF suffered 2358 patients (56.6%), abdominal - 1455 (34.9%), thoracal - 354 (8.5%). Results: The results show that amyloidosis was developed in 6.67 % of patients during second, in 9.93 % - during third, in 10.8 % - 4-th, in 13.4 % - 5-th, in 14.1 % - 6-th, in 16.1 % - 7-th, and in 11.5 % - during 8-th decades of life. Our investigations show also that no performed tonsillectomy (according to decade beginning with second: 7.4 %, 6.4 %, 8.0 %, 28.6 %), no performed appendectomy (5.2%, 8.7%, 13.1%, 16.5%, 12.5%), no performed herniotomy (5.2%, 8.5%, 5.4%, 11.4%, 11.1%), no underwent ileus (3.4%, 7.9%, 6.0%, 18.5%, 6.7%) do not influence significantly on the probability of amyloidosis in FMF patients. And in case of estimation of all performed operations the contingent of FMF patients being underwent the surgical operations do not differ from the general contingent of FMF patients (5.4%, 8.3%, 11.3%, 16.7%, and 11.1%). Thus the different surgical pathologies do not influence on the frequency of development of amyloidosis in patients with FMF at least in Armenia.

(abstract 112)

Three novel missense amino acid mutations in MEFV gene of Turkish FMF patients.
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Familial Mediterranean Fever (FMF) is an auto-inflammatory disorder, prevailing in the Mediterranean basin and characterized by recurrent bouts of febrile serositis by self-limited recurrent bouts of fever and painful episodes of sterile serositis that typically involve the peritoneum, pleura, and synovia. A less frequent but most severe complication is progressive amyloidosis that may affect several organs, especially the kidney, leading to end stage renal failure. The FMF disease causing gene, MEFV( 16p13.3) consist of 10 exons and encodes a protein of 781 amino acids called Pyrin . Pyrin belongs to a class of proteins involved in the regulation of apoptosis and inflammation.. Pyrin is the founding member of a growing family of proteins containing a pyrin domain a death domain–like structure known to interact with other pyrin domain–containing proteins, that are involved in the regulation of apoptosis and NF-kB activation. Mutations interfere with the role of the pyrin domain, allowing an uninterrupted inflammatory cascade.According to the Infevers database, 165 MEFV mutations and polymorphisms have been identified so far most of them confined to exon 10 of the gene.In this study we are notify 3 novel nucleotide change in MEFV gene which cause of three missense amino acid substitution in Pyrine protein.Nucleotide sequence in four exons of MEFV gene was analysed after PCR amplification with direct DNA sequencing method. Direct sequencing was carried out with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits v. 3.2 in the ABI 3100 automated DNA sequencer . The first mutation(c.453G>C; p.R151S) was identified in 2th exon of MEFV gene in child with typical FMF criteria. The second mutation (c.1516A>G; p.I506V) was identified in 5th exon of MEFV gene in patient with atypical FMF criteria. The third mutation( 2126T>G; p.L709R) was identified in 10th exon of patient with atypical FMF criteria.The fact that mutations are associatedwith spontaneous inflammatory attacks indicates that the protein is a relevant regulator of the inflammatory process. The mechanisms that trigger FMF attacks and the underlying reasons for the localization of the ensuing inflammation have been established. FMF attacks reflect abnormal trafficking of PMNs or altered chemotactic signaling from monocyte/macrophages.Turkish patients with FMF have a unique spectrum of mutations including a newly described mutation with a typical
or non-typical phenotype.

(abstract 217)

Is Familial Mediterranean Fever an important disease in Sweden?
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Introduction Out of 1.5 million inhabitants in the western region of Sweden, 2-3 percent have migrated or are born to parents that have migrated from the countries with high prevalence of Familial Mediterranean Fever (Eastern Mediterranean countries). The prevalence of FMF in western Sweden is undocumented as well as the clinical picture and importance of this disease. Among Swedish health-care workers the knowledge and awareness of FMF is increasing. The purpose of the study was to characterize FMF in western Sweden. Patients and methods In the western region of Sweden, patients with FMF are mainly cared for in three closely collaborating centers. Out of these the Queen Silvias Pediatric Hospital at Sahlgrenska University Hospital in Gothenburg has developed to resource centre for FMF and autoinflammatory diseases in general. During the last years patients with autoinflammatory diseases have continuously been registered in the five main hospitals in the region. We have retrospectively analysed the case records of patients with FMF from this registry. For patients with suspected or clinical FMF, genetic analyses have been carried out in Ankara, where the 12 common MEFV mutations have been investigated. Results We identified 41 patients with FMF in the region. The median age at the first symptoms were 4 years and the range were 3 months to 37 years. The median age at diagnosis was 10 years and the range 2 to 44 years. The median time from the first symptoms to diagnosis, were 4 years (range < 1 year to 34 years). The frequencies of the most common symptoms were in descending order; fever 100%, peritonitis 90%, pleuritis 22% and arthritis 12%. The most common ethnic origins of the patients were, including mother and fathers side: Turkey (n=30), Lebanon (n=24), Syria (n=10) and Armenia (n=6). Of the 36 patients tested for mutation in the MEFV gene 28 (78%) were homozygotes or compound heterozygotes, 5 (14%) were heterozygotes and in 3 (8%) no mutation were found. Conclusion According to our preliminary data we have identified 41 people living with FMF in western Sweden, this is most likely an underestimation. Our data indicates that FMF is an important diseases in Sweden. The time to diagnosis need to be shortened. We conclude that in today’s globalized world, Swedish healthcare workers have to adapt to a new context, were infectious diseases and genetic diseases, not previously seen, are a reality.

(abstract 101)

Familial Mediterranean fever caused by homozygous E148Q mutation complicated by Budd-Chiari Syndrome and polyarteritis nodosa.
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A Caucasian boy presented at the age of 3 years with intermittent night sweats, fever and
arthralgia. He was well between fever episodes. Initial investigations were normal. Aged 5 years he developed acute abdominal pain with ascites, and doppler ultrasound revealed hepatic vein thrombosis. He was thus diagnosed with Budd-Chiari syndrome, underwent successful angioplasty and began low-dose aspirin. Intermittent fevers, of unconvincing periodicity and of 1-5 days duration persisted, with abdominal pain but without rash. Autoimmune screening was negative. He re-presented at the age of 12 years with ongoing fever and abdominal pain and elevation of acute phase markers. Abdominal ultrasound showed hepatosplenomegaly. Selective visceral arteriography was consistent with vasculitis. He was treated with corticosteroids, azathioprine and low dose aspirin with prompt symptomatic improvement. The patient then stopped all medications and re-presented with night sweats, myalgia, abdominal pain and anorexia. Neutropaenia and lymphopaenia were coincident with fever episodes. Bone marrow aspirate was normal. A therapeutic trial of colchicine was commenced with immediate resolution of all symptoms and normalisation of blood tests. Genetic testing revealed homozygous mutation at exon 2 (E148Q) of the MEFV gene, supporting the clinical diagnosis of Familial Mediterranean Fever (FMF). Pyrin E148Q has an allele frequency of ~20% in Asian populations in which the overwhelming majority of heterozygotes and homozygotes appear to be healthy; certainly FMF seems very rare in these large populations. Pyrin E148Q is by contrast rare in Caucasians, in which its significance remains far from clear; it may contribute or exacerbate non-FMF inflammatory disorders. The association here of homozygosity for pyrin E148Q in a Caucasian patient with severe FMF lends further support to the possibility that it may have greater pathogenic potential in Caucasians. Our case is of further interest in that the FMF was complicated by two serious complications: Budd-Chiari syndrome and vasculitic change on arteriography mimicking polyarteritis nodosa (PAN). Budd Chiari syndrome has not hitherto been reported as a complication of FMF. We postulate the combination of fever, dehydration and vascular inflammation predisposed to hepatic vein thrombosis. PAN may be associated with FMF in populations where FMF is highly prevalent. The pathogenic potential of pyrin E148Q in Caucasians merits further study.

(abstract 136)

Profile of Cytokines, Growth Factors and Chemokines During Attacks of FMF

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Attacks of Familial Mediterranean fever (FMF) represent one of the most devastating states of inflammation. FMF is an autoinflammatory disease caused by mutations in the gene coding for pyrin, which lead to accentuated innate immune responses involving the IL1 and probably Th1 pathways. Our aim is to assess the characteristics of inflammation in FMF patients during an acute attack by evaluating some growth factors, chemokines and cytokines. Six patients (median 11 years (7-16)) were sampled 12-36 hour after the onset of a typical FMF attack. All had elevated acute phase reactants. All six had M694V/M694V homozygote mutations in the MEFV gene. Cytokines (IL-1β, IL-2, IL-6, IL-10, TNFα, IL-12, IL-13, IL-17, IFNγ, IL-1ra, G-CSF, GM-CSF), chemokines (MIP-1α, MIP-1β, CXCL10, CCL11, CXCL11, CXCL5), growth factors (EGF, FGF, VEGF, HGF) and CD40 ligand were measured by a commercially available multiplex beads immunoassay based on Luminex platform (Fluorokine MAP Multiplex Human Cytokine Panel, R&D Systems, Minneapolis, USA). IL1Ra (median 1047 pg/ml), TNF alpha (median 3.05 pg/ml) and IL6 (median 19.14 pg/ml) levels were elevated whereas IL2, IL10, IL12, IL17 and IL13 were not detectable in any of the samples. IL1beta and IFNg were elevated in only one sample each. EGF, VEGF and HGF were markedly elevated as well. Among the chemokines that were measured MIP1b, CCL11
(exotaxin), CXCL11 (I-TAC) and CXCL5 (ENA-78) were all detectable at a varying range. CD40 L was also elevated with a median of 5063 pg/ml. IL1Ra is induced by inflammatory stimuli with IL1 and reflects the IL1-dependent inflammatory response. IL1beta may not be detectable because of the short life and predominantly local production. IL10, IL13 and 17 were not detected supporting the lack of the TH2 and Th17 pathways in FMF. On the other hand IFN was not markedly elevated either. Chemokines were elevated reflecting the neutrophil inflammation and recruiting in this disease. The growth factors were thought to be elevated as an inflammatory response phenomena; HGF is known to be the main inducer for acute phase reactants from the liver. It was interesting to note that CD40 L is also significantly increased in these patients. This molecule may be serving as a link between the innate immune response and adaptive on during the attacks of FMF.

(abstract 107)

FMF AND PHENOTYPE/GENOTYPE RELATIONSHIP: REPORT OF AN ITALIAN FAMILY
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We describe three people affected by FMF, members of the same family, all descendent from people born in Puglia carrying a rare mutations in Italian FMF patients. Clinical diagnosis has been done on the basis of Tel-Hashomer criteria, Severity score has been calculated employing the system proposed by Pras in 1998. The father has a severity score index of 3 (mild severity), while the daughter and the son has a score of 5 and 8 respectively (moderate severity). The 3 patients have been investigated for MEFV mutations by genome sequencing of exons 2, 3, 5 and 10. Father and children are compound heterozygous, carrying two FMF mutations: R761H on exon10 and E148Q on exon2. The mother has no mutations. This let us suppose that the two mutations found are disposed in “cis” way on the same chromosome and an autosomal dominant inheritance. Otherwise the 3 patients had either a mild disease or a moderate-severe disease. Due to its genetics heterogeneity in alleles and loci, FMF frequently presents heterogeneity of the clinical picture, which is extremely evident in genotype/phenotype correlation. R761H/E148Q genotype has been described in one patient of Italian origin, who presented fever with severe arthritis and myalgia. The R761H is an extremely rare mutation described in symptomatic Armenians, Turks and Syrians, often associated with other mutations (K695R,K695K,M694V), defining a condition of compound heterozygous and a clinical picture resembling phenotype of homozygotes for those mutations described to cause symptoms. Patients carrying heterozygous condition have been described to be affected by FMF, suggesting a dominant pattern of transmission. The role of the E148Q variant remains controversial: several authors suggested that this variant is a low penetrance mutation, others that E148Q might be a functional polymorphism with a phenotypic effect only in cis-association with a disease-causing MEFV mutation. The possibility that the non-carrier chromosome causes FMF expression by a mutation in the non-coding region of its MEFV was explored by investigating other SNPs, common in the general population and not FMF-associated. The analysis identified three SNPs in the exon 2 of the non-carrier chromosome of the unaffected mother inherited by both sons. These polymorphisms could be determinant in causing a different allele expression. This condition has been observed in some polymorphisms investigated in other genetic disease as Porphyry and Long QT Syndrome.
MONITORING OF RENAL DAMAGE IN A COHORT OF SICILIAN PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER (FMF)

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever and serositis. Amyloidosis is the most crucial complication of FMF.

Methods: Between 2002 and 2007, 28 patients (11 girls, 18 boys) median age 316 months, (range 8-624 months) in whom FMF was clinically diagnosed and with a follow-up period of at least six months were included in the study. The mean age of the patients at the time of the onset of the symptoms was 110+-106 months (range 3-360). On investigation of MEFV, genetic mutations more frequently detected were M680I (12/28) and M694V (8/28). Age of starting colchicine was 267+-180 months (range 48-602). The patients were followed at 6 month intervals; at each visit, physical and blood tests (renal function) were performed. Daily urinary protein excretions were calculated.

Results: 5 patients showed a proteinuric stage (9.04+-3.04 mg/mq/h), without fever attacks, but two of them were non compliant with the treatment. There were no significant differences (age at onset, initial dose of colchicine and serum amyloid A levels) in the two groups. The FMF patients with elevated proteinuria showed a short time between the age of onset and the starting of colchicine than ones with normal proteinuria. Two patients with pathological proteinuria did not have MEFV gene mutations.. After the increase of colchicine, there was the complete resolution of proteinuria in all patients.

Conclusions: our study suggests the importance to study renal function in patients with FMF. Colchicine is an effective medication in the prevention and treatment of amyloidosis; in fact we report three cases in which colchicine (1,5 mg/day) reversed proteinuria. In contrast to other reported studies, there was no correlation between amyloidosis and M694V homozygosity in this cohort. Ten patients of our population who received a delayed diagnosis (median age of diagnosis 40 years) don’t show renal damage. Complex alleles, modifier loci, genetic heterogeneity, additional environmental and epigenetic modifiers have been advocate as potential misleading of FMF phenotype and genotype correlation.

ECHOCARDIOGRAPHIC FEATURES OF CARDIAC INVOLVEMENT IN FAMILIAL MEDITERRANEAN FEVER

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Familial Mediterranean Fever (FMF) is an autosomal recessive trait characterised by recurrent attacks of fever, polyserositis, pain and compiliation with AA nephropathic amyloidosis. A range of stress factors and certain mutational genotypes (homozygous state of Met694Val mutation of FMF gene (MEFV), cause severe course of the disease. We have studied echocardiographic issues of cardiac involvement in FMF and identified four cases of predominant cardiac amyloidosis which is not characteristic for FMF and other chronic inflammatory disorders. In all cases end-diastolic dimension of the left ventricle and left ventricular ejection fraction were within normal limits, hypertrophy of the interventricular septum and posterior wall of the left ventricle and granular sparkling of myocardium suggestive for amyloidosis were detected. Diastolic dysfunction of both ventricles characteristic for abnormal relaxation and restriction were revealed. In case of restrictive pattern of intracardiac blood flow administration of colchicine at a dose of 1,8 mg/day over 2 years
led to transformation of the restrictive pattern into abnormal relaxation. Extensive investigation of renal functions failed to reveal signs of renal insufficiency. It is noteworthy that in these cases mutational analysis of MEFV gene revealed Met694Val mutation in homozygous state. None of the patients were receiving regular colchicine therapy before diagnosis of amyloidosis. Echocardiography revealed hyperechogenity of papillary muscles in some FMF patients with proteinuric, nephrotic and uremic stages of systemic amyloidosis. This feature is of importance for early detection of cardiac involvement in amyloidosis. Moderate prolaps of anterior leaflet of mitral valve suggestive for inflammatory dysplasia of connective tissue should be referred as a common feature of cardiac involvement in FMF with and without amyloidosis. We have used isometric (handgrip) stress echocardiographic test to assess diastolic function of both ventricles and the state of pulmonary blood flow at preamyloidal and proteinuric stages of amyloidosis in FMF. Shifts of isovolumetric relaxation time of the left ventricle, peak velocity of left and right atrial filling and acceleration time of pulmonary flow are considered as an informative findings allowing to reveal early signs of diastolic dysfunction and pulmonary hypertension at preamyloidal and proteinuric stages and to evaluate the state of heart during colchicinotherapy. Our observations allow to emphasize the necessity of regular (1-2 per year) isometric stress echocardiographic assessment of FMF patients in preamyloidal and amyloidal stages and regular use of colchicine at a doses of 1-2 mg/day for prevention of chronic heart failure and amyloidal affection of the heart in FMF.

(abstract 20)

Rilonacept (IL-1 Trap) for Treatment of Colchicine Resistant Familial Mediterranean Fever: A Randomized, Multicenter Double-Blinded, Alternating Treatment (N of 1) Trial

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Background: There is no current alternative for the 5-15% of FMF patients who are resistant or do not tolerated colchicine. Since pyrin has an important role in IL-1β regulation, and IL-1 inhibition is effective in other autoinflammatory diseases, we hypothesize that IL-1 inhibition will decrease the number of FMF attacks in these patients. Methods: We will use rilonacept, a fusion protein that binds and neutralizes IL-1. Recruited subjects will have at least one heterozygote MEFV mutation and active FMF with at least 1 attack per month despite receiving adequate colchicine or are intolerant of colchicine. We will use a single-subject alternating treatment design (N of 1) with subjects receiving in random order two 3-month courses of rilonacept at 2.2 mg/kg (max 160 mg) by weekly SC injection and two 3-month courses of placebo. Subjects with 2 FMF attacks during a treatment course may transfer in a blinded manner to the other treatment arm until the end of that course. The alternating treatment design is suited for FMF, since in previous trials attacks may occur almost immediately after colchicine is discontinued. Based on other rilonacept studies the carry-over effect is not expected to last more than 3-4 weeks. Results will be analyzed by traditional probability and Bayesian statistics. 17 subjects with a minimum age of 4 years will be recruited, yielding a power >80%. We chose 7 U.S. sites based on populations of ethnic groups associated with increased prevalence of FMF and the National Institutes of Health (NIH) FMF clinic. The primary aim is to assess the efficacy of rilonacept in decreasing the number of FMF attacks. Secondary aims include determining the proportion of subjects who have no attacks, differences in the FMF severity score, acute phase reactants and quality of life between treatment arms and differences in inflammatory gene expression before and after treatment. Using intramural NIH resources, we will bring subjects for study visits from centers that are not study sites. The study’s principal investigator will work part time at the NIH, to coordinate among the NIH and extramural study sites. The study is funded by the U.S. FDA Orphan Drug Program and will start enrolling patients in May-June 2008. Conclusion: This study may offer an alternative treatment for colchicine
resistant FMF and support the pathogenesis of IL-1 in FMF. This study design may serve as a template for studies of new biologics for rare autoinflammatory diseases.

(abstract 203)

THE FREQUENCY OF ANKYLOSING SPONDYLITIS AMONG FAMILIAL MEDITERRANEAN (FMF) PATIENTS FOLLOWED IN A UNIVERSITY HOSPITAL


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Background: An increased frequency of sacroiliitis has been reported in FMF. The aim of this study is to determine the frequency of spondylarthropathies (Spa) among our FMF patients. Methods: A questionnaire involving 5 questions about inflammatory back pain, peripheral arthritis and enthesitis was developed. It had a sensitivity of 100% for detecting axial and/or peripheral joint involvement in the 70 patients tested with various rheumatological conditions. FMF patients were screened with this form and those who answered positively to at least 1/5 questions were called to the clinic for a detailed history, physical examination, sacroiliac x-rays and evaluation of enthesopathy with ultrasound when necessary. If the detailed history or physical examination in the clinic did not confirm the presence of inflammatory back pain, peripheral arthritis lasting longer than 6 weeks or enthesal pain patients were considered not to have a Spa. For those with suspected findings, sacroiliac x-rays were taken and examined twice, by 3 experienced rheumatologists in a blinded manner. Thus 6 observations were present for each x-ray. Sacroiliitis was considered if the x-ray was graded 2 and over according to New York criteria in at least 4/6 observations. If the grade was 3 or 4 in 4/6 observations, patient was accepted to have definite sacroiliitis, thus AS. The remaining patients were further evaluated with sacroiliac MRI and HLA B27 on 3 conditions: 1) inflammatory back pain even if x-rays are normal 2) physical findings suggesting sacroiliitis or enthesopathy 3) sacroiliitis, where the grading was less than 3, in 4/6 observations. Results: 712 patients were recorded in our FMF outpatient clinic between 1995-2007. 486 could be reached, 262 of them were questioned on the phone and 224 during their routine controls. 142/486 gave a positive answer to at least 1 of the 5 questions. Until now 89/142 were further evaluated in the clinic. 36/89 were diagnosed as not having a Spa after a detailed history and physical examination. 11 were diagnosed as AS. The remaining 42 patients are being further evaluated for HLA B27 status and sacroiliac MRI. Conclusion: Although the results are not yet conclusive, among 89 FMF patients with a history of inflammatory back pain and/or protracted arthritis and/or enthesitis, 11 patients (12%) had definite AS. The role of selection bias in this high ratio should also be considered because the data originates from a rheumatology clinic.

(abstract 207)

Clinical, Laboratory and Radiological Findings of patients with Familial Mediterranean Fever associated Ankylosing Spondylitis (FMF-AS)


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OBJECTIVES: An association between familial Mediterranean fever (FMF) and ankylosing
spondylitis (AS) has previously been recognized. The aim of this survey is to describe clinical, laboratory and radiological findings of FMF patients with bilateral sacroiliitis and to compare these findings with those of ankylosing spondylitis patients followed in the same clinic. METHODS: 22 FMF-AS (male/female: 11/11, mean age: 28 ± 10 years) and 40 AS (male/female:31/9, mean age: 40 ± 9.6) patients were surveyed. After a chart review, patients were called to the clinic. A detailed history was taken including demographic features, disease duration, presenting symptoms, family history, characteristics of FMF and arthritis attacks, presence of deformities, and concomitant inflammatory bowel disease, psoriasis and uveitis. HLA B27 positivity was analyzed. The presence of enthesopathy was evaluated using B mode and power Doppler ultrasonography. RESULTS: In 8/22 FMF-AS patients, FMF and AS was diagnosed at the same time, in 12/22 patients FMF was diagnosed 9.08 ± 4.88 years before AS and in 2/22 patients whose presenting symptom was hip pain, AS was diagnosed 4 and 26 years before FMF. AS symptoms started earlier in FMF-AS patients when compared to AS patients (age at AS onset: 18.8 ± 6.8 vs 25.5 ± 7.8; p=0.015). Mean duration of follow-up was 8.8 ± 7 years for FMF-AS and 14 ± 9.5 years for AS patients. A family history of FMF and amyloidosis were more common among FMF-AS patients (p< 0.001 and p< 0.01 respectively). The frequency of amyloidosis, uveitis, inflammatory bowel disease and psoriasis was similar among the 2 groups. Peripheral arthritis, especially with short duration and with redness over the joint was more common among FMF-AS patients. The frequency of axial involvement and deformities was similar except that chest expansion was better in FMF-AS patients (P=0.037). Enthesitis score was significantly higher in AS patients (p< 0.01). The frequency of HLA B27 positivity was similar among the 2 groups (9/22 vs 24/37). CONCLUSION: FMF-AS patients have an earlier onset of AS symptoms, more frequent peripheral arthritis, better chest expansion and less enthesopathy when compared to AS patients. HLA B27 positivity was present in almost one half of FMF-AS patients in contrast to the former contention. The disease seems to follow a milder course in FMF-AS patients, but this might be related to a shorter duration of follow-up in these patients.

(abstract 215)

FEATURES RELATED TO OTHER AUTOINFLAMMATORY DISEASES ARE FREQUENT AMONG FMF PATIENTS


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Background: Autoinflammatory diseases share a common spectrum of symptoms like recurrent abdominal or chest pain and fever. Certain features on the other hand such as hearing loss, periorbital edema, cold sensitivity are attributed to certain AIDs. However the presence and frequency of the latter group of signs and symptoms among FMF patients have not been studied. Aim: To evaluate the frequency of signs and symptoms related to other autoinflammatory diseases in FMF patients. Methods: Definite FMF patients fulfilling tel-Hashomer criteria who attended 4 different rheumatology departments, taking care of both children and adults were asked to fill out a standard questionnaire which included questions about various symptoms of other autoinflammatory diseases. Results: 161 patients answered the questionnaire. 64 (40%) reported that their attacks were precipitated by cold. 28 (17%) reported sore throat, 13 (8%) cervical lymphadenomagey and 11 (7%) both during attacks. 7 (4%) reported redness in the eyes and blurred vision during attacks. Periorbital edema during attacks was reported by 18 (11%). 19 (12%) reported hearing loss. Mean age at onset of hearing loss was 41.3±10.9 years. Rash occurred during attacks in 24 (15%), between attacks in 12 (8%) and both in 10 (6%) patients. In the majority of
patients, rash was over the legs (72%). 60 (37%) reported severe muscle pain with stiffness of a muscle group during attacks. 28 also reported (17%) erythema over the involved muscle. 39 patients (24%) had attacks lasting longer than 1 week. Headache was present during the attacks in 75 (45%). 37 (23%) had more than 3 oral ulcers per year. 62 (39%) reported diarrhea during attacks. 135 patients (84%) reported that they benefitted from colchicine. Erysipelas like rash over the involved joint was reported by 61 (38%). 15 (9%) had episodes of arthritis which lasted longer than 1 month and 35 had (22%) experienced simultaneous arthritis in 3 or more joints. Conclusion: This survey showed that FMF patients share many of the clinical signs and symptoms characteristic of other autoinflammatory disorders. However the results depend on patient reports which may be misleading without objective evaluation, especially in symptoms such as hearing loss. A larger group of patients with various inflammatory conditions should be tested to determine sensitivity and specificity of these signs and symptoms to each of the autoinflammatory diseases.

(abstract 8)

SERONEGATIVE SPONDYLARTHITIS AS CLINICAL FEATURE OF FAMILIAL MEDITERRANEAN FEVER

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Familial Mediterranean fever (FMF) is an autosomal, recessively inherited disease, affecting people of Armenian, Jewish, Arabic, and Turkish ancestry. The disease is the prototype of the periodic febrile syndromes. Its hallmark is short attacks of fever and painful manifestations in the abdomen, joints, chest, and other organs. Chronic and protracted manifestations, particularly nephropathic amyloidosis, chronic arthritis, and protracted myalgia, may also occur in the disease. Articular syndrome is the third on frequency clinical feature of FMF after serositis and fever, forming a triad of the major diagnostic criteria. The incidence of affection of iliosacral joints and spine, according to the literature data is 17-32 %. There is a certain pathogenetic generality between FMF, ankylosing spondylarthritis and a group of seronegative spondylarthritides. The purpose of the present study is the evaluation of frequency, clinical features, an X-ray pattern, definition of an antigen HLA-B27 in patients suffering with FMF who had a clinical pattern of sacroilitis. Thirty patients with FMF are examinated. Interrelation of men and women is 3:1. The abdominal form are at 12 patients, mixer form at 14 patients and at 4 patients – the thoracic form. Age of the patients is variated from 18 to 45. Clinical symptoms of a sacroilitis have been revealed at 8 male patients (26,6%). All 8 patients had pain syndrome and expressed of rigidity in a lumbar part of spinal column with limitation of moving. The symptoms of Shober’s and Thomayer’s are positive at 8 patients. At six patients the lumbar lordosis are absent and only in one cases revealed the absence of lumbar lordosis and thoracic kyphosis - «a posture of the beggar» was marked. The X-ray examination all 8 patients has revealed a pattern of a sacroilitis: sharp narrowing of an articular space (at 5 patients), an illegibility of osteal edges of ileosacular joints (at 2 patients), and a complete obliteration of an articular space (at 1 patients). At 6 patients one-side, and at 2 – bilateral sacroilitis is revealed. At 4 patients are revealed the distraction of spinal column in the part D 12 – L2. HLA-B27 has been revealed in all patients with a clinical pattern of sacroilitis in a range of the control. Thus, the characteristic diagnostic criteria of spondylarthritis in FMF are the X-ray proved unilateral sacroilitis, the predominance of affection of spinal part of spinal column, the seronegativity of HLA B27 antigen, the absence of the eye symptomatic (iridis, iridocyclitis), the predominance of mail gender, the low progressing of the pathological process. Seronegative spondylarthritis is one of the clinical forms of articular syndrome of FMF and can be included in a group of seronegative spondylarthritides.
The clinical spectrum of 94 French patients carrying a single mutated MEFV allele

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Although familial Mediterranean fever (FMF) is an autosomal recessive disorder, mutation analysis in our laboratory showed that 15% of clinical FMF patients had only a single mutated allele in the MEFV gene. The reason why FMF carriers may develop the disease is still unclear. In addition, the presence of at least one FMF allele has been shown to be associated with several inflammatory diseases. Aim of the study: To assess the clinical characteristics of French FMF patients carrying a single MEFV mutation Method: A retrospective chart review of patients with FMF symptoms who were referred to the national reference centre for auto-inflammatory diseases. Genetic testing: Systematic screening of exon 2 and 10 was performed in MEFV gene. A subset of patients was also investigated for other hereditary recurrent fevers Results: Clinical and biological data was available for 94 patients (47 males, 47 females). Forty-two % of them were Jews, 17% Arabs, 8% Italian, 6% French, 3% Turks, 3% Armenian and 11% of other origin. Familial history of FMF could be found in 23%, MICI in 10%, amyloidosis in 3% and Behçet in 3%. The mean age of onset was 11.8y [3months-47y], median: 2y. Most (98%) suffered from fever during attacks. While fever was higher than >39°C in 80% of the patients, duration and frequency of an attack varied (3d: 36%; >2/m: 15%, 1-2m: 48% 90% of them. Associated diseases in these patients were PFAPA (4), Ankylosing spondylitis (5), Crohn’s disease (1) and Castleman disease (1). Discussion The clinical picture of French patients carrying a single MEFV mutation resembles that of homozygote patients. Most of them required colchicine treatment. This study displays a wide variety of associated diseases in both patients and their families. The occurrence of PFAPA syndrome raises the question of the accurate treatment while 2 patients responded well to colchicine, and another one was cured after tonsillectomy. Complete screening of both MEFV and other auto-inflammatory gene mutation is required to increase our understanding of disease expression in our patients.

Observation of juvenile chronic arthritis in children with MEVF gene mutations in Armenia

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Acute recurrent or chronic destructive arthritis are among manifestations of Familial Mediterranean fever (FMF). Chronic arthritis damage large joints of legs; sacroileitis and enthesis also are presented. Differentiation of these manifestations with Juvenile Chronic Arthritis (JCA) is important, especially if JCA coexists with FMF. The peculiarities of JCA in 12 patients with MEVF mutations were studied. All of them were males with average age of 12 y. HLA B-27 allele and MEVF mutations were determined in all patients. Four FMF gene mutations were revealed: 7 compound heterozygotes (M694V and M680I), 2 homozygotes (M694V), 2 heterozygotes (M694V), one heterozygote (R761H). HLA B-27 allele was identified in 7 patients. In 7 cases JCA
was the first manifestation, then FMF joined as rare attacks of thoracalgia or nontypical attacks. 5 patients with typical attacks of FMF during following 2-3 years developed JCA mostly with signs of sacroileitis. 4 patients had nontypical course of FMF with arthritis the only symptom. Arthritis with polyserositis (abdominalgia, thoracalgia) was detected in 8 patients. All these patients had severe JCA - polyarthritis or oligoarthritis with involvement of hip, knee or ankle joints and X-ray confirmed sacroileitis. Arthritis was resistant to aggressive treatment, had high laboratory activity. NSAIDs and DMARDs were not effective and only steroid therapy led to the achievement of relative clinical and laboratory remission. In most cases positive effect of colchicinotherapy on JCA course was observed. It made possible to withdraw steroids and control JCA by DMARDs and NSAIDs. FMF other symptoms resolved due to colchicinotherapy. We suggest to investigate for FMF all JCA patients from affected by FMF ethnic groups, especially with sacroileitis, large joints involvement of lower extremities, enthesitis resistant to aggressive treatment with DMARDs and NSAIDs. In these cases arthritis could be the first, early and the only manifestation of FMF. The patients should be asked for isolated febrile attacks, haemorrhagic vasculitis, rare episodes of X-ray confirmed pleurisies and family history of FMF. They also should be tested for MEFV mutations. Early detection of MEFV mutations, diagnosis of FMF and starting therapy by colchicine improves the course of arthritis and prevent FMF complications. FMF patients with resistant course of arthritis (sacroileitis and large joint arthritis) should be tested for HLA-B27.

(abstract 29)

Small molecules originating from microbes in blood of patients with familial Mediterranean fever versus patients with other gastrointestinal disorders


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The contribution of microflora to the severity and progression of intestinal inflammation during familial Mediterranean fever (FMF) was investigated. Previously, we have shown microecological breakage in FMF. In the present work we attempted to reveal prevalent bacterial markers (small molecules originating from microbes) in blood, which could participate in the launch of inflammatory process in FMF. The blood samples of the following groups were analyzed: FMF patients in attack, n=29; FMF patients in remission, n=65; healthy controls, n=48; patients with non-genetic gastrointestinal disorders (GI), n=14. For detection of specific markers of bacteria the gas chromatography-mass spectrometry (GC-MS) method (in mass-fragmentography regimen) with the high sensitivity mode of selected ion monitoring was applied. Comparative analysis of 36 bacterial markers (oxi-, iso-, anteiso-, unsaturated, cyclopropanoic acids, and aldehydes) present in the blood of all investigated groups was performed. The results indicate a cardinally biased and unusual profile of bacterial markers for all diseased groups investigated. The highest concentrations of bacterial markers were observed in the blood of FMF patients in remission period, whereas in the attack period the corresponding values were reduced. Notably, the results obtained for GI patients cardinally differed from other investigated groups. Discriminant analysis (DA) revealed that different stages of FMF were distinctly characterized by the set of markers of bacterial origin and differed from healthy controls and GI patients. The DA data indicate the possibility to differentiate acute stage of FMF and GI exacerbation based on the set of markers of bacterial origin. The results of DA of pairs of studied groups indicate that there is no complete coincidence of significant markers for discrimination of different pairs of groups, which reflects the multiplicity of bacterial agents involved in disease activity. Notably, none of the bacterial markers specific for FMF was
detected. The revealed alterations of markers originating from both anaerobes and aerobes in the blood of FMF patients seem to reflect a metabolic imbalance and indicate the peculiarities existing in the microbial-ecological status in FMF. The results suggest that in FMF – known to be characterized by genetically determined excess reactivity – an inflammatory potential is realized when the level of the bacterial load in remission period amounts to critical, i.e. a triggered launching of an inflammatory response of the organism occurs.

(abstract 186)

**Prevalence and phenotype associated with pyrin E148Q in Northern European patients investigated in a UK specialist fever clinic**

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Background: The pyrin variant E148Q is present in up to 20% of individuals in some ethnic groups, but is thought to have a healthy population frequency of only ~1% among northern Europeans. The wider significance of this particular variant remains unclear. Methods: Search of our fever clinic database identified 221 northern Europeans (excluding Ashkenazi) in whom pyrin E148Q had been sought. All had undergone screening of MEFV that included a minimum of exon 10 and the 5’ end of exon 2. Results: Pyrin E148Q was present in 34 northern Europeans. 12 of these individuals had FMF or probable FMF of whom 10 were members of 3 different families with compound alleles associated with dominant FMF, and 2 had an associated exon 10 mutation presumed to be on the opposing allele. 3 patients had a mutation in another fever gene: 2 had TRAPS associated with the C33Y variant, and the other had NLRP3 V198M associated with a lifelong fever syndrome. 17 individuals were E148Q heterozygotes and 2 were homozygotes. Of the 17 heterozygotes, 16 were referred for investigation of possible fever syndromes and the other had unexplained AA amyloidosis. The median age at onset of symptoms was 19 yrs; 88% reported fevers, 69% had arthralgia/myalgia and 54% had episodes of abdominal pain. Symptomatic episodes lasted longer than 7 days in more than 50%, and were accompanied by median CRP of 39mg/L compared with 3mg/L when asymptomatic. Only 2 out 7 patients treated with colchicine appeared to benefit. The 2 northern European E148Q homozygotes were male and both presented before adolescence with fever, myalgia and apthous ulceration; one also had had liver disease and polyarteritis nodosa (PAN) but his fever symptoms responded to colchicine. Of note 2 other men in the series also had life threatening vasculitis (lupus nephritis, ANCA negative vasculitis). Conclusion: The 15% prevalence of pyrin E148Q identified in this mixed series of northern Europeans referred to a fever clinic is substantially higher than is thought to occur in the healthy northern European population, suggesting a contribution to disease. The associated phenotype was variable, but prolonged episodes of fever and myalgia/arthralgia accompanied by an acute phase response were common, and where tested usually failed to respond to colchicine. As in other typical ethnic groups, pyrin E148Q coupled with recognised pathogenic exon 10 mutations is sufficient to cause FMF in northern Europeans.

(abstract 50)

**Prolonged remission in familial Mediterranean fever**

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Background: Familial Mediterranean fever (FMF) is a genetic disease, with a diverse phenotype, ranging from severe, frequent, multi-site bouts of serositis, to a subclinical disease. High severity of the disease has been associated with North African Jewish origin of the patient, and with homozygosity to the MEFV mutation M694V. In contrast, mild form of the disease characterizes patients from Iraqi and Iranian extraction, patients bearing only one MEFV mutation and patients with late onset disease. Objective: To define and characterize prolonged remissions in FMF. Methods: Cases were 25 FMF patients, who experienced a remission, lasting at least 3 years, while off colchicine. They were identified using our computerized database on about 8000 patients, and compared to 25 FMF control patients, recruited consecutively, while coming for their regular follow-up visit in the National Center for FMF. Clinical, demographic and genetic data were abstracted from the patient files, completed through direct or telephone interview, and analyzed using Fisher’s exact test or Student’s t test as appropriate. Results: The mean duration of remission was 13±8.9 years. Of 45 demographic, clinical, and genetic parameters analyzed, only 2, severe phenotype (in 3 cases vs.11 controls, p=0.012) and homozygosity to the M694V MEFV mutation (0 vs. 7, p= 0.009), appeared to be significantly less common in cases. A trend for a lower rate of Jewish North African origin of at least one parent (7 cases vs.13 controls, p=0.095) and lower rate of leg pain (6 vs. 12, p=0.09) were also noted in cases. Some other markers of a severe disease, such as chronic arthritis (0 cases vs.3 controls), anemia of chronic disease (1 vs.5), arthritis attacks (5 vs. 10) were also less common among cases but not significantly so. Conclusions: A prolonged remission may be manifested in a subset of FMF patients with a milder form of the disease. With regards to disease severity, based on historical controls, patients who experience episodes of prolonged remission, might be posed between patients with delayed disease onset and patients from the Iraqi-Jewish stock. While off colchicine, patients with a prolonged remission, should be carefully monitored for possible subclinical inflammation.

(abstract 92)

**Familial Mediterranean Fever in Ashkenazi Jews: The Mild End of the Clinical Spectrum**

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**ABSTRACT**

**Background and Aim:** The present study sets out to characterize familial Mediterranean fever (FMF), a recessive disorder characterized by episodes of febrile serositis, in Ashkenazi patients, a Jewish ethnic subgroup in which FMF has been scarcely described before. Patients and Methods: A retrospective analysis, comparing all 57 FMF patients of Ashkenazi Jewish extraction followed at the National Center for FMF of the Sheba Medical Center in Israel to age and sex matched, patients of Iraqi (62 patients) and North-African (NA, 61 patients) origin, followed at the same center. The three groups were compared for demographic, clinical and genetic parameters. Mor criteria were used to determine disease severity Results: Age at disease onset and at disease diagnosis was similar among Ashkenazi and Iraqi patients (21-25 years at onset and 34, on average, at diagnosis) as opposed to onset before age 10 and diagnosis at 16, in the majority of NA patients (p=0.001). Family history of FMF was present in only 29% of Ashkenazi patients as opposed to 82 and 77% of the Iraqi and NA patients respectively (p=0.001). Frequency of abdominal, chest and febrile attacks were similar among the 3 groups while joint attacks were experienced less frequently in Ashkenazi (22.8%) compared to Iraqi and NA patients (32.3 and 83.6% respectively, p=0.05). Involvement of more than 2 attack sites during the course of the disease was more common among NA than among Ashkenazi patients (25.4% vs. 22.8%, respectively, p=0.05). 42% of the Ashkenazi patients suffered from mild manifestations and 5% suffered from severe disease before the institution of colchicine therapy, as opposed to 2 and 36%, respectively, in NA Jews (p=0.0001), and 18 and 11%, in Iraqi Jews (p= 0.5). A good response to
colchicine was noted in a similar proportion of Ashkenazi and Iraqi patients (82-84%) as opposed to only 56% of NA patients (p=0.0001). Proteinuria, renal failure and amyloidosis were most frequent among the NA patients (18, 6.6 and 9.8% compared to 5.3, 0, 3.5 and 1.6, and 0% in Ashkenazi and Iraqi patients, respectively). Conclusion: Ashkenazi FMF patients comprise a unique subset, posed at the mildest end of the demographic, clinical, genetic and treatment spectra of FMF

(abstract 94)

**MEFV Mutation Carriage in Israeli Jewish Subjects from Ethnicities with Low Risk for Familial Mediterranean Fever**

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Aim: To determine the frequency, type and effect of common MEFV mutation carriage in Jewish subpopulations with low prevalence of FMF. Methods: The study group was comprised of 163 Non-FMF Jewish adults of Bucharian, Turkish, Georgian, Yemenite and Bulgarian origin. The prevalence of the most frequent MEFV mutations in the Israeli Jewish population, namely: M694V, V726A and E148Q, was assessed using PCR amplification and restriction enzyme analysis. The association of mutation carriage with a personal history of FMF-like phenomena as well as various inflammatory and non-inflammatory diseases, was evaluated. Results: A high MEFV mutation frequency was found among Jews of Bucharian, Georgian and Bulgarian origin (20 %) while intermediate and low rates were detected in Jews of Turkish and Yemenite extraction (14 and 8 %, respectively). M694V and/or E148Q were more common than V726A among subjects of Bucharian, Georgian and Yemenite origin (p=0.05). The three mutations were equally distributed among Turkish subjects while Bulgarians carried either V726A or E148Q, but not M694V. An increased frequency of pericarditis, arthritis, erysipelas and Behçet's disease was reported in MEFV mutation carriers as opposed to lower rates of hypertension. Conclusion: An unexpectedly high rate of MEFV mutations was observed in Israeli Jewish subjects extracted from ethnicities with a low prevalence of FMF. MEFV mutation carriage, in this group, is associated with various inflammatory disorders.

(abstract 97)

**CT findings in familial Mediterranean fever patients during attacks and in attack-free periods**

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Background and aim: Acute abdominal attacks are the hallmark of the recessively inherited familial Mediterranean fever (FMF), experienced by over 95% of the patients at some point over the disease course. As the abdominal FMF attack is frequently accompanied by peritoneal inflammation and a striking acute phase response, it may be clinically indistinguishable from other acute abdominal conditions. The present study aims at characterizing abdominal computerized tomography (CT) findings of FMF patients during abdominal attacks and remission in a blinded manner, in addition to evaluating the role of tomography in confirming the clinical suspicion of an acute FMF attack. Patients and Methods: Two expert radiologists, blinded to the clinical data, particularly disease activity and final diagnosis of the patients, reviewed successive abdominal CT studies, performed
on FMF patients admitted to the hospital over a 3 year period. Scans were evaluated for the presence of pericardial effusion, organomegaly, peritoneal and mesenteric findings, nodal enlargement, bowel abnormalities and vascular wall pathology including the presence of calcifications. An unblinded FMF clinician related the unison radiological findings with the clinical data abstracted from the patients' hospital and clinic charts. Results: CT studies of 32 FMF patients (19 M/12 F, mean age 41.5±15.7), were analyzed. The diagnosis on discharge was abdominal FMF attack in 15 patients, other acute abdominal conditions such as colitis, diverticulitis, small bowel obstruction, renal colic and pelvic inflammatory disease in another 11 and non-acute abdominal conditions, usually investigation of chronic abdominal pain, elevated liver enzymes, amyloidosis, anemia etc. in 6. The magnitude and variety of pathology visualized in scans performed during acute FMF attacks and in attack-free periods was comparable. Conclusion: This report, the largest to-date to record CT findings of FMF patients, and the first to do so in a blinded fashion, suggests that CT findings during acute abdominal attacks of FMF patients are scarce and non-specific. We therefore conclude that the primary role of CT scanning, in the emergent setting, is in the exclusion of non-FMF causes of acute abdominal pain.

(abstract 187)

RETROPERITONEAL FIBROSIS REGRESSION AFTER COLCHICINE THERAPY

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Retroperitoneal fibrosis (RPF) is a fibro-inflammatory process affecting the retroperitoneal structures. For RPF treatment, steroids, tamoxifen and immunosuppressors have been proposed. We describe two cases with RPF associated with Familial Mediterranean Fever (FMF) in a patient and recurrent pericarditis in the other one. In both cases the colchicine alone was effective to control the RPF. Case 1. A 48-year-old male, affected by RPF treated with corticosteroid and tamoxifen, with history of periodic fever and abdominal pain ascribed to relapsing cholangitis, was admitted to our hospital in February 2006, complaining of myalgias. The history revealed recurrent fevers since childhood, diagnosed as rheumatic fever. In 1999, the patient underwent cholecystectomy for gall stones, followed by a surgical complication treated by choledocho-jejunostomy. After discharge, the fever episodes were attributed to cholangitis. The patient underwent insertion of a biliary stent, without benefit. In 2004, an abdominal NMR showed a periaortic RPF, confirmed by histology. A ureteral stent was inserted and steroids, cyclophosphamide and subsequently tamoxifen were started. After admission, we observed a fever attack with increase of acute phase reactants, for two days, disappearing without antibiotics. The patient’s history, the fever attacks with abdominal pain, led us to hypothesize FMF; the genetic test for MEFV showed the mutation R42W. All drugs, ineffective for RPF, were stopped and colchicine was started. After 2 years follow-up, colchicine prevented fever attacks and induced complete RPF resolution. Case 2. A 41-year-old male, with a history of severe recurrent essudative pericarditis, associated with orbital, mediastinic and retroperitoneal fibrosis, was admitted to our hospital for a relapse of the pericarditis. The therapy included high dose of prednisone, indomethacin, azathioprine and tamoxifen, but the colchicine addition allowed to taper all the other drugs until stopping them. Currently, the patient is asymptomatic over 2 years with colchicine alone and RPF is significantly reduced. We suggest that FMF should be investigated in case of Fever of Unknown Origin with retroperitoneal involvement. The coincidental association of RPF in two patients with FMF and recurrent pericarditis, allowed us to observe the benefit of colchicine therapy for RPF. Therefore the colchicine could be proposed as therapeutic tool in the treatment of RPF.
FEVERS OF UNKOWN ORIGIN (FUO): A COST ANALYSIS


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The "Costs of Illness analysis" aims to evaluate the economic impact of a disease on different social components. The costs of illness are direct, given to patients and families, easily quantifiable and indirect, charged to society, not expressed as monetary terms. We tried to evaluate retrospectively the costs of undiagnosed disease in a population of 60 patients presented to our clinic for FUO. By a questionnaire we collected demographic and clinical data, number of hospital admissions, private specialists visits, visits at outpatient clinics, Day-Hospital admissions, prescriptions, duration of the treatments, surgical therapies, complications of their febrile disease, investigations paid directly by the patients and those provided by National Healthcare System, number of times they did the minimal diagnostic work-up for FUO, school or work days lost, travel expenses to care access. Costs were estimated using the standard reimbursement fees and charges for services and procedures from the Italian National Healthcare System (DRG 2006 19th ed.), the index of prices for public pharmacies (2006 ed.) and the websites of public or private transportation companies. For the part of indirect costs that is not expressed in monetary terms, we performed a quantification of the losses, in terms of productivity or patient unsatisfaction. It was assumed a relation between unproductivity, unsatisfaction and Gross National Product (GNP). We calculated the presumed relation between lack of patient productivity-satisfaction, and their impact on the GNP. The average diagnostic delay was 14.7 +7 years. Febrile attacks last from 2 to 15 days in 68% of cases, and occurring more than 2 times a month in 25% of cases. One patient had continuous fever for 8 years. Patients had a total of 490 public and private medical consultations, 250 hospital admissions, 43 unnecessary surgical interventions, 75 Day-Hospital admissions, 513 examinations at public hospitals and 424 at private laboratories; work absenteeism among parents and patients was 49.420 days. Above all, a long time without a diagnosis was shown to have an impact on society estimated at 15% to 30% of the GNP and at 40-60 % on Health Expenditures per capita. It is evident that reducing the time of diagnosis for FUO is a crucial in order to increase National Healthcare System efficiency. Appropriate medical expertise and guidelines are necessary in order to improve the cost /efficiency ratio.

FREQUENCY OF MEFV MUTATIONS' DIAGNOSIS IN GENETIC LABORATORIES:
THE INFLUENCE OF EXPERT CLINICAL SELECTION


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The diagnostic value of molecularly analyzing the Familial Mediterranean Fever (FMF) gene
(MEFV) has been well established in patients selected on the basis of ethnic background or clinical criteria; molecular tests allowing the routine diagnosis of FMF have now been developed in many laboratories in generally unaffected countries, such as France and Italy, too. Indeed, in these generally unaffected countries, where the frequency of mutations is not high, the selection of patients with periodic fever by an expert would increase the cost /efficiency of MEFV testing.

Objectives: We investigated the influence of the expertise of centres specialised in diagnosis and treatment of FMF on the frequency of mutations found in the Genetics Laboratories of two Italian Universities. Methods: A retrospective study was conducted on 500 patients referred for FMF genetic tests between 2002 and 2007 in two Italian genetics laboratories (Parma University and Rome Catholic University); they received blood samples of patients selected by “expert clinical centres” and a number of other medical centres not specifically dedicated to FMF diagnosis, “other centres”. Tel Hashomer and/or AR criteria were adopted in the “clinical expert centres”. Statistical analysis was performed by Pearson X² tests. Results: 61.8% (89/145) of the patients having one or two MEFV mutations were found among those selected by “expert clinical centres”, whereas, 44.56% (82/184) of the patients having one or two MEFV mutations were found among those sent by “other centres”. The p-value was p = 0.09, very close to statistical significance. Conclusion: MEFV mutation screening showed a higher frequency in a larger proportion of patients referred by “expert clinical centres”; an extrapolation of larger samples would probably show a definite statistical significance. To maximize genetic diagnosis, a decision tree for FUO, including Tel Hashomer and AR criteria, should be developed, and associated with the advice of an expert practitioner able to give further indications about hereditary periodic syndromes other than FMF.

(abstract 185)

Familial Mediterranean fever: simultaneous observation of 2 abnormal MEFV transcripts in Lebanese FMF patients


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Introduction: Familial Mediterranean fever is an autoinflammatory autosomal recessive disease particularly frequent around the Mediterranean basin. Mutations in the MEFV gene on chromosome 16p13.3 were found responsible for this disease. However, DNA screening studies showed the presence of only one MEFV mutation in a number of clinically diagnosed FMF patients. In this study, the MEFV transcript was screened for any alternative splicing in 29 Lebanese FMF patients carrying only one MEFV mutation. Patients and Methods: The 29 patients included in the study were found heterozygous for one MEFV mutation after screening of the MEFV coding genomic sequence. Total RNAs were extracted from each patient using the phenol chloroform method, then transcribed into complementary DNA (cDNA) using random primers. MEFV cDNAs were then amplified by Polymerization Chain Reaction (PCR) and sequenced on an ABI Prism 3130 genetic analyzer. Results and discussion: In seven of the patients, 2 abnormal transcripts were observed, in addition to the normal expected one. One of them lacked exon 7 and the other one exons 7 and 8. Studies to determine the origin of these abnormal transcripts are pending. Such findings could explain the clinical diagnosis of FMF in these patients.

(abstract 23)

Alternative complement pathway in Familial Mediterranean fever
The human complement system is an important branch of innate immunity and comprises approximately 35 known plasma and membrane-bound proteins involved in efficient activation and tight regulation of the system. The activation of complement cascade by three pathways generates opsonins, inflammatory mediators and cytolytic protein complexes that play an essential role in tissue damage and normally function to eliminate foreign pathogens and to opsonise necrotic and apoptotic cells. However, the undesirable complement activation contributes to the pathology of many inflammatory diseases. During the attacks of Familial Mediterranean fever (FMF) multitudes of systemic events are triggered, most of which promote an autoinflammatory state. The complement is one of the several factors that contribute to pathogeneses in this disease. The detection of complement in FMF is essential for the understanding of disease pathomechanisms, especially from the point of view of immunity. In present study we examined the activation of alternative pathway (AP) and key regulators of AP: factor B (fB) and factor D (fD) in the blood serum of 19 FMF patients (males 10, females 9; mean age ± S.D. 28 ±10years). The control group consisted of 23 healthy volunteers (males 12, females 11; mean age ± S.D. 31 ±11years) without FMF positive family history. A hemolytic assay was based on the standard 50% complement hemolysis test for AP of human serum complement. Furthermore, correlations of acute phase reactants such as C-reactive protein (CRP) with AP activity and foregoing complement factors were determined. In the serum of FMF patients the mean values of AP, fB and fD were significantly lower compared with the healthy subjects. However, no significant correlation between these factors in respect to the mean values of CRP was detected. Our study is continuing to expand the knowledge of the complement system in FMF. The obtained data evidence that AP activation probably is not involved in the process of activation of C3 and subsequent generation of C3d and C5b-9 membrane-active complex (MAC). It is quite possible, that the initiator of the activation of complement system is the formation of antibodies to modified proteins and subsequent formation of immune complexes, which are known to be increased at this pathology from our previous reaserches, which in his turn results in activation of complement by classical pathway. Therefore, it is reasonable to propose that significant hypoactivation of AP in FMF may be conditioned by higher concentration of CRP on targets via its interaction with inhibitor of complement AP activation factor H and C4BP. New studies of the complex interplay between the complement cascade and FMF-associated immune response may be a therapeutic target in the treatment of this disease.

Further investigations of the activation of complement system in FMF and correlation of the measured parameters with FMF gene mutations and clinical picture will make it possible to provide a critical approach to identify the new strategies of FMF therapy.

Pathogenic circulating immune complexes in patients with familial Mediterranean fever before and after colchicine therapy

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Familial Mediterranean fever (FMF) is the most prevalent member of autoinflammatory diseases worldwide. FMF is characterized by unexplained recurrent attacks of inflammation, which respond favorably to colchicine treatment. Secondary (AA) amyloidosis is the main and potentially lethal
complication of the disease. Although the possibility of multiple immunological mechanisms has been studied, however the actual molecular mechanism involved in the development of the inflammatory response occurring in FMF and amyloidosis FMF-associated is unresolved. The aim of the present study includes the study of immune response of organisms of patients with FMF complicated or not complicated by renal amyloidosis, as well as the effect of colchicine on the indicators of immune status of organism. The important components of the inflammatory immune response that seem to play a key role in FMF-associated immune response and may be considered as targets in FMF therapy is circulating immune complexes (CICs). The main task of the present study was to determine the levels of pathogenic CICs in the blood of patients with FMF complicated or not complicated by renal amyloidosis, before and after colchicine therapy. In our study 32 FMF affected subjects (males/females 20/12; mean age ± S.D. 28 ±10 years), 29 FMF affected subjects with renal amyloidosis (males/females 19/10; mean age ± S.D. 34±11 years) and 28 healthy volunteers (males/ females 16/12; mean age ± S.D. 31 ± 11 years) were involved. All the subjects were Armenians born in Armenia. Two subfractions of CICs were isolated from the serum samples of the patients and healthy subjects by precipitation with 3% and 4% polyethilenglycol (6kDa). Concentration of isolated CICs was determined by a spectrophotometric assay. Later so-called factor of the size (K) of CICs was calculated. K=K2/K1, where K1 and K2 were concentrations of CICs, isolated accordingly by 3 % and 4 % PEG-precipitation. K>1.5 value corresponds to pathogenic CICs. The results obtained indicated that the most of CICs in colchicine-free FMF patients (85%) and patients with renal amyloidosis (87%) represented pathogenic CICs, while in healthy subjects the pathogenic CICs made only 9% from a common population of CICs. After two months of regular colchicine treatment, no significant difference was detected between the patients involved and the healthy subjects. This study demonstrated that regular colchicine treatment results in suppression of formation pathogenic CICs. The effect of colchicine on these parameters suggests its immunomodulator potential. The results will help to define the peculiarities of molecular pathomechanisms involved in inflammatory response in the pathogenesis of FMF and assess the influence of colchicine on this response. The data of our study open the perspective of development of targeted methods of verifying the efficiency of colchicine treatment in FMF and prophylactics of amyloidosis.

(abstract 25)

**Alternative complement pathway in Familial Mediterranean fever**


1) Institute of Molecular Biology of Armenian National Academy of Sciences; 2) The First Department of Internal Medicine of the Yerevan State Medical University; 3) N1 University Hospital of Pharmacotherapy and Clinical Pharmacology of the Ministry of Health of Armenia

The human complement system is an important branch of innate immunity and comprises approximately 35 known plasma and membrane-bound proteins involved in efficient activation and tight regulation of the system. The activation of complement cascade by three pathways generates opsonins, inflammatory mediators and cytolytic protein complexes that play an essential role in tissue damage and normally function to eliminate foreign pathogens and to opsonise necrotic and apoptotic cells. However, the undesirable complement activation contributes to the pathology of many inflammatory diseases. During the attacks of Familial Mediterranean fever (FMF) multitudes of systemic events are triggered, most of which promote an autoinflammatory state. The complement is one of the several factors that contribute to pathogeneses in this disease. The detection of complement in FMF is essential for the understanding of disease pathomechanisms, especially from the point of view of immunity. In present study we examined the activation of alternative pathway (AP) and key regulators of AP: factor B (fB) and factor D (fD) in the blood
serum of 19 FMF patients (males 10, females 9; mean age ± S.D. 28 ±10 years). The control group consisted of 23 healthy volunteers (males 12, females 11; mean age ± S.D. 31 ±11 years) without FMF positive family history. A hemolytic assay was based on the standard 50% complement hemolysis test for AP of human serum complement. Furthermore, correlations of acute phase reactants such as C-reactive protein (CRP) with AP activity and foregoing complement factors were determined. In the serum of FMF patients the mean values of AP, fB and fD were significantly lower compared with the healthy subjects. However, no significant correlation between these factors in respect to the mean values of CRP was detected. Our study is continuing to expand the knowledge of the complement system in FMF. The obtained data evidence that AP activation probably is not involved in the process of activation of C3 and subsequent generation of C3d and C5b-9 membrane-active complex (MAC). It is quite possible, that the initiator of the activation of complement system is the formation of antibodies to modified proteins and subsequent formation of immune complexes, which are known to be increased at this pathology from our previous reaserches, which in his turn results in activation of complement by classical pathway. Therefore, it is reasonable to propose that significant hypoactivation of AP in FMF may be conditioned by higher concentration of CRP on targets via its interaction with inhibitor of complement AP activation factor H and C4BP. New studies of the complex interplay between the complement cascade and FMF-associated immune response may be a therapeutic target in the treatment of this disease. Further investigations of the activation of complement system in FMF and correlation of the measured parameters with FMF gene mutations and clinical picture will make it possible to provide a critical approach to identify the new strategies of FMF therapy.

(abstract 111)

Familial Mediterranean Fever and HLA markers

Tsulaia M. (1), Polianskaia I. (0), Beglaryan A. (2), Ayrapetyan H. (2), Sarkisian T. (2), Pagava K. (1)*

1) State Medical University, Tbilisi, Georgia; 2) Center of Medical Genetics and Primary Health Care, Yerevan, Armenia; 3)

AIM: To reveal a possible association between HLA markers and Familial Mediterranean Fever (FMF).

MATERIALS AND METHODS: HLA markers had been investigated in 72 children with clinically defined FMF according to the Tel-Hashomer criteria (without MEFV mutations testing). The age of patients was 3-16 y. HLA antigens of A and B classes using the microcytotoxic test in a total population of lymphocytes, HLA-DR antigens - by a prolonged test in B-lymphocytes. The following antigens were identified: locus A: A1, A2, A3, A11, Aw19, A28; locus B: B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, Bw22, B27, B35, B40; locus DR: DR1, DR2, DR3, DR4, DR5, DR27. The same investigations were performed in 93 healthy donors (46 females, average age 31.1±1.0; 47 males, average age 32.0±1.1). In addition, blood samples from members of 21 nuclear families were tested for the above-mentioned genetic markers. Each family included at least two affected persons from different generations incl. at least one affected child (in all 27 affected and 24 unaffected family members). RESULTS: In these group of patients the confidential positive connection was demonstrated in relation to B5 (RR=2.4) and B7 (RR=3.5). Correspondingly in females - to A1 (RR=2.7) and B7 (RR=5.7), in males – to A9 (RR=2.7) and B5 (RR=2.9); besides, the negative association was demonstrated in relation to DR1 (RR=0.19) in males. Familial analysis revealed HLA haplotype particular to each family which was connected to the FMF and has been segregated from the affected parents and/or grandparents to the affected children. Among 7 Armenian FMF patients with ankylosing spondilitis-like syndrome HLA B27 was detected in three patients with more severe destructive joint problem (especially hip joints). CONCLUSIONS: Our data suggest the hypothesis of a linkage between genes controlling FMF and
Familial Mediterranean Fever and HLA markers

Meri T. (1), Irina P. (0), Ara B. (2), Hasmik A. (2), Tamara S. (2), Karaman P. (1)*

1) State Medical University, Tbilisi, Georgia; 2) Center of Medical Genetics and Primary Health Care, Yerevan, Armenia; 3)

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Familial Mediterranean Fever and HLA markers

Pagava K. (1)*, Tsulaia M. (1), Polianskaia I. (0), Beglaryan A. (2), Ayrapetyan H. (2), Sarkisian T. (2)

1) State Medical University, Tbilisi, Georgia; 2) Center of Medical Genetics and Primary Health Care, Yerevan, Armenia; 3)

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The age of patients was 3-16 y. HLA antigens of A and B classes using the microcytotoxic test in a total population of lymphocytes, HLA-DR antigens - by a prolonged test in B-lymphocytes. The following antigens were identified: locus A: A1, A2, A3, A11, Aw19, A28; locus B: B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, Bw22, B27, B35, B40; locus DR: DR1, DR2, DR3, DR4, DR5, DR27. The same investigations were performed in 93 healthy donors (46 females, average age 31.1±1.0; 47 males, average age 32.0±1.1). In addition, blood samples from members of 21 nuclear families were tested for the above-mentioned genetic markers. Each family included at least two affected persons from different generations incl. at least one affected child (in all 27 affected and 24 unaffected family members).

RESULTS: In these group of patients the confidential positive connection was demonstrated in relation to B5 (RR=2.4) and B7 (RR=3.5). Correspondingly in females - to A1 (RR=2.7) and B7 (RR=5.7), in males – to A9 (RR=2.7) and B5 (RR=2.9); besides, the negative association was demonstrated in relation to DR1 (RR=0.19) in males.

Familial analysis revealed HLA haplotype particular to each family which was connected to the FMF and has been segregated from the affected parents and/or grandparents to the affected children. Among 7 Armenian FMF patients with ankylosing spondilitis-like syndrome HLA B27 was detected in three patients with more severe destructive joint problem (especially hip joints).

CONCLUSIONS: Our data suggest the hypothesis of a linkage between genes controlling FMF and HLA genes. We propose to study the probable intragenic cooperation causing the poor FMF course. As next step we will perform more precise molecular investigations of the FMF cohort in Georgia. Collaboration between our two centers will help to reveal the possible correlation between MEFV and HLA alleles and genotypes.

(abstract 117)

Amyloidosis of familial Mediterranean fever is the most frequent renal biopsy finding in children in Armenia


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Objective: To compare the results of renal biopsies of native kidneys in patients with FMF with those obtained in the other pediatric patients examined from 1993 to 2007. Patients and methods: Of all 212 patients aged 1-18 years who underwent percutaneous renal biopsy, 27 % had familial Mediterranean fever (FMF). Diagnosis of FMF was based on clinical findings and genetic analysis. None of the patients had been on regular colchicine treatment. The main indication for biopsy was the nephrotic syndrome, present in 82 % of patients with FMF and in 45 % of the 155 remaining patients. – Biopsies were evaluated by light microscopy in Yerevan and Zurich and by electron microscopy (except for amyloidosis) and immunohistochemistry (last 36) in Zurich. Results: The most common histological lesion was renal amyloidosis (22%), followed by focal segmental glomerulosclerosis (FSGS, 9%), lupus nephritis (7%), and various types of glomerulonephritis. Of the 47 patients with amyloidosis, 43 were nephrotic and 4 had non-nephrotic proteinuria. Notably, 10 (4 nephrotic) of the 57 patients with FMF had nephropathies other than amyloidosis: Minimal change nephrotic syndrome (4), acute post-infectious glomerulonephritis (2), extrarenal vasculitis (2), FSGS (1) and chronic interstitial nephritis (1). Of the 2 patients with vasculitis, one (with a purpuric rash) had a GN with few fibrocellular crescents without immune complexes, consistent with ANCA-associated GN, and the other (with protracted myalgias) had crescentic membranoproliferative GN type I. Conclusions: The large number of renal amyloidosis due to FMF is striking.
Therapy with colchicine has meanwhile been strongly intensified. However, an important minority of FMF patients had other renal lesions that were probably coincidental (in 8 of 10), thus underlining the importance of renal biopsy. This study would not have been possible without international collaboration.

(abstract 91)

**The age related peculiarities of colchicine therapy in children with Familial Mediterranean fever (FMF)**

Petrosyan R. (1)*

1) First

Background: It is well known fact that colchicine is the most effective and reliable drug in treatment of Familial Mediterranean Fever. Along with that its full pharmacological mechanism is still unknown. The goal of this research was to find out the relationship between the patients age, allelic condition of MEFV and the daily dosage of colchicine which relieves fully the attacks of diseases. The sample consisted of 110 children being under the follow up in Republican Children FMF Centre of Armenia. All the children were aged from to 15 years. Student’s statistics was used to evaluate the significance of the dosage difference obtained in different age groups. All the patients were divided into three major groups: younger age children – to 4.5 years, middle age - 4.6 to 6.5 years, older age - above 6.6 years. It was found the average daily dosage of colchicines per one kg of body weight was 0.037+0.0029 mg/kg in younger group, 0.0316+0.002 mg/kg in middle aged and 0.034+0.028 mg/kg in older aged group. The variations between the daily dosage made 0.0187+0.00084 to 0.058+0.0014mg/kg showing no statistically significant difference between the age groups. Anyway the dosages calculated in all the groups were much more higher used before and they corresponded to those reported by the leading FMF centers in the world. While splitting the above mentioned groups of patients into the subgroups dependant on the FMF allelic condition we found that the homozygous and compound heterozygous of younger group required significantly (∆)

(abstract 93)

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(abstract 96)

The age related peculiarities of cholchicine therapy in children with Familial Mediterranean fever (FMF)

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(abstract 71)

Is E148Q a Benign polymorphism or a Disease Causing Mutation?


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Is E148Q a Benign polymorphism or a Disease Causing Mutation? Dina Marek1, Yaakov Berkun2, Shai Padeh2, Almogit Abu1, Haine Resnik Wolf1, Mordechai Pras4, Avi Livneh3,4, Elon Pras1 1The Danek Gartner Institute of Human Genetics, 2Safra Children Hospital, 3Department of Medicine F, 4Heller Institute of Medical Sciences, Sheba Medical Center, Tel Hashomer, Israel; Affiliated to the Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel Familial Mediterranean fever (FMF) is an autoinflammatory disease characterized by recurrent attacks of fever and serositis. The disease has been shown to be caused by mutations in the MEFV gene. E148Q is one of the most common changes detected in this gene. Originally E148Q was considered a disease causing mutation but recent studies have raised doubts and suggested it to be a benign polymorphism. Tchernitcho et al. found a similar frequency of E148Q in 233 FMF patients (3.62%) compared to their unaffected relatives (3.75%) and recently has shown that there is no preferential
transmission of E148Q from heterozygous patients to their affected offspring's. We used data from 4 previous studies and compared the frequency of E148Q in Israeli FMF patients with definite disease to a very large group of Israeli control subjects. Three of the studies estimated the frequencies of the 3 most prevalent FMF sequence variations M694V, V726A and E148Q in Israeli control population and one study described the prevalence of these three mutations in a group of 412 Israeli patients with definite FMF according to the Tel Hashomer criteria. The E148Q allele was found in 58 of 824 FMF alleles (7.03%) compared to 163 of 2802 control alleles (5.8%), p=.228). Using the same data sources a much larger difference was found for the M694V mutation (391 of 824 FMF alleles compare to 82 of 4188 control alleles, p=.00001) and for the V726A mutation (122 of 824 FMF alleles compare to 141 of 4018 control alleles, p=.00001). Although we found a small difference between the groups it did not reach statistical significance. Since there are no functional studies to assess the role of sequence variations in MEFV, discriminating between benign polymorphisms and disease causing mutations rely on epidemiological data. Despite the large size of our sample we could not find evidence that would support the notion that E148Q is a mutation and not a polymorphism.

(abstract 188)

**Nontraditional Mutational Mechanisms in Familial Mediterranean Fever**

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1) University of Iowa, Department of Pediatrics

Familial Mediterranean fever (FMF) is the archetypal autoinflammatory syndrome. It is an autosomal recessive disease whose responsible gene, MEFV, encodes for pyrin/marenostrin. Pyrin is thought to downregulate neutrophil-mediated inflammation by inhibiting IL-1 processing. Clinical heterogeneity is the hallmark of FMF and is likely influenced by MEFV genotype. Using traditional mutational analysis, nearly 70 clinically-relevant point mutations or small deletions in MEFV have been identified, but in an appreciable portion of FMF patients one or more mutant alleles are not identified. The majority of the studies employ direct sequencing in addition to other mutation detection techniques, which would not detect exonic deletions or duplications. Multiplex Ligation-dependent Probe Amplification (MLPA), is a high throughput technique designed to detect moderate to large deletions or duplications. We hypothesize that MEFV exonic deletions or duplications contribute to clinical variability of FMF. The Arab FMF population has the highest proportion of unidentified mutations (up to 50%). Therefore, it may provide the best chance of determining if non-traditional mutational mechanisms are important in FMF. Our lab has collected data on a cohort of 200 unrelated and clinically well-characterized Arab FMF patients, and 200 matched controls. Exon 10 of MEFV was sequenced in 48 patients; 34 out of 96 mutant alleles (35%) were identified: 11 homozygous M694V, 2 homozygous M694I, 2 homozygous V726A, and 1 compound heterozygote M694V/M694I. Two patients had only one detectable mutant allele, M694V in one and M694I in the other. Of the 48 patients, four patients were found to have abnormal MLPA results: exon 1 duplication, exon 2 deletion, exon 3 and exon 10 deletion, and exon 1 and exon 8 deletions. Polymorphisms at the site of probe hybridization may lead to apparent, although false positive, deletion. We are currently sequencing the exons to exclude this, as well as employing other methods to confirm these findings. FMF remains a serious disease with significant morbidity and continued mortality. Although the diagnosis remains a clinical one, the ability to reliably identify mutations in all affected individuals would allow earlier diagnosis and treatment. Our data suggest that MEFV deletions or duplications may play a role in disease susceptibility. Understanding the full spectrum of MEFV mutations will facilitate current genotype-phenotype correlation studies.
Two years continuous intravenous colchicine therapy for treatment of familial Mediterranean fever (FMF) unresponsive to oral colchicine

Objective: Use of intravenous (IV) colchicine to supplement oral therapy in FMF patients resistant to standard therapy over a period of several months has been previously reported. In the present report we evaluate the efficacy and safety of weekly (IV) colchicine, in addition to oral colchicine therapy, administered for a period of two years to a patient with FMF and near total small bowel resection, who was previously unresponsive to standard oral colchicine prophylaxis. Methods: A 35 year old Jewish female FMF patient of North African origin, with unrelated polycystic kidney disease, who was of M694V homozygous genotype, continued with frequent FMF attacks despite oral colchicine, 2-3 mg/day. She suffered a catastrophic mesenteric vein thrombosis which resulted in ischemic/necrotic small bowel. As a result, she underwent a near total small bowel resection. No evidence of vasculitis was found on pathological examination. Further investigations included a search for a coagulopathy which revealed factor V Leiden heterozygocity. The patient was treated with additional weekly IV colchicine, 1 mg per infusion, for two years, along with total parenteral nutrition. She was evaluated periodically for all disease manifestations and specifically for frequency of FMF abdominal, chest, joint and skin attacks, laboratory studies especially monitoring erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP), and adverse effects. Results: With the IV treatment, there was complete remission of her febrile abdominal and thoracic attacks; joints attacks (arthritis) and erysipelas-like skin lesion attacks, however, were unrelieved during the study period. Recurrent abdominal pain without fever was reported. It was apparently unrelated to the FMF, but linked instead to documented esophagitis and renal stones and remitted with therapy appropriate to these diagnoses. Significant reduction in ESR and CRP was observed under IV colchicine therapy. Complete blood counts, liver and renal function tests were unaffected by the treatment. The IV colchicine treatment was apparently safe and well tolerated, without reports of side effects and with no detected local chemical phlebitis at IV sites nor evidence of amyloidosis. Conclusions: This is the longest treatment with weekly IV colchicine, in addition to oral colchicine, 2 years, reported in the English literature. On documented followup, safety was confirmed and partial efficacy, on abdominal and thoracic febrile attacks, was demonstrated in a patient with FMF previously refractory to oral colchicine.

Intravenous colchicine treatment for six months: adjunctive therapy in familial Mediterranean fever (FMF) unresponsive to oral colchicine.

Objective: Regular oral colchicine treatment may not control the typical febrile attacks of FMF in about 5-10% of compliant patients. Supplementary intravenous (IV) colchicine has been suggested as efficacious therapy in this setting. We report herein on the efficacy and safety of weekly IV colchicine for six months, in addition to oral colchicine therapy, in 5 oral colchicine-resistant
patients. Methods: Five patients with frequent FMF attacks despite maximal oral colchicine therapy were treated with weekly IV infusions of 1 mg colchicine for 6 months. They were evaluated at intervals for the number of abdominal and pleuritic febrile attacks, joints flairs, and erysipelas - like skin lesions, as well as white blood cell count, erythrocyte sedimentation rate (ESR), and C reactive protein (CRP) and this data was compared with results of these parameters in the 6 months preceding the trial therapy. Results: A fifty percent reduction in the frequency of febrile abdominal and chest attacks was achieved in 6 months. Statistically significant (paired T-test) differences were observed in white blood cell count, ESR, CRP (p< 0.05). Joint attacks and erysipelas – like skin lesions were unrelieved during the study period (p=0.28). The treatment was safe and well tolerated, without side effects relating to the infusions nor evidence of colchicine toxicity. FMF patients before / under colchicine IV therapy Patients genetics Abdominal Joints/ WBC ESR CRP age/sex /thoracic erysipelas febrile - like attacks attacks 39/F V726A 7/0 2/2 12700/8810 35/14 149/18 E148Q 28/M M694V 12/8 6/5 16200/7120 60/30 104/23 M694V 23/M M694V 6/1 0/0 17900/7520 80/25 45/25 M694V 34/M M680I 11/7 3/3 13000/8500 65/44 25/0.3 M680I 20/M ND 5/0 5/0 17000/9460 55/20 200/4 Paired T-test difference P

(abstract 40)

Intravenous colchicine treatment for six months: adjunctive therapy in familial Mediterranean fever (FMF) unresponsive to oral colchicine.
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Objective: Regular oral colchicine treatment may not control the typical febrile attacks of FMF in about 5-10% of compliant patients. Supplementary intravenous (IV) colchicine has been suggested as efficacious therapy in this setting but other FMF manifestations were not assessed. We report herein on the efficacy and safety of weekly IV colchicine for six months, in addition to oral colchicine therapy, in five oral colchicine-resistant patients. Methods: Five patients with frequent FMF attacks, despite maximal oral colchicine therapy, were treated with weekly IV infusions of one mg colchicine over a six month period. They were evaluated at intervals for the number of abdominal and pleuritic febrile attacks, arthritis episodes and erysipelas - like skin lesions, as well as white blood cell count (WBC), erythrocyte sedimentation rate (ESR), and C reactive protein (CRP) and this data was compared with results of these parameters in the 6 months preceding the trial therapy. Results: The following are the patients' age / sex, genetics (pyrine mutations) and data referring to results in the six months before / under colchicine IV therapy as to – number of abdominal and thoracic febrile attacks, number of joint and erysipelas- like skin attacks, WBC (cells per ml), ESR (mm per hour) and CRP (mg per dl): Patient A 39/ F, V726A/E148Q, 7/0, 2/2, 12800/8810, 35/14, 149/18 Patient B 28/M, M694V/M694V, 12/8, 6/5, 16200/7120, 60/30, 104/23 Patient C 23/M, M694V/M694V, 6/1, 0/0, 17900/7520, 80/25, 45/25 Patient D 34/M, M680I/M680I, 11/7, 3/3, 13000/8500, 65/44, 25/0.3 Patient E 20/M, Not Done, 5/0, 5/0, 17000/9460, 55/20, 200/4 Paired T-test differences were calculated as: P

(abstract 56)

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(abstract 106)

Desensitization to colchicine in a patient with Familial Mediterranean Fever

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Background. Colchicine toxicity is well known in patients with familial Mediterranean fever (FMF) and Behcet’s disease (BD). Toxic adverse events can be avoided not using colchicine in combination with drugs interacting with CYP3A4 and/or P-glycoprotein. Moreover, in order to improve gastrointestinal colchicine tolerance, a lactose-free diet and the treatment of intestinal bacterial overgrowth and/or Helicobacter Pylori-infection are recommended. Less frequently colchicine treatment interruption may be due to a hypersensitivity reaction. Since colchicine is the gold standard for FMF therapy and therefore the drug of choice for prophylaxis against FMF attacks and FMF-associated amyloidosis, desensitizing treatment may be considered in patients with colchicine hypersensitivity. Methods. We report of a 22-year-old female, affected by FMF. Once diagnosis was given, oral colchicine 1 mg/day was started, with immediate improvement and gradual regression of all her symptoms. Several further episodes of fever and pleuritic pain led to increase her oral daily dose of colchicine and starting the intravenous administration. At the cumulative daily dose of 3 mg, the patient developed a generalized itching eritematous rash with oedema of the glottis, that promptly remitted after the therapy was discontinued and intramuscular corticosteroids were administered. Prick, intradermal and patch tests performed with colchicine resulted negative. On the basis of a positive oral challenge test (fever, vomiting, diarroea and abdominal pain at the threshold dose of 1 mg) a nonallergic hypersensitivity (intolerance) to colchicine was diagnosed. Since the drug was indispensable for the patient, she underwent a 9-day oral desensitizing treatment. Progressively increasing doses of colchicine solution (0.5 mg/ml) were administered every 30 minutes, starting from 0.1 ml of a solution diluted 1:10000 in saline to reach
the final dose of 3 mg on the 9th day. Results. The patient completed the desensitizing treatment without side effects reaching the highest dose of 3 mg. She is currently on oral therapy with 2 mg colchicine daily without problems. Conclusions. Another paper reported a successful desensitization to colchicine in a similar case, but the dose reached was lower (0.25 mg). Further studies are needed to assess efficacy and safety of these treatments, however colchicine desensitization can be considered a useful tool in FMF-patients who developed hypersensitivity reactions.

(abstract 3)

**Familial Mediterranean Fever in Iranian Children, First Study from Iran**

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1) ARUMS( Ardabil University of Medical Sciences )

Familial Mediterranean Fever in Iranian Children, First Study from Iran: Familial Mediterranean fever, which is the prototype of the hereditary periodic fever syndromes, is common in the countries around the Mediterranean Sea. "Familial Mediterranean fever" is the common form of the "Hereditary Periodic Fever Syndromes" which has autosomal recessive pattern and presents with self-limited periodic fever and serositis in its classic form. Jewish, Armenian, Arab, and Turkish people or in other words people who have Mediterranean originality are affected by this disease. MEFV is the responsible gene in this disease and it is located on the short arm of chromosome 16. The mutation of this gene leads to a defect in synthesis of a protein known as pyrin-Marenostrin.

Northwest of Iran is near to Turkey and people who live there have Turkish origin. This is the first report of FMF in children who live in northwest of Iran. Although it is a common disorder in north-west of Iran, interestingly it was not a familiar syndrome to physicians in this area. Methods: This research is a descriptive study from October 2004 to October 2007 at pediatric rheumatology clinic. According to the Tel-Hashomer criteria, FMF had been diagnosed. All of the patients had been examined, interviewed (and their parents when it needed) and have filled out a questionnaire in pediatric rheumatology clinic. Findings: 43 patients inter to this study, all were under 18 years old and minimum age was 1.5 years. 25 patients were female and 18 were male (M/F ratio was 0.7). Abdominal pain (60.5%) and fever (11.6%) were the main clinical symptoms in this group. The most common symptoms in these patients were systemic symptoms (97.7%) GI (95.3%) and musculoskeletal (55.8%) respectively. The most common period of pain was 12-72 hours. Majority of the patients had hospital admission for diagnostic work up (85%) and some of them (32%) had surgical operation erroneously. More than 90% of the patients had taken different drugs before diagnosis and 20% had positive family history of FMF. The parents of patients were first degree relatives in more than 50% and in 59.5% delay in diagnosis was more than 3 years. All took Colchicine as a first choice therapy, and more than 95% had good response to colchicine. Result: Not surprisingly, FMF is a common chronic and periodic auto inflammatory disorder in northwest of Iran, although it is not a familiar disease to physicians in Iran. Key words: FMF, periodic fever syndrome, children, colchicine

(abstract 211)

**A SURVEY OF CO-MORBIDITY AND SOCIOECONOMICAL STATUS IN PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER (FMF)**

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1) Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey
Objectives: To describe the frequency of co-morbidities in addition to socioeconomical and educational status in patients with FMF comparing with diseased and non-diseased controls.

Methods: Consecutive 144 (75 M/ 69 F) patients with FMF, 79 (4 M / 75 F) with systemic lupus erythematosus (SLE), 142 (84 M/ 58 F) patients with Behcet's syndrome (BS) and 96 (57 M/ 39 F) non diseased controls selected among hospital workers, all aged 18 years and over, participated in the study. A formal questionnaire was prepared to assess the co-morbid conditions, socioeconomical and educational status of the study group and co-morbid conditions and deaths among first-degree relatives. Results: There were significantly more females among SLE patients due to inherent disease conditions compared to other groups (P < 0.001). FMF and BS patients were relatively younger compared to patients with SLE and HC. Patients with FMF were more likely to be single (P = 0.05) and to have a higher educational level (P < 0.001). Patients with BS had significantly lower monthly income compared to other study groups (P < 0.001). The rate of employment, which was assessed only among males, was also similar among patients with FMF, BS and controls (P = 0.2). The frequency of diabetes mellitus, chronic bronchitis, recurrent upper respiratory tract infections, cardiovascular disease, stroke, hyperlipidemia, peptic/duodenal ulcer, psoriasis, allergic skin disorders, deafness, hepatitis, tuberculosis and cancer was not different among the study groups. While SLE patients were more likely to have hypertension and urinary tract infections, FMF patients were more likely to have diarrhea and history of penicillin prophylaxis. History of FMF and recurrent use of antibiotics were more likely to be frequent among relatives of FMF patients. History of skin allergic disorders was less frequent among relatives of BS patients. History of cancer was more frequent among relatives of SLE patients. Family history of death in any first degree relative was less frequent in the FMF group compared to other study groups. Conclusion: The frequency of co-morbidities such as diabetes mellitus, ischemic heart disease or stroke were not different among patients with FMF, SLE and controls and their first degree relatives. Low prevalence of death among relatives of FMF patients deserves further prospective studies.

(abstract 132)

**Longevity and MEFV mutations: a study in mutation carriers**

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Introduction: The high carrier rate of MEFV mutations in the Mediterranean populations may suggest an advantage for heterozygous subjects in combat with lethal pathogens. In the era of modern medicine this advantage may still extend longevity. Adversely, a continuously alerted innate immune may precipitate or aggravate inborn trends for inflammatory diseases, leading to a shorter lifespan. Aim: To determine the association between MEFV mutations and longevity and examine the disease profile of mutation carriers. Methods: 168 Israeli subjects aged 90 years and above hospitalized at the Sheba Medical Center were screened for the common MEFV mutations: M694V, V726A and E148Q. Their mutation rate was compared to that in historical control cohorts and their disease profile was portrayed by questionnaire. Results: The cohort included 114 (68%) women and 54 (32%) men, average age 94 yrs. 130 (77%) of the patients were of Ashkenazi origin. There were 39 (23%) MEFV mutation carriers, most (30 carriers) had the E148Q mutation, 8 had the V726A mutation and 1 had the M694V mutation. In addition, one subject had two mutations, V726A and E148Q, with no FMF symptoms. Old Ashkenazi Jews had significantly more MEFV mutations than historical Ashkenazi controls (29/130 vs. 81/1582, OR 2.7. 95% CI 1.7-4.2, p=0.0001) and this attributed to a 2 fold higher rate of the E148Q mutation in both genders. The major life threatening diseases (myocardial infarct, cerebro-vascular accident and diabetes) as well
as the common infectious diseases (pneumonia and urinary tract infection) did not differ in distribution between carriers and non-carriers. The rate of malignant diseases was low irrespective of the mutation status. However, hypothyroidism, a condition whose rate increases with old age was 2 fold higher in the MEFV mutation carriers (20% in carriers, 9.3% in non-carriers, OR=2.4, 95% CI 0.9-6.4, p=0.09). 2 carriers but none of the non-carriers had osteoarthritis (p=0.055).

Conclusions: The higher rate of the mild, E148Q mutation in old Ashkenazi Jews than in historical controls, suggests that it may contribute to their longevity. However, the mutation carriers did not appear to be protected from the major mortality causing diseases or from infectious diseases. A possible connection between the E148Q mutation, increased hypothyroidism and longevity remains to be further explored. The effect of the more severe, non-Ashkenazi MEFV mutation, M694V, remains to be examined

(abstract 52)

**Rare MEFV exon 10 mutations in Israel**


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Introduction: By large, the screen for MEFV mutations in patients suspected for FMF is confined to the three most common mutations in Jews: M694V, V726A and E148Q. In Arab patients, two more mutations, M680I and M694I are considered common, and added to the routine mutation screen. However, about 45% of the MEFV gene alleles of FMF patients do not bear these FMF associated mutations. Aim: To determine the significance, spectrum, and manifestations of other MEFV mutations in Israeli subjects with suspected FMF. Methods: Sequencing of exon 10 of MEFV was performed on DNA samples from 138 Jewish and 83 Arab patients with manifestations suggesting FMF. Re-evaluation for possible FMF, using established criteria, was performed in subjects in whom “other” mutations were found. Results: In addition to the common mutations, found in about 30% of the alleles of both Jewish and Arab referrals, five other mutations were identified: G632S, R753H, K695R, A744S and R761H. These mutations were carried by 9/276 suspected Jewish MEFV alleles (3.3%), and by 1/166 (0.6%) suspected Arab MEFV alleles. The Jewish subjects with these mutations were of Ashkenazi, Bulgarian, Buchari, Iranian, Syrian, and Kochini extractions, all considered atypical for FMF. Based on clinical criteria, 6 of the 9 rare-mutation carriers were diagnosed with FMF, 2 with one rare mutation and 4 with 2 FMF mutations, one of which was a rare mutation. In the remaining 4 of the 10 rare-mutation carriers, clinical evaluation still continues. Conclusions: These results suggest that other mutations are by far rarer than the common mutations, and are not likely to account for the absence of MEFV mutations in the majority of MEFV mutation-negative Israeli FMF patients. Further studies are needed to determine the penetrance of these rare mutations.

(abstract 51)

**The prevalence of familial Mediterranean fever gene mutations in patients with rheumatic heart disease**

BACKGROUND: Rheumatic heart disease (RHD) represents the most severe manifestation of Acute rheumatic fever (ARF). Although there is much evidence for the role of group A streptococci in the etiology of ARF, the pathogenesis of ARF and RHD remains an enigma. It is intriguing why only a small proportion of infected individuals actually develops ARF and consequently RHD. The host response to streptococcal antigens is under genetic influence, and it is therefore pertinent to search for genetic markers that influence the development of RHD. In this regard, Mediterranean fever (MEFV) gene mutations might be candidate, since genetic defect in familial Mediterranean fever (FMF) is proposed to be the impaired control of inflammation in response to certain recognized and unrecognized stimuli. ARF has been considered in the differential diagnosis of FMF because these two diseases have some clinical and laboratory features in common. There are also autopsy reports of rheumatic mitral stenosis in patients with FMF and amyloidosis. Moreover, a history of ARF during childhood is not infrequent among patients with FMF. However, it is not clear whether or not this coexistence reflects a true relationship between two similar diseases.

OBJECTIVE: The aim of the present study was to investigate the role of the MEFV gene mutations in the genetic susceptibility to RHD in Turkish patients and establish the relationship of these alleles with the pattern of valve damage.

METHODS: A total of 31 patients (21 women, 10 men; age range, 18-50 years; mean age, 34 ± 14.8 years) with an echocardiographically documented predominant mitral stenosis were enrolled in this study. Diagnosis of valve lesions was made by echocardiography, catheterization or both. Patients with predominant mitral regurgitation or isolated aortic or tricuspid valve disease were excluded. None of the patients had the diagnosis of FMF. Genetic analysis was carried out by the NanoChip® Molecular Genetics Workstation investigating two hot spots (exon 2 and 10) for MEFV mutations.

RESULTS: Four of the patients were found to have heterozygote MEFV mutations. Three of these mutations were E148Q/- and one was V726A/-.

CONCLUSION: In the light of our preliminary results, we may conclude that the frequency of MEFV mutations in patients with RHD are not higher than the normal population. Further studies with larger sample sizes are needed for better understanding the possible relationship between these two disorders and to clarify whether specific mutations play role in the pathogenesis.

(abstract 58)

Is there a link between MEFV gene mutations and gouty arthritis?


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INTRODUCTION: Gouty arthritis is a common medical problem, affecting at least 1 percent of men in Western countries. The classic symptoms of gouty arthritis are recurrent attacks of acute, markedly painful monoarticular or oligoarticular inflammation, but polyarthritis and chronic arthritis can occur. Colchicine is an effective treatment for acute attacks of gout. Furthermore it is used to reduce the recurrence of acute attacks of gout during initiation of antihyperuricemic therapy (1). On the other hand, familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by recurrent attacks of fever and serositis. FMF is caused by mutations in MEFV gene. Recurrent attacks of arthritis is a presenting symptom of 15% of FMF patients of Turkish origin. Attacks of FMF responds to colchicine and it is also used in the prevention of FMF attacks (2). The aim of this study was to examine the frequency of MEFV gene mutations and its relation with arthritis attack in gout patients.

Methods: A total of 26 gout patients (diagnosed according to the clinical criteria of
Wallace) were included in this study. Subjects were questioned for the presence of the Tel-Hashomer criteria for diagnosis of FMF. Sex, age, number of gout attacks, diuretic use, history of renal stone and presence of tophus were also collected. Genetic analysis was carried out by the NanoChip® Molecular Genetics workstation investigating two hot spots (exon 2 and 10) for MEFV mutations. Results: All participants were male. The mean age of subjects were 51.4 (Range 21-79) years. 16 patients had less than 5 attacks, 4 patients had 5-10 attacks, one patient had 10-15 attacks and 2 patients had more than 15 attacks. Three of the gout patients were diagnosed as polyarticular gout. One patient had tophus, five of the subjects had a history of renal stone and ten patients had a history of diuretic use. There were no subjects who had clinical features of FMF. Three out of twenty-six patients had mutations in MEFV gene (11.5%). None of them were homozygotes. Two patients were heterozygotes for pyrin M694V and one were heterozygotes for pyrin E148Q. There were no correlations between the MEFV gene mutations, number of gout attacks, history of renal stone and history of diuretic use (p>0.05) Discussion: In this study we found 11.5% of the gout patients had MEFV gene mutations. It has been reported that healthy turks had a 20% frequency of MEFV gene mutations (3). According to these findings we may speculate that MEFV gene mutations in the Turkish gouty patients are not increased and the presence of mutations are not associated with the frequency of gout attacks.

(abstract 63)

MEFV MUTATIONS IN AN ELDERLY POPULATION


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Introduction Two main observations suggest that MEFV gene is involved in inflammatory pathway in general: 1) Increased MEFV mutation frequencies in other chronic inflammatory diseases such as Behçet’s disease (1-3) and rheumatoid arthritis (4); 2) Decreased mRNA product of MEFV gene in healthy mutation carries (5) and during acute inflammation other than FMF (6). We hypothesized that the frequency of such mutations in the elderly population who have remained free of disorders with chronic inflammation, like FMF or Rheumatoid Arthritis would be lower than in the general population. We have therefore analyzed the frequency of most common MEFV mutations in such a group of healthy elderly population. Methods An ‘elderly group’ was chosen among the patients from the outpatient clinics of the division of Geriatrics of the Cerrahpasa Medical Faculty. Patients with a negative history for inflammatory disorders and normal physical examination who led a normal life style selected into the study group (N=165). A rheumatologist as well as a geriatrician were involved in patient selection. Total genomic DNA was isolated from blood, and PCR-RFLP analysis was performed using established protocols. Patients serums were analyzed for inflammation markers, high sensitive C-reactive Protein (hsCRP) and Romatoid Factor (RF) levels in the hospital central laboratory. Chi-squared test was used for comparison in non-parametric analyses. Results and Discussion Demographic properties of the elderly population are as follows: 73% women 27% men; average age 74, (%95 C.I=67-80, SD=6.553); hypertension in 74%, osteoporosis in 53% osteoarthrosis in 29%; 91% have hsCRP < 5 mg/dL and 88% have RF < 15 IU/mL. The frequency of five common mutations (M694V: 1,86%, M694I: 1,83%, M680I: 0,62%, V726A: 2,16%, E148Q: 5,18%) (carrier rate 23%) were found not to be significant when compared
to Turkish historical controls. However, subgroup analyses for inflammation markers such as; RF and hsCRP levels versus mutation subgroups showed that carriers of E148Q mutation had significantly higher RF levels compared with non-carriers (X²= 7.114, df=1, p=0.008). Increased prevalence of clinical RA and RF positivity with age are well known observations. Our findings suggest that these might be related to MEFV mutations.

References

Mutation analyses of the MEFV gene in 11 Familial Mediterranean fever patients and 54 healthy control subjects in Japan.


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Background: Familial Mediterranean fever (FMF) has been considered a rare disease in Japan. A lack of specific diagnostic methods made it difficult to diagnose FMF before the causative gene (MEFV) was identified. Thirty-four FMF cases confirmed by the presence of MEFV mutations have been reported in Japan until now. The mutations identified in these Japanese patients (L110P, E148Q, P369S, R408Q, and M694I) differ from the five founder mutations common in FMF patients from Mediterranean countries (E148Q, M680I, M694V, M694I, and V726A). Since treating our first FMF patient in 2005, we have diagnosed 11 FMF patients by screening for these mutations.

Material & Methods: The mutation analyses were performed to detect the presence of L110P, E148Q, P369S (R408Q), and M694I using an amplification refractory mutation screening system (ARMS) and RFLP methods. We examined the mutations in patients with recurrent fever and abdominal or chest pain with elevated inflammatory reactions, and in 54 healthy anonymous control subjects.

Results: The FMF patients were 8 males and 3 females with a mean age of 40.8 years (range: 21 to 77 years). Mutation analyses revealed nine compound heterozygous E148Q/M694I mutations and two homozygous P369S and R408Q mutations with homo- and heterozygous E148Q mutation. The allele frequencies of L110P, E148Q, P369S (R408Q), and M694I in the healthy volunteers were 5.6%, 24.1%, 2.8%, and 0.0%, respectively. Eight control subjects (14.8%) had homozygous or compound heterozygous mutations of these mutations (L110P/E148Q; 4 cases, L110P/E148Q/E148Q; 1 case, E148Q/P369S/R408Q; 2 cases, L110P/E148Q/E148Q/P369S/R408Q; 1 case).

Discussion: Most of the Japanese FMF patients had the M694I mutation. The allele frequency of M694I in the Japanese patients was 47.7%, which was much higher than that in Mediterranean FMF patients (0 to 7%). Although other mutations (L110P, E148Q, P369S, and R408Q) were frequently detected in normal Japanese subjects, some of the typical FMF patients also had these mutations. We should follow these subjects with mutations for the development of FMF-like symptoms.

Prevalence and significance of mutations in the familial Mediterranean fever gene (MEFV) in patients with COPD

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Purpose: Familial Mediterranean fever (FMF) is a recessively inherited disorder caused by the mutations of the MEFV gene. As the MEFV gene plays an important role in the regulation of neutrophil-related inflammatory processes, the mutations of this gene are thought to alter the clinical manifestations of other inflammatory diseases. COPD is also characterized by the inflammation of small airways caused by cigarette smoking and the enhanced inflammatory reactions seen in MEFV mutation carriers may thus play a role in altering the responses to cigarette smoking in the small airways. In this study we examined the frequency of selected MEFV mutations in COPD patients and analyzed the relationship between the presence of mutations and the clinical pictures of COPD. Materials & Methods: We analyzed the presence of MEFV mutations in 102 COPD patients, 54 healthy anonymous control subjects, and 19 smokers with normal pulmonary function tests. We examined the L110P, E148Q, P369S, and M694I mutations using RFLP and an amplification refractory mutation screening system (ARMS). Results: COPD patients consisted of 94 males and 8 females with a mean age of 73.7 years. Overall prevalence of mutation carriers among healthy controls and COPD patients revealed no significant differences (48.3% vs. 44.4%). Among the COPD patients, serum IgE level (IU/ml) was significantly lower in patients with mutations than in those without mutations (134.0 vs. 400.8, p=0.0234). COPD patients on home-oxygen therapy were more frequently mutation carriers than the patients not on home-oxygen therapy (76.9% vs. 47.2%, p=0.0452). Allele frequencies of E148Q and P369S and the frequency of homozygotes/compound heterozygotes were significantly higher in COPD patients on home-oxygen therapy than in those not on home-oxygen therapy. FEV1.0 is likely to decline more rapidly in patients with MEFV mutations than in those without mutations. The mean annual declines of FEV1.0 (ml/year) of normal, heterozygote, and homozygote/compound heterozygote groups were -2.7, -29.1, and -43.7, respectively. COPD patients had a significantly higher allele frequency of E148Q and were more frequently carriers and homozygotes/compound heterozygotes of the MEFV mutation, when compared with normal smokers. Conclusion: These data appear to suggest that MEFV mutations may be responsible for the development of COPD in smokers and may modulate the clinical pictures of COPD.

(abstract 11)

Carriers of MEFV mutations and symptoms related to FMF


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Clinical and genetic investigations of more than 8000 Armenian patients suffering from Familial Mediterranean Fever (FMF, OMIM 249100) demonstrated significant correlation between spectrum of MEFV (MEditerranean FeVer) mutations and clinical severity including of renal amyloidosis. Using PCR and reverse-hybridization molecular technology for diagnostics of FMF (ViennaLab Labordiagnostika GmbH, Austria) we have determined the symptoms associated with MEFV mutations in heterozygote carriers, believed to be suffering from FMF, and compared with affected homozygotes and compound heterozygotes. Among 450 healthy controls the asymptomatic carriers of MEFV mutations (1:5) were detected with the following frequencies: M694V (4.7%); V726A (4.6%); M680I (1.8%); R761H (0.2%); F479L (0.4%); P369S (4.9%); E148Q (3.4%). This distribution was compared with the data obtained from FMF patients: M694V (50.6%), V726A (22.3%), M680I (18.7%), R761H (3.2%), M694I (0.4%); E148Q (2.2%), F479L (1.3%). MEFV mutations were detected in 98.65% of FMF patients (77% of exon 10 mutations): compound heterozygotes (83.7%), only one mutation carriers (12.9%), or 3-4 mutations carriers(0.3%). No
MEFV mutations were detected in 3.12% of patients with definite (0.99%) or probable (2.13%) FMF. In carriers of one MEFV mutation the most prevalent and severe cases are caused by the presence of a single M694V or M680I mutation, which was associated with fever, abdominal, thoracic, and joint pain, skin symptoms, myalgia (protracted fibril), and renal amyloidosis rarely. The highest relative risk for the FMF development in carriers of M694V (1.9) and M680I (1.8) mutation in comparison with the carriers of other MEFV mutations is statistically significant. The following FMF mutations in V726A, M680I, R761H, E148Q, F479L heterozygotes still cause definite FMF. Carriers of M694I and R42W mutations usually show no clinical symptoms. As we mentioned above, P369S mutation is the most common in the healthy population (4.9%) but is less frequently represented in the patients (0.2%). This suggests a reduced penetrance of P369S mutation. Among healthy controls we detected 10 genotypes with P369S complex alleles, 7 didn’t show clinical FMF manifestation, 3 manifested mild disease, suggesting that P369S might ameliorate the phenotypic effect of exon 10 mutations. In 17 individuals with suspicion on FMF P369S mutation was revealed: 7 carriers, 8 compound heterozygotes (1 P369S/F479L, 7 P369S/E148Q) and 2 complex alleles. A744S rare mutation was revealed in 3 heterozygotes and 3 compound heterozygotes (2 A744S/M680I, 1 A744S/M694V). In all FMF patients with P369S and A744S mutations such symptoms as arthralgia and mialgia were detected. E148Q mutation was detected in 382 cases (4.73%), including 288 FMF patients (75%) (82 heterozygotes, 14 E148Q/V726A, 14 E148Q/M694V, 36 E148Q/M680I, 2 E148Q/R761H, 2 E148Q/E148Q, 2 E148Q/M680I) and 4 displayed complex alleles (P369S/E148Q/R761H; P369S/E148Q/M694V; E148Q/V726A/F479L, E148Q/R761H/M694V). Using a population-based study of healthy persons and FMF patients, we provide evidence that these three alleles are a disease-causing mutations and not are a benign polymorphisms in Armenian population. We suggest that in some cases other factors along with MEFV genotype, such as environmental or possibly other genetic factors play role in the determination of the severity of the inflammatory attacks in FMF.

(abstract 12)

DIFFERENT SPECTRUM OF MEFV MUTATIONS IN FMF PATIENTS


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8500 individuals were tested for MEFV mutations, including 6618 patients with genetically defined FMF (77.8%), 450 healthy controls, 1432 family members and carriers. DNA screening revealed a homozygous M694V mutation in MEFV confirming more severe phenotype and a limited response to colchicine at nephrotic stage of renal amyloidosis. FMF patients with other genotypes still have a good chance to ameliorate the nephrotic syndrome and to maintain renal function. In 90% of our FMF patients, Colchicine is effective to keep the inflammation under control. We have observed many FMF cases with concurrent manifestation: FMF along with epilepsy (M694V/M694V; V726A/M680I); SJögren syndrome (M694V/M694V); monozygotic twins (heterozygous carriers for M680I mutation); one of them with FMF, and the other – non-FMF, but with epilepsy; bronchial asthma (M694V/V726A, V726A/M680I, M680I); thalassemia (M694V/M694V); hyperthyroidism (M694V/M680I); Tourette syndrome (M694V/M694V); ulcerative colitis (M694V/M694V); renal amyloidosis and multiple sclerosis (M680I/M680I). Neurological features are accompanied along with administration of colchicides. For FMF children the onset of the disease presented by monoarthritis is peculiar phenomenon. About 20% of FMF patients (predominantly M694V homozygotes) have ankylosing spondilitis-like syndrome. We suggest that increased frequency of MEFV mutations is associated with other pathogenic manifestations and large number of these patients will be necessary to study these associations. Notwithstanding this phenomenon, genetic analysis has laid the foundation for understanding a variety of disease
mechanisms leading to autoinflammatory syndromes, including FMF. We have detected 20 FMF patients (0.3%) with 3 or 4 MEFV mutations, who were clinically not distinguishable from MEFV homozygotes or compound heterozygotes. Among 206 individuals with no MEFV mutations, 151 cases were characterized as “probable FMF” with recurrent febrile attacks and 55 patients as “definite FMF” indicated the possibility of the existing FMF-like syndromes. In this study 2415 familial cases (28.4%) were observed. Pedigree analysis of 200 families suggests that parent-to-offspring transmission of mutations was autosomal recessive in 91.5% and pseudo-dominant in 8.49%. Meta-analysis across all published and our data, including results and multiple reports, obtained from different populations, and the evidence for a functional consequence could be helpful to reveal association of MEFV pathogenic risk alleles with other syndromes.

(abstract 152)

Search for large rearrangement in the MEFV gene using Multiplex Ligation Probe Amplification (MLPA) and Quantitative PCR

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Familial Mediterranean Fever (FMF) is an hereditary autoinflammatory disease characterized by recurrent short attacks of fever, abdominal pain, pleuritis, arthritis, and erysipelas-like erythema. FMF is caused by mutations in the MEFV gene 1,2. Routine techniques only allow detection of punctual mutations. However, it would be interesting to develop new strategies to detect large rearrangements such as exon deletions or duplications, and to screen the whole gene in one single PCR reaction. In this study, we used the Multiplex Ligation Probe Amplification (MLPA) technique described by Schouten et al3 to investigate all 10 exons and the promoter of the MEFV gene. We selected 49 patients with typical FMF symptoms displaying only one mutation in the MEFV gene. DNA from an asymptomatic individual was included in each experiment as a negative control. No positive control of copy number variant in the MEFV gene is currently available to our knowledge. DNAs were analyzed in duplicate using the Salsa MLPA kit Po94 MEFV (MRC-Holland). Chromatographs were interpreted using the software Genemapper V4.0 (Applied biosystems). No copy number changes could be evidenced in the exonic regions. In one patient, a decreased fluorescent peak was observed in the promoter amplicon as compared to the negative control. We eliminated a possible mismatch due to a SNP by sequencing the region encompassing the MLPA probe. The possible promoter deletion in this patient was further investigated by real-time quantitative PCR using the light-cycler LC-480 (Roche). A primer pair was chosen in the X chromosome to validate this approach since this patient was a male, and the negative control was a female. We could not confirm the promoter deletion. Our results demonstrate that a second technique is valuable, and shows that copy number changes of the MEFV gene are unlikely in FMF patients. 1 The French FMF Consortium: A candidate gene for familial Mediterranean fever. Nat Genet 1997; 17: 25-31. 2 The International FMF Consortium: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 1997; 90: 797-807. 3 Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G: Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002; 30: e57.

(abstract 115)

Clinical Periodic Fever Syndromes in 51 Greek children: the diagnostic value of molecular
analysis


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Background: The diagnosis and management of Periodic Fever Syndromes (PFS) is sometimes difficult when solely based on clinical manifestations. The aims of the study were: a) The detection of mutations or genetic alternations in children with PFS attending our Center, b) The correlation of genetic mutations/alternations with the disease phenotype. Materials and Methods: The established mutations for Familial Mediterranean Fever (FMF) were investigated in the whole 51 children with FMF phenotype using until the end of 2004, PCR digestion and ARMS; from January 2005 we applied Non Isotopic Cleavage Assay (NIRCA) followed by sequencing, in order to study the whole MEFV gene for established mutations, genetic alternations or polymorphisms. In those who were negative for FMF mutations the analysis was extended to Hyper IgD Syndrome (HIDS) and TNF Receptor Associated Periodic Syndrome (TRAPS) genes. Results: In 40/51 children more than 1 of the known mutations of the MEFV was detected. In 8 more children rare mutations/genetic alterations were identified, namely G632S, I641F, K695R (exon 10, n=3 patients) and the genetic alternations R202Q in homozygocity (exon 2, n=2 patients) and E230K (exon 3, n=3 patients). Finally, in 3 children no mutation was detected for FMF; 2/3 agreed to be investigated for further genetic analysis that proved unfruitful for HIDS or TRAPS. The 3rd patient aged now 20 years, and free of PFS for more than 5 years, refused any further investigation. Their final diagnosis in all 3 patients according to the follow-up disease course was incomplete or atypical PFAPA syndrome. The commonest mutations of MEFV were the M694V (33/48) and the M680I (17/48) of the FEMV. The M694V mutation was highly significantly correlated with rheumatic manifestations during the FMF attacks (p=0.003). The commonest compound heterozygocity identified was M694V/M680I. The investigation of 51 children with PFS by the contemporary molecular methods, mainly for FMF and in negative for FMF cases for HIDS and TRAPS, led to the genetic confirmation of the FMF diagnosis in 48/51 and to the correlation of certain clinical manifestations with the presence of a distinct MFEV mutation. In 3/51 patients no mutations for FMF, and in 2/3 for HIDS or TRAPS were detected.

(abstract 113)

Multiplex Ligation-dependent Probe Amplification, not a valuable adjunct in FMF diagnostics.

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Background: Familial Mediterranean Fever (FMF), is an autosomal recessive disorder, caused by mutations in the MEFV gene, encoding PYRIN. Despite the identification of over 60 disease associated MEFV mutations, the clinical diagnosis cannot be genetically confirmed in many patients. In these cases FMF associated mutations can be identified in less than two MEFV alleles. This does not rule out that deletions in the MEFV gene are involved in FMF. A Multiplex Ligation-dependent Probe Amplification (MLPA) has been developed for FMF (MRCHolland). This technique allows the detection of exon deletions or duplications as well as entire gene deletions. Methods: To estimate the contribution of single or multiple exon MEFV gene copy-number changes
to the MEVF mutation spectrum, we analysed 91 FMF patients. This group of patients was previously sequenced and selected for having only one FMF allele (77 patients) or being homozygous (or hemizygous) for a mutation and/or several polymorphisms (14 patients). Results: In the 91 FMF patients screened not a single deletion/duplication was detected. This result suggests that single and multi-exon MEVF gene copy-number changes do not contribute substantially if at all to the MEVF mutation spectrum. Conclusion: Standard inclusion of MLPA in genetic testing of patients with FMF has no added value.

(abstract 109)

**Severe dysmenorrhea: an unusual presentation of Familial Mediterranean Fever**

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Familial Mediterranean Fever (FMF) is an autosomal recessive disease (MEFV gene) characterized by recurrent fever and inflammatory serositis. Although majority of patients have random pattern of attacks, some reports described precipitating factors. A literature review indicated that FMF attacks occurring only during menstruation are rarely seen. We report the cases of three patients with severe dysmenorrhoic pain as unusual clinical presentation of FMF. They were 3 females with a mean age at onset of 12 years. They never had typical attacks of fever and abdominal or chest pain, but they suffered from regular and severe dysmenorrhoic pain. Leukocytosis and C-reactive protein (CRP) elevation were noted during these attacks in all patients. Unlike dysmenorrhoea, none of these patients' attacks responded to non-steroidal anti-inflammatory drugs. The diagnosis of FMF was based on typical clinical and laboratory features. On investigation of MEFV, M694V was the most frequent mutation. All patients responded well to colchicine, and amyloidosis was not documented in any patients. In conclusion, we suggest that gynecologists must be aware of FMF in the differential diagnosis of dysmenorrhea or endometriosis especially in the people of Mediterranean origin.

(abstract 110)

**Tonsillar exudate in patients with Familial Mediterranean Fever: a review of ten cases.**

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Familial Mediterranean Fever (FMF) is an autosomal recessive disease (MEFV gene) characterized by recurrent fever and inflammatory serositis. PFAPA syndrome is a periodic fever with adenitis, pharyngitis and aphthous stomatitis. We reviewed the files of 10 patients diagnosed with FMF who presented, at the onset, clinical symptoms similar to PFAPA syndrome rather than to FMF. The diagnosis of FMF was based on typical clinical and laboratory features. On investigation of MEFV, M694V was the most frequent mutation. All patients responded well to colchicine, and amyloidosis was not documented in any patients. In conclusion, we suggest that gynecologists must be aware of FMF in the differential diagnosis of dysmenorrhea or endometriosis especially in the people of Mediterranean origin.
patients responded well to colchicine, and amyloidosis was not documented in any patients. The frequency of attacks slowly increased with disease progression. and clinical profile of attacks changed. In addition to intermittent fever, all patients had abdominal pain, two had headache and tonsillar exudate with adenopathy disappeared. In conclusion, in the early stage of disease, FMF could be confused with PFAPA in our patients. Many atypical cases of FMF emerge: we report the tonsillar exudate as its early and unusual presentation in childhood.

(abstract 79)

**MEFV MUTATIONS: AN IMPORTANT GENETIC PREDISPOSING FACTOR IN HENOCH-SCHÖNLEIN PURPURA**


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Objectives: Henoch-Schönlein purpura (HSP) is the most common vasculitides of childhood period. Both genetic and environmental factors are suggested to play role in the development of HSP. The aim of our study was investigate the prevalence of MEFV gene mutations in Turkish patients with HSP but without any symptoms of familial Mediterranean fever (FMF). In addition, we assess the differences of the special characteristics of HSP between the patients with and without mutations.

Methods: Eighty pediatric patients (44 male and 36 female), who were followed between September 2006 and January 2008 were enrolled. All patients fulfilled the EULAR/PRES endorsed consensus criteria for the diagnosis of HSP. Blood for mutation analysis was obtained either at the time of the diagnosis of HSP or during follow up visits in previously diagnosed patients. None of the patients had the diagnosis of FMF in their past history and in the follow up period. Six predominant mutations (p.M694V, p.M680I, p.M694I, p.V726A, p.K695R, p.E148Q) in the MEFV gene were studied.

Results: The mean age at the diagnosis of HSP was 7.8±2.8 (range 2 to 13.5) years. Twenty seven (33.8%) patients without FMF were found to be heterozygous for one of the screened MEFV mutations; p.M694V in 16, p.M680I in 5, p.V726A in 3 and p.E148Q in 3 patients. Clinical features of HSP did not differ between patients with and without mutations. However, the frequencies of elevated erythrocyte sedimentation rate and CRP values were found to be significantly higher in patients who had MEFV mutations.

Conclusions: Alterations in the MEFV gene are important susceptibility factors for the development of HSP. Upregulation of the inflammatory response that was reported in healthy carriers of FMF predisposes them to the development of HSP and other vasculitides. It seems that in the future further genetic studies may identify other likely causative inflammatory genes that might have roles in the pathogenesis of these mysterious vasculitides.

(abstract 214)

**Characterization of the human CIAS1 promoter**

Mutations in the CIAS1 (NLRP3) gene have been identified in a continuum of inflammatory disorders including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID). These disorders are collectively referred to as cryopyrinopathies because CIAS1 codes for the protein Cryopyrin. However, there are several patients with classic cryopyrinopathy phenotypes that do not have readily detectable mutations in CIAS1 coding regions. It is known that CIAS1 expression is highly regulated, but there has been no formal characterization of the CIAS1 promoter. We hypothesized that variations in the CIAS1 promoter sequence may have significant effects on disease state in patients with cryopyrinopathies, as well as more common inflammatory diseases since variants in non-coding regulatory sequence can have significant effects on gene expression or function, and in some cases may be associated with disease. We first determined the transcriptional start site of CIAS1 and analyzed the DNA region upstream of the transcriptional start site for potential transcription factor binding sites. Luciferase reporter plasmid constructs were created to assay the promoter activity of several areas of the promoter region and confirm the regulatory function of specific binding sites. Finally, we sequenced the promoter regions from cryopyrinopathy patients and normal controls without coding region mutations, looking for variations unique to these patients. Three different alternative splice forms for the 5' end of the gene were confirmed and two distinct regions with significant promoter activity were found. Within these distinct regions several potential transcription factor binding sites were identified. We also identified several unreported sequence variations in the promoter region in normal controls, and found one unique single nucleotide polymorphism (SNP) near a transcriptional factor binding site in a mutation negative FCAS patient that was not identified in over 200 matched controls. Cloning of this unique SNP into luciferase reporter plasmid constructs resulted in greater than two fold increased promoter activity, suggesting that this variant may play a role in disease in this patient. From this report additional studies can now be done to further characterize the CIAS1 promoter and sequence variants, which will lead to a better understanding of the regulation of CIAS1 expression and its role in disease.

Microarray-based gene expression studies of systemic inflammation in patients with cryopyrin-associated periodic syndromes (CAPS)


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CAPS are rare autoinflammatory diseases characterized by various degrees of systemic inflammation. Most CAPS patients have gain of function, missense mutations in the CIAS1 gene (encodes cryopyrin), which mediates the activation of IL-1β and IL-18. CAPS patients respond dramatically to treatment with anakinra. To better understand the pathogenesis of CAPS and identify new candidate genes, we searched for differentially expressed genes (DEGs) in CAPS patients vs. controls and in NOMID patients before and after treatment with anakinra. Gene expression-based models were developed to differentiate CAPS patients from healthy and from individuals with other autoinflammatory disorders. We collected PBMCs from 22 CAPS patients, 30 TRAPS patients, 8 HIDS patients, 6 PAPA patients, and 34 healthy controls. These samples were hybridized to Affymetrix HG_U133A 2.0 microarrays. The statistics were corrected for multiple testing and used to identify DEGs between the various groups of samples. Ontological
classification and pathway analysis using Ingenuity Pathway Analysis (IPA) software was utilized to understand relationships between the DEGs. Linear discriminant analysis (LDA) was used to build models that are predictive of CAPS. These models were evaluated by cross validation and by testing independent CAPS samples. We identified 1,880 DEG’s in CAPS patients vs. controls. In our analysis of 16 NOMID pre vs. post anakinra samples, 263 DEGs were identified. To enhance confidence in the list of anakinra-responsive genes, the 2 gene lists were merged and we identified the overlapping set of 173 transcripts. 127/173 genes were down-regulated by anakinra. Among interesting genes were SNCA, SOD2, BCL2L1, STAT3, several integrins and heat shock proteins. 23/173 anakinra-responsive genes were validated by qRT-PCR. 1707/1880 DEGs identified in the CAPS patient vs. control analysis were not responsive to anakinra. These genes may be part of pathways that are not directly regulated by IL-1β or they may not respond to dose of anakinra given. Finally, the genes, which underlie the CAPS specific models, may be used to understand the mechanism of disease since they are able successfully to classify samples from NOMID patients both on and off anakinra.

(abstract 165)

Initial Characterization of a Mouse Model of Cryopyrinopathy


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Mutations in the human CIAS1 gene are responsible for a continuum of autoinflammatory disorders, including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID). CIAS1 (NLRP3) codes for the protein cryopyrin (NALP3), which interacts with other proteins in a complex called the inflammasome, and is involved in interleukin-1 (IL-1) production. There is evidence suggesting that mutations in CIAS1 lead to a gain of function for cryopyrin resulting in increased IL-1 and systemic inflammation. In order to further study disease pathogenesis, knockin mice were generated with mutations corresponding to L353P (observed in FCAS) and A352V (observed in MWS). The DNA construct used included a floxed neomycin resistance cassette, which prevented expression of the mutant allele. Mice were mated to Cre Zp3 (oocyte expression) and Cre Lysozyme (monocyte and neutrophil expression) to remove the cassette. Serum was obtained for cytokine analysis by Luminex. Tissues were fixed with formalin and stained for histologic analysis. Gross phenotype becomes apparent at day 2 with obvious growth retardation and skin abnormalities including pustules, scaling, and absent hair growth that progress during the first week of life. Death occurs within 8 or 9 days. Multiple serum cytokines were markedly elevated in the mutant mice compared to wild type littermate controls, including proinflammatory cytokines such as IL-1 and IL-6, chemokines such as KC (IL-8), and regulatory cytokines such as IL-17 and IL-10. IL-1 was markedly decreased relative to controls. Pathologic analysis revealed patchy subcutaneous and epidermal neutrophilic infiltrates in the skin. In addition, neutrophilic infiltrates were observed in conjunctiva, joints, and muscle. Perivascular neutrophils were also detected in the meningeal lining. Although the knockin mouse phenotype appears to be more severe than what is observed in MWS and FCAS patients, the character and distribution of neutrophilic tissue inflammation and profiles of serum cytokines are similar, suggesting that this model can be used to study the pathogenesis of cryopyrinopathies and explore novel therapeutic interventions.
A SPORADIC CASE OF CINCA-NOMID SYNDROME DUE TO A NOVEL AND SOMATIC MUTATION IN THE CIAS1 GENE.


Chronic Infantile Neurological, Cutaneous and Articular (CINCA) syndrome, also known as Neonatal-Onset Multisystem Inflammatory Disease (NOMID) is a rare autoinflammatory disease located at the severest end of disease severity spectrum of cryopyrinopathies. These diseases are associated with germline, dominantly inherited mutations in CIAS1 gene. Most of CINCA-NOMID patients are sporadic cases, and mutational analysis of CIAS1 gene usually revealed disease-causing mutations in 55-60% of them. This genetic heterogeneity raised the question concerning the genetic defect(s) underlying in the remaining patients. Methods: Clinical and laboratory data were collected through a specific questionnaire. Genomic DNA was extracted from peripheral blood leukocytes and mutational analysis of autoinflammatory diseases-associated genes was performed by different molecular biology methods. Results: We present the case of a 4 year-old male Spanish patient, with no familial history of disease, who was clinically diagnosed of CINCA-NOMID syndrome by the presence of an early-onset urticaria-like rash, a symmetrical knee arthropathy and a facial dismorphism. CNS manifestations were the less severe in his clinical picture, mainly characterized by a mild headache, with no signs of intracranial hypertension, papilledema, seizures or mental retardation. Mutational analysis of CIAS1 gene revealed a potential G-to-C transversion at 907 position, which should provoke the novel missense D303H amino acid exchange. However, the intensity of each nucleotide peak in the chromatogram was very different, corresponding the higher intensity to the wild-type allele. This change was repeatedly detected in sequences from independent PCRs, excluding reasonably a technical problem. The presence of the potential mutated allele was looked for by other than sequencing methods such as RFLP, subcloning and PCR-SSP strategies, and finally enabled us to identify and to isolate the new mutation-harbouring allele. This D303H missense mutation was not present among a panel of 300 control chromosomes from Spanish healthy blood bank donors. Conclusion: We described at the first time the novel missense D303H mutation in the CIAS1 gene in a Spanish patient afflicted by the CINCA-NOMID syndrome, which has been identified as a somatic mosaicism. These data, together other previously reported, highlighted the important role of somatic mutations as disease-causing mutations in several diseases other than cancer.

Brain Multiple Sclerosis-like lesions in a patient with Mückle-Wells syndrome (MWS)

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Brain Multiple Sclerosis-like lesions in a patient with Mückle-Wells syndrome (MWS) Sandrine Compeyrot-Lacassagne, Tu-Anh Tran, Séverine Guillaume-Czitrom, Isabelle Marie, Isabelle Koné-Paut Division of Pediatrics and Pediatric Rheumatology, CHU Bicêtre, APHP, 74 rue du Général Leclerc, 94270 Le Kremlin Bicêtre, France We report the case of a 45 year-old female with MWS (heterozygous R260W mutation in the CIAS1 gene) who underwent a systematic brain MRI in the
setting of a therapeutic trial. Brain MRI showed FLAIR signal abnormalities in the periventricular white matter involving mainly the corpus callosum and the temporal lobe. These lesions were consistent with demyelinization as it may be seen in multiple sclerosis (MS). The patient did not display any neurological symptoms apart from usual headaches and her neurological examination was unremarkable. Cerebral fluid could not be obtained at the time of the imaging. These MS-like lesions although never described in MWS have been reported in one patient with another cryopyrin-associated periodic syndrome (CAPS) (c.1043C>T mutation in the CIAS1 gene). Unlike our patient, this patient had neurological symptoms in keeping with MS (Lequerré et al, Rheumatology 2007; 46(4):709-14). Furthermore, an association between MS and Tumor Necrosis Factor Receptor-associated periodic syndrome (TRAPS) has also been reported (Kümpfel et al, Arthritis and Rheumatism 2007; 58(8):2774-83). More generally, it appears that unlike “classical” autoimmune diseases, immunopathogenesis is played out at the sites of inflammation in autoinflammatory diseases such as CAPS. Our case raises awareness of this neurological complication in MWS and suggests that patients with MWS should be screened for neurological involvement in order to optimize their management.

(abstract 77)

Acute blindness as the presenting symptom in chronic recurrent autoinflammatory syndrome associated with a novel mutation in exon 3 of the CIAS1 gene

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Neurological and ocular manifestations are well known in chronic infantile neurologic, cutaneous, articulare syndrome (CINCA), but so far complete loss of vision has not been described. We report the clinical, genetic and laboratory characteristics and the response to anakinra in a case of CINCA. This boy had urticarialike rash from the first few weeks of life. From the age of 6 months, he had periods of fever lasting from 2 weeks to 3 months with fever free intervals lasting from 2 weeks to 4 months. He was treated with antibiotics for respiratory problems during the first week of life and several times thereafter. Episodically, the parents noted some redness of the eyes, particularly when fever. He had a tendency to irritability and discomfort and could never sleep through the night. At the age of 17 months he woke up with complete blindness of both eyes. Eye examinations showed mild uveitis that could not explain the loss of vision and papilloedema on the left side. The cervical, axial and inguinal lymph nodes were enlarged and the gait was stiff. The cerebrospinal fluid contained 38x10^6 WBC/l measured twice 6 weeks apart. Corticosteroids, antibiotics and antiviral treatments had no effect. At age 19 months he developed daily fever, increasing arthralgia and widespread rash. He had recurrent severe headache. Episodes of raised intracranial pressure were demonstrated, demanding a lumbar shunt. Visual evoked potentials (VEP) showed no response and he could not see any objects. Anakinra 2 mg/kg/day was started at age 23 months. He responded promptly with reduced stiffness, improved physical activity, no fever, no rash and no more sleep disturbances. The vision gradually improved, VEP signals appeared and he can now see objects on 3 meters distance. Laboratory values before/after anakinra were: ESR 60/20 mm/h, CRP 225/2 mg/l, blood WBC 32/19 x10^6/l,blood neutrophiles 22/7 x10^6/l and serum calprotectine 37100/3047 μg/l (range 100-900). Sequence analysis of the CIAS1 exon 3 showed a heterozygote mutation L571F that has not been reported before. Both parents were analysed and they did not carry the mutation. Conclusion: Acute blindness was the presenting symptom in this 17 months old child child with urticaria, periodic fever, aseptic meningitis, increased intracranial pressure, papilloedema, uveitis, conjunctivitis, arthralgia, glandulopathy, response to anakinra and a CIAS1 mutation consistent with CINCA.
Cryopyrin Associated Periodic Fever Syndromes and a New CIAS1 Mutation in Turkish Patients

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Background. Familial Mediterranean fever (FMF) is the commonest type of autoinflammatory disorders, and its prevalence is considerably high in Turkey. Cryopyrin associated periodic fever syndromes (CAPS) are consisted of Neonatal Onset Multisystem Inflammatory Disorder (NOMID), Muckle Wells Syndrome (MWS) and Familial Cold Autoinflammatory Syndrome (FCAS), and seen very rarely. We herein report 2 patients with NOMID and 3 patients with MWS of Turkish origin. Patients and Methods. All 5 patients were referred to us for the differential diagnosis of FMF or juvenile idiopathic arthritis, and because of their CAPS-associated clinical features, we isolated genomic DNA from whole blood and sequenced third exon of the CIAS1 gene. Results. One of the patients with NOMID was 16-year-old boy, who described recurrent attacks of fever and urticarial rash since age 1, arthritis attacks and elevated acute phase response since age 2. He had bony overgrowth in both knees, frontal bossing and papilledema. With the sequencing of the CIAS1 gene, we identified heterozygous missense Y441H mutation, which has not been reported before in any CAPS patient. The other NOMID patient was a 4-year-old girl with the typical features of recurrent fever, urticarial rash and arthritis attacks, and we found a heterozygous D303N missense mutation. This patient responded very well to anakinra treatment, and she is currently doing well with every other day injections. Other 3 patients with MWS were from one family. Index case was a 46-year-old male patient with a history of fever, urticarial rash and conjunctivitis attacks since age 5 and sensorineural hearing loss; and we identified a heterozygous D303N missense mutation. His 10-year-old dizygotic twins were also carrying D303N mutation, and they both had recurrent attacks of fever and skin rash. Father responded well to anakinra injections, but he did not want to continue anakinra treatment following a macroscopic hematuria attack. A cystoscopic biopsy confirmed AA type amyloidosis. Conclusion: We herein report novel Y441H mutation in a NOMID patient of Turkish origin. Anakinra was previously shown to be effective and fast acting in controlling inflammatory activity and symptoms in CAPS patients. Efficacy of every-other day anakinra treatment in one of our NOMID patients may suggest that IL-1 blockade could also be titrated according to the severity of inflammatory findings.

Good response to IL-1β blockade by anakinra in a CINCA/NOMID patient without CIAS1 mutations


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Background: CINCA/NOMID is an autoinflammatory disease, defined by the triad of urticarial...
rash, neurological manifestations and arthropathy accompanied by recurrent fevers and systemic inflammation. Increasing neurological deficits result from aseptic meningitis. Autosomal dominant inheritance is suspected although sporadic cases are reported. 60% of CINCA patients carry mutations in the CIAS1 gene on chromosome 1q44. Treatment with recombinant interleukin-1 receptor antagonist (anakinra) has shown improvement in a cohort of 18 patients (12 carried CIAS1 mutations). Corticosteroids could be reduced in all cases. Case: We report a 23-year-old female CINCA patient showing good response to anakinra treatment. Since her first days of life she suffered from urticarial rash, arthralgias, arthritis and febrile episodes. Aseptic meningitis, chronic headaches, mental retardation, sensorineural hearing loss and a complicated cataract resulting in glaucoma developed. Treatment with corticosteroids and colchicine was insufficient. Methods: The following parameters were measured at 0, 3 and 6 months of anakinra therapy: complete blood count, CRP, ESR, serum and CSF cytokine profiles, CSF cell count, cognitive function tests, audiograms and brain MRI. The CIAS1 gene was sequenced. Results: CIAS1 DNA sequence was normal. Brain MRI revealed symmetric hypointense areas in the globus palli, prominent transparenchymal vessels and areas of dysmyelination, all of which did not change under anakinra treatment (1-1.5mg/kg/24h). Likewise, cognitive function and hearing loss did not improve. In contrast, fever, urticaria, arthralgias and headaches resolved within 24 hours. Laboratory abnormalities (leukocytosis, anemia, thrombocytosis, CRP) improved within three days. CSF leukocyte counts decreased significantly within three months but remained elevated. Dramatic improvements were seen in the levels of serum and CSF TNF alpha, IL-6 and IL-8, whereas IL-10 levels remained normal. IL-1ß could not be detected in serum or CSF. Corticosteroids were discontinued after 6 months. Conclusions: This case demonstrates that anakinra can reduce inflammation-related symptoms and laboratory parameters in CINCA patients without CIAS1 mutations to the point that corticosteroids can be discontinued. Alterations in brain parenchyma may be treatment resistant, possibly secondary to permanent damage from chronic aseptic meningitis with iron and calcium deposits in amyloid-containing areas.

(abstract 219)

Anakinra in Muckle-Wells-Syndrome (MWS) – experience up to 29 months

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MWS is a dominantly inherited autoinflammatory disease characterized by rashes, fever, arthralgia, ocular manifestations, progressive sensorineural deafness and systemic amyloidosis. Treatment with anakinra, a soluble IL-1 receptor antagonist has been shown to be safe and effective [1]. Here we describe the effects of up to 2,5 years of treatment with Anakinra in 11 patients with Muckle-Wells-Syndrome (MWS). 11 patients (3 male / 8 female; age 5-67 years) were diagnosed with MWS by characteristic clinical presentation and identification of CIAS1 mutations. Anakinra was started at 100mg sc per day in adults and 1 - 6 mg/kg/d in children according to symptoms. A 20-point semi-quantitative MWS disease activity score (MWS-DAS) was calculated as the sum of the symptoms: fatigue, fever, headache, eye symptoms, hearing loss, rash, oral ulcers, abdominal pain, arthralgia/arthritis, renal symptoms, with symptom absent = 0, mild =1 and severe =2. MWS-DAS, physicians’ global assessment score (PGAS) on a 10-point visual analogue scale and inflammatory parameters (ESR, CRP, SAA) were determined before Anakinra treatment and after 15 (4-29) months. Treatment with Anakinra was effective in all patients, although not all patients became symptom free. Data are presented as median and range before Anakinra and at follow-up. MWS-DAS decreased from 7 (3-10) to 2 (1-4) and PGAS was reduced from 4 (3-8) to 1 (0-2).
Inflammatory parameters also decreased: ESR [mm/h] was 24 (6-52) and came down to 8 (5-18), CRP [mg/dl] decreased from 1.9 (0.01-2.76) to 0.1 (0.01-1.73) and SAA [mg/l] was reduced from 31.5 (3.3-91.3) to 5.2 (1.1-13). Systemic amyloidosis decreased in two patients. Progress of hearing loss could be stopped and hearing improved to normal in one patient. Adverse events included injection site reactions, increased rate of infections, decrease in levels of immunoglobulins, weight gain. No severe adverse events were recorded. Compliance was compromised in two patients because of the need for subcutaneous injections. Anakinra is safe and effective in the treatment of Muckle-Wells-Syndrome at follow-up up to 2.5 years. However not all patients experience complete remission. Dose increase is limited by strong local irritations caused by high doses. Daily subcutaneous injections are a drawback particularly in children. Reference: [1] Hawkins PN et al. N Engl J Med. 2003, 348:2583-4

Long term benefices of IL1 receptor antagonist, anakinra, in 10 CINCA patients


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Cryopyrin associated periodic syndromes(CAPS) are rare autoinflammatory diseases that include familial cold autoinflammatory syndrome(FCAS),Muckle-Wells syndrome(MWS) and chronic infantile neurologic articular syndrome(CINCA syndrome).They present a continuum of severity with overlapping symptoms as fever,rash,articular involvement associated with acute-phase inflammatory response:CINCA is the most severe disorder with significant morbidity up to premature death in severe cases.Almost all patients have chronic aseptic meningitis and progressive perceptive deafness.Ocular inflammation is also frequent and half of the patients present characteristic bony overgrowth arthropathy.CAPS are due to autosomal dominant mutations of CIAS1 gene, a gene that encode for cryopyrin.This protein plays a critical role in IL-1 processing, a cytokine that is increased in these disorders.Anakinra, an IL-1 receptor antagonist, is very efficient to control clinical symptoms and inflammatory markers of CAPS.Hearing loss improved in some patients and neurological involvement was also shown to improve in CINCA.However, data about long term benefice are still spare. We have treated 10 patients with CINCA (median age: 8.5 years, range 0.25 to 20) with anakinra (1 mg/kg/day) for 19 to 40 months (median 31). We confirmed the dramatic and rapid improvement of clinical and biological markers that lasted overtime. Improvement of CNS involvement was present in all patients but variable: headaches and papillary oedema normalised durably in 6 patients and improved in 4. CSF parameters (pleiocytosis, CSF pressure and protein level) controlled after 6 months of treatment improved in all patients even though the difference was not statistically significant as compared to baseline. In 4 patients with partial neurological response, dose of anakinra was increased up to 3 mg/kg/day with additional benefits in 1. Higher doses (3 mg/kg twice daily) were also needed in 2 young babies with severe disease, because of persistence of symptoms and inflammatory markers.These unusual doses could be due to disease severity, rapid clearance of anakinra in infants or both. Two of the seven patients with hearing loss at baseline improved, deficit remained stable in the others. All patients gained substantial height and weight. Overgrowth arthropathy present in one patient didn’t improved after...
32 months of therapy suggesting that these anomalies are not driven by IL-1.

A novel NLRP3 variant in an Italian patient with Muckle-Wells syndrome: characterization of IL-1β processing and secretion before and after anakinra treatment


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Over 70 missense mutations in the NLRP3 gene are associated with hereditary autosomal dominant autoinflammatory diseases including FCAS, MWS and CINCA, representing a spectrum of signs and symptoms of increasing severity. Mutations result in disregulation of IL-1β processing and increased secretion of this cytokine. Recently, it has been shown that monocytes from patients with NLRP3 mutations display a dramatic increase in LPS-induced IL-1β secretion compared to controls (up to 40 ng/ml vs ≤ 5 ng/ml in 3h), whereas exogenous ATP fails to further induce IL-1β release (Gattorno et al, Arthritis and Rheum 2007; 56:3138-3148). We describe a 35-year old man presenting with mild renal failure due to renal amyloidosis and recurrent fever, arthralgias and urticarial skin rash since his first year of life. Attacks were not triggered by cold exposure and were self-limiting, lasting from 5 to 7 days and occurring every 40 days. The patient developed nephrotic syndrome aged 33 and a kidney biopsy showed AA amyloidosis. CRP and SAA levels were increased between attacks. Family history was uninformative. He had been previously treated with corticosteroids with partial remission of symptoms whereas colchicine was paradoxically associated with recrudescence of attacks. Genetic screening for mutations in the NLRP3 gene showed the presence of a G to A transition at the first nucleotide of codon 280, resulting in Asp to Asn substitution. Sequencing of MEFV and TNFRSF1A revealed no mutations. The mutation was not found in 200 control chromosomes. Treatment with anakinra was started in October 2007 at 50 mg/day, the dose being reduced according to creatinine clearance. SAA promptly normalized after one week and remained within reference range since then. Complete remission of attacks was observed while proteinuria and renal failure are stable after three months of therapy. In vitro IL-1β production and secretion by the patient’s monocytes, evaluated before treatment, showed a defective response to ATP-induced stimulation, consistent with the results obtained in other CINCA and MWS patients bearing NLRP3 mutations. However, unlike in the other CINCA/MWS patients, the levels of IL-1β secreted in response to LPS and other TLR ligands were not higher than in healthy controls (< 2 ng/ml in 3 h). Further characterization of IL-1β processing and secretion under anakinra treatment in this patient is ongoing.

Long-term follow up of patients with CINCA syndrome: efficacy and tollerabilty of Anakinra treatment.


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We report the long-term follow up of 15 Italian patients affected by Chronic Infantile Neurological Cutaneous and Articular (CINCA) syndrome. 11 patients were treated with the IL-1 receptor antagonist (subcutaneous injections at a daily dosage of 1 mg/Kg in children and up to 100 mg in adults), 2 refused the therapy, and the others did not require treatment because of a mild presentation of the disease so far. In treated subjects, inflammatory signs (fever, rash, articular involvement, conjunctivitis, uveitis) disappeared or showed substantial improvement. A rapid normalization of acute phase protein levels (ESR, CRP), immunoglobulins and haemoglobin concentration also occurred, in most cases within the first month of treatment. The neurologic symptoms ameliorated too: the frequency of cephalia episodes significantly diminished; papilledema disappeared in 4 out of 11 treated patients and persisted in all 3 untreated patients. In one subject, out of the five presenting perceptive deafness, the sensory defect improved. Moreover, a case with very severe iridocyclitis completely resolved. As expected, not inflammation-correlated signs such as dysmorphisms (typical facies) and bone alterations constantly persisted. Anakinra showed very good tolerability and no patient withdraw therapy. We reported only two side effects: local erythema at the site of injection in 2 out of 11 subjects, and oral aphtosys (not previously reported among side effects), refractory to treatment, in another patient. Regarding the 4 untreated patients: the one with very mild phenotype is still doing well, the other patients worsened in the last year and will start the Anakinra therapy as soon as possible. The subjects that refused the treatment worsened during follow up. Other treatments, such as corticosteroids or NSAIDs, were not able to prevent the worsening or onset of further symptoms. In summary, our study demonstrates that a long lasting treatment with Anakinra appears to be safe and highly effective in patients affected by CINCA syndrome, improves natural history and prognosis, with rare and mild side effects. Fine tuning of the therapy, especially regarding the dosage and the route of administration is needed. We hope that in future a long-acting administration of Anakinra, instead of a daily subcutaneous injection, will bring an important benefit in the quality of life of CINCA patients.

Experience of a dedicated fever clinic and laboratory service at the UK National Amyloidosis Centre (NAC).


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Background: In 2004 we established a formal clinical service for patients with hereditary periodic fever syndromes (HPFS) within the National Amyloidosis Centre. Patients are either referred directly for clinical evaluation, or undergo initial genetic screening on receipt of two page proforma detailing the clinical features. Methods: The basic genetic screen encompasses FMF (MEFV exons 2 and 10), TRAPS (TNFRSF1A exons 2-6), HIDS (MVK exons 9 and 11) and CAPS (NLRP3/CIAS1 exon3). The precise genotyping was determined by the NAC physicians either in clinic or on review of the proformas and if necessary it was extended to include additional exons when indicated on a case by case basis. Since 1 January 2005, 374 patients were referred directly to the clinic, and proformas and accompanying samples were received on a further 659 patients, totalling 1033 cases. MEFV was screened in 725 patients (70%), TNFRSF1A in 470 (45%), MVK in 270 (26%) and NLRP3/CIAS1 in 188 (18%). Results: Mutations were found in 271 patients (26%): 176 patients had amino acid variation in MEFV (73% had a single mutation and 27% had
two mutations), 36 in TNFRSF1A, 24 in MVK (69% had a single mutation and 31% had two mutations) and 35 in NLRP3/CIA1S. 12 novel mutations were identified. Three out of four patients with the novel NLRP3/CIA1S mutations (Del436T, L571F and I572F) were referred with a classical diagnosis of CINCA, whilst the fourth patient with the novel variant L264H was categorised clinically as MWS. Patient with H22R, the novel TNFRSF1A variant had typical features of TRAPS. Similarly two novel MVK mutations (L29fs c.86delT and P286L) were found in individuals with unexplained fevers, rash, diarrhoea, ulcers and elevated IgD. R143P, the new mutation in MEFV was, in addition to E148Q found in a patient with clinical phenotype of FMF, another novel compound A595VR717S was associated with rather unspecific attacks of fever.

Conclusion: Since setting up the HPFS clinical service in 2004 the demand for genetic testing has been growing and in 2007 we have seen a 10% increase in referrals. We plan to extend our genetic analysis to screen two further exons (4 and 6) of NLRP3/CIA1S in individuals with clinical symptoms of CINCA or MWS in whom we have not identified amino acid variation in exon 3. We are also setting up screening of NOD2 for patients with suspected Crohn’s Disease and Blau Syndrome.

(abstract 41)

Nationwide surveillance of chronic infantile neurological cutaneous and articular syndrome in Japan.


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Although a number of CINCA/NOMID patients has been reported in East Asian countries, epidemiologic surveillance of the syndrome has not been performed. Therefore, we conducted nationwide surveillance of CINCA/NOMID in Japan. Primary survey sheets were sent to 559 hospitals with pediatrics division in Japan. Collection rate was 67.4%. Twenty Japanese CINCA/NOMID patients were reported from these hospitals. As a secondary survey, we assessed detailed clinical manifestations of the reported CINCA/NOMID patients using a questionnaire and performed physical examination if necessary to confirm the diagnosis and reassessed the CIA1S genotypes if necessary. We recently reported that some CIA1S mutation-negative patients have CIA1S mutations as low-level mosaicism (Saito M, et al. Blood Epub ahead of print, 2007). Therefore, for those without detectable CIA1S mutations, we surveyed if these patients have latent mosaicism of CIA1S. The clinical manifestations of the patients were compared with the status of CIA1S mutations such as heterozygote, mosaicism or no CIA1S mutations detected, and the tentative functional activities of the detected CIA1S mutations were evaluated by the ASC-dependent NF-kB transactivation in HEK293FT cells and the induction of necrotic cell death in THP1 human monocytic cell lines. These analyses could reveal clinical characteristics of CINCA/NOMID patients in an Asian country and genotype-phenotype correlations in Japanese population with relatively homogeneous ethnic backgrounds.

(abstract 95)

A clinical prediction rule to exclude cryopyrin associated periodic syndromes in patients with recurrent symptoms
Introduction– Cryopyrin associated syndromes (CAPS) are autosomal dominant autoinflammatory diseases caused by mutations in the cryopirin gene (CIAS1/PYPAF1/NALP3). Neonatal Onset Multisystem Inflammatory Disease (NOMID) presents with distinguishing permanent features. By contrast, manifestations of Familial Cold Autoinflammatory Syndrome (FCAS) and of Muckle-Wells Syndrome (MWS) are episodic, except deafness. Our aim was to define a clinical criterion able to exclude MWS or FCAS without genetic testing in some patients.

Methods– Clinical data were extracted from the request formularies sent to a French reference laboratory with blood samples for CAPS genetic testing. Patients with a suspicion of NOMID, a cryptogenic inflammatory amyloidosis, or a family member already tested positive were excluded. A recursive partitioning algorithm was applied to find the most discriminatory composite clinical criterion satisfied by all patients with a mutated CIAS1 allele. Positive patients had the following characteristics (medians for quantitative variables and percentage for binary variables): onset at 1.5 years old, deafness 72%, cold sensitivity 68%, attacks lasting 2.5 days with fever in 44%, urticarial rash in 100% and joint pain in 86%. The composite criterion [urticarial rash AND (onset < 20 years old OR attacks < 4 days)] was the most discriminatory. It had a sensitivity of 100% [95% confidence interval: 93-100], a specificity of 77% [70-82] and a negative likelihood ratio of [0-0,17].

Discussion– A negative composite clinical criterion was sufficient to rule out FCAS and MWS in our population. If genetic testing had been limited to patients fulfilling this criterion, the total number of tests would have decreased by 60% without missing a single positive patient. The criterion is also very sensitive in patients excluded from the derivation cohort. Two patients among 14 tested because of cryptogenic amyloidosis had a mutated CIAS1 allele and both fulfilled the clinical criterion. Among 34 tested patients who already had a positive family member, 31 were positive and clinical data were available for 18 of them, all of whom fulfilled the criterion.

Conclusion– Genetic testing for FCAS and MWS might be limited to patients who experience urticarial rash and whose attacks began before the age of 20 or last less than 4 days.

(abstract 122)

**Long-Term Safety of Rilonacept (IL-1 Trap) in Cryopyrin-Associated Periodic Syndromes (CAPS): Results from a 1-Year Study**

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Background: The efficacy and safety of weekly sc 160 mg rilonacept over 24 weeks in 47 patients (pts) with either Familial Cold Autoinflammatory Syndrome (FCAS) or Muckle-Wells Syndrome (MWS) was demonstrated in a pivotal combination of 2 double blind (DB), placebo-controlled studies with an intervening single-blind rilonacept treatment period. The most frequently reported adverse event (AE) during the initial 6-week blinded phase in rilonacept pts was mild to moderate injection site reaction (48%) followed by upper respiratory infection (26%), sinusitis (9%), nausea (4%), UTI (4%) and diarrhea (4%). Three pts withdrew, all on rilonacept: 1 for noncompliance, 1 for joint pain and 1 for pre-existing hepatitis, and one pt was reported with a serious AE (sciatica).

Here, we report on the safety of rilonacept during a subsequent 24-week open label extension (OLE). Methods : Pts who completed the 24-week blinded studies enrolled in a 24-week OLE and...
received rilonacept 160 mg sc weekly. Pts were assessed at OLE week 6, 12, 18 and 24. Anti-rilonacept antibodies were measured by two highly sensitive assays. Results: The DB study population consisted of 16M and 31F with a mean age of 51 yrs, 44 with FCAS and 3 with MWS. 44/47 pts enrolled into the OLE from the DB studies. Commonly reported AEs over the 24-week OLE included injection site reaction (36%), nasopharyngitis (11%), sinusitis (7%), UTI (7%) and bronchitis (5%). One pt was reported with serious AEs of hypokalemia, hyponatremia and pulmonary embolism and was withdrawn, but then requested and was granted study re-entry. Two deaths occurred in a subsequent second 24-week open-label extension phase: one due to pneumococcal meningitis in a 70 year old pt, and another due to coronary atherosclerosis in a 37 year old pt. Small to moderate reductions in neutrophil and platelet counts, and increases in cholesterol reported in rilonacept pts during the initial 24-week blinded phase were maintained during the 24-week OLE. Anti-rilonacept antibodies, detected in 24%-40% of pts, were without apparent clinical effect. Few clinically significant abnormal labs were reported. Conclusions: Treatment with rilonacept was generally well tolerated. Other than injection site reactions, there was no clear pattern of AEs in rilonacept treated pts. Changes in neutrophils, platelets and cholesterol were consistent with reversal of IL-1 induced inflammation.

(abstract 163)

The TLR-4 ligands MRP-8/14 correlate to disease activity in Muckle-Wells patients during IL-1Ra therapy


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Background: Muckle-Wells syndrome (MWS) is a dominantly inherited autoinflammatory disease characterized by rashes, fever, arthralgia, progressive sensorineural deafness and systemic amyloidosis. The clinical presentation and the detection of a CIAS1 mutation are used to establish the diagnosis. The pro-inflammatory Damage Associated Molecular Pattern (DAMP) molecules Myeloid-Related Protein (MRP)-8/14 have been recently identified as ligands and activators of TLR-4 and correlate to disease activity in several inflammatory diseases. Objectives: To study therapy response to IL-1Ra and to compare classical inflammation markers and MRP8/14 for their usefulness as biomarkers in a cohort of 21 MWS patients. Methods: Serum concentration of MRP-8/14 determined by ELISA and classical inflammation markers ESR, CRP and SAA were analysed in 21 patients who had documented molecular diagnosis of CIAS 1 mutation and presented with the clinical features of MWS before starting therapy. 12/21 patients were treated with IL-1Ra and inflammation markers were analysed throughout the course of therapy. Results: MRP 8/14 levels were higher in patients before IL-1Ra therapy during uncontrolled inflammation (3555±585ng/ml) and dropped significantly in successfully treated patients (1590±350ng/ml; p=0,001), but were still elevated in comparison to healthy controls. ESR (p=0,013) and CRP (p=0,008) reflected response to therapy on a lower significance level, while SAA showed no significant difference (p=0,182). MRP8/14 correlated significantly to disease severity (r=0.521, p=0,018), physicians global assessment (r=0.478, p=0.03), CRP (r=0.553, p=0.017), and ESR (r=0.576, p=0.012). Conclusion: Measurement of MRP8/14-levels in MWS might be a valuable tool to reflect disease activity and response to anti-inflammatory therapy. MRP8/14-levels in clinically successful treated patients still are clearly elevated, which might indicate subclinical inflammatory activity.
Hydatidiform moles

(abstract 39)

Homzygous NLRP7 mutation in a Moroccan woman with recurrent reproductive wastage.


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The NACHT, LRR and PYD domains-containing (NLRP) proteins are key intracellular regulators of apoptosis and inflammation. Some members of this family have been involved in autoimmune and autoinflammatory diseases. In 2005, the NLRP7 gene was associated with recurrent and/or familial hydatidiform moles (HM). A 25-year-old woman was referred for genetic advice. She had experienced 4 reproductive failures. The first pregnancy was terminated for personal reasons at 8 weeks of gestation, and was complicated 3 weeks later by intraperitoneal haemorrhage due to trophoblastic tissue on the uterine wall. Treatment included vascular coagulation of trophoblast (coelioscopy), and methotrexate administration. The second pregnancy was a blighted ovum spontaneously eliminated at 4 weeks of gestation without complications. The two last pregnancies were complete hydatidiform moles without embryonic structure on pathology examination. Treatment included aspiration, βhCG level monitoring (for both) and methotrexate administration (for the second). Pelvic ultrasound was normal. Biological explorations showed iron deficiency anaemia, without immunological or thrombophilic abnormalities. Cytogenetic studies showed a paracentric inversion of chromosome 14 for the woman and a normal karyotype for her husband. There was no history of consanguinity or known reproductive troubles in the patient's family. To search for a possible genetic origin of her recurrent reproductive failures, we extracted DNA from her peripheral white blood cells and sequenced all exons and intronic boundaries of the NLRP7 gene. We discovered that she was homozygous for the p.Arg693Trp (c.2077C>T) mutation in exon 5, that was previously reported in a German family (MoGe2) with recurrent HM, but no other kind of reproductive failure (Qian, et al., 2007). We also conducted high resolution melting curves experiments. DNA from our patient either crude or mixed with wild-type DNA (ratio 1:1), positive samples (homozygous and heterozygous genotypes from the German family kindly provided by Dr R Slim), and a reference (wild-type DNA) were amplified in parallel experiments. Curves obtained from our patient’s crude DNA were superimposable with the homozygous MoGe2 sample, and the mixed DNA shown the same pattern as the heterozygous MoGe2 sample. NLRP7 mutations have been reported in 7 families, and 6 isolated patients (Kou, et al., 2007; Qian, et al., 2007). This result supports that the recurrent reproductive wastages in our patient were due to mutations in this gene, and extends the small number of cases described so far. Moreover, this is the first patient with North-African origin. In conclusion, our data confirm that p.Arg693Trp is responsible for recurrent HM, but may also be involved in other reproductive pathological conditions. Mutation screening of NLRP7 should be proposed systematically in this kind of pathology. Kou Y, Shao L, Peng H, Rosetta R, Del Gaudio D, Wagner A, Al-Hussaini T and Van den Veyver I. (2007). A Recurrent Intragenic Genomic Duplication, other Novel Mutations in NLRP7 and imprinting defects in Recurrent Biparental Hydatidiform Moles. Mol Hum Reprod. Qian J, Deveault C, Bagga R, Xie X and Slim R. (2007). Women heterozygous for NALP7/NLRP7 mutations are at risk for reproductive wastage: report of two novel mutations. Hum Mutat 28(7):741.
Inflammasome and autoimmunity

(abstract 14)

**Familial panniculitis, Sensorineural hearing loss and Arthropathy: A new association**


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AIM To describe two siblings with early onset panniculitis, sensorineural hearing loss and arthropathy. Case Report The older patient is eight years old who was well till the age of six months; she started to have hyperpigmented and diffuse progressive skin lesions. At the age of three years she was found to have sensorineural hearing loss. She had generalized body aches; and was found to have flexion contractures of small joints of both hands and right knee. Skin biopsy showed plasma cell rich septal panniculitis. She had elevated inflammatory markers. The rest of work up was inconclusive. The younger patient is six years old. At the age of eight months she had scattered patchy skin hyperpigmentation. This was followed by sensorineural hearing loss. Similar to her sister she has generalized body aches; she had left knee flexion contracture. Work up was similar to her sister. Both were treated empirically with ibuprofen, prednisone, Methotrexate and Etanercept for few months. No significant improvement accomplished. CONCLUSION This is the first report of autosomal recessive plasma cell panniculitis, sensorineural hearing loss and arthropathy. To the best of our knowledge this is a new association.

(abstract 15)

**Familial panniculitis, Sensorineural hearing loss and Arthropathy: A new association**

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(abstract 16)

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Familial panniculitis, Sensorineural hearing loss and Arthropathy: A new association

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EFFECTS OF TNF BLOCKADE BY INFLEXIMAB ON THE CENTRAL INFLAMMASOME COMPONENT NALP3 IN RHEUMATOID ARTHRITIS PATIENTS.


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Background: The NALP3 inflammasome is a proinflammatory protein complex involved in regulation of innate immunity and the production of IL-1. The proinflammatory cytokines, IL-1 and TNF, are key molecules in the pathogenesis and persistence of rheumatoid arthritis (RA). Blockade of TNF in these patients has had profound therapeutic effects. This study investigates the expression of NALP3 in peripheral blood and synovial tissue of RA patients and the effects of TNF blockade by infliximab on this pathway. Methods: The RA patients studied (n=23) had all failed at least two traditional disease modifying anti-rheumatic drugs, before being initiated onto infliximab. Messenger RNA (mRNA) expression analysis was carried out on peripheral blood mononuclear cells (PBMC), pre- and 2 hours post-infusion, at weeks 0, 2, and 14. The Disease Activity Score (DAS28) classified patients as being good, moderate (n=12) and non-responders (n=11) to infliximab. Quantitative PCR techniques were used to detect NALP3 mRNA levels from PBMC of these patients. NALP3 mRNA levels were also examined in synovial tissue biopsies, taken pre- and post-infliximab treatment, in an additional group of patients (n=5). Results: Infliximab had marked effects on the expression of NALP3; there was a significant decrease in the NALP3 transcript levels in the responders, who were found to have lower levels of NALP3 transcript (p=0.04), prior to treatment (baseline). The NALP3 mRNA levels then declined further after treatment, and there were significant differences between baseline and pre- (p=0.007) and post-infusion (p=0.03) at week 14. However, preliminary findings in synovial biopsies of 3 responders and 2 non-responders indicate that the changes observed in peripheral blood are not replicated in synovial tissue, as there was an increase rather than decrease in NALP3 levels observed in patients who responded.

Conclusions: Previously we have reported on the effects of infliximab on NALP3 mRNA levels in peripheral blood, and these findings have been confirmed. At baseline there are lower NALP3 mRNA levels in the blood of ‘responder’ RA patients, which is reduced with treatment. The data suggests that there is a biological role for the NALP3 inflammasome in response to infliximab. Infliximab induces a decrease in the NALP3 transcript levels in PBMCs from RA patients responding to infliximab, and this is not observed in RA patients who don’t respond to infliximab therapy.

(abstract 118)

Gene polymorphisms in the NALP3 inflammasome are associated with interleukin-1 production and severe inflammation - relation to common inflammatory diseases?


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Background: This study was done to assess whether a patient with arthritis and antibiotic-resistant fever carries mutations in the genes encoding the NALP3 inflammasome. NALP3, ASC and TUCAN are components of this inflammasome, which triggers caspase-1-mediated interleukin-1beta (IL-1beta) release. Activating mutations in the gene encoding NALP3 (NLRP3) have recently been linked to familial periodic fever syndromes. Methods: Genetic analysis of NLRP3 and the gene encoding TUCAN (CARD-8) was performed on genomic DNA from the patient and from a population-based collection of DNA (806 subjects). For in vitro studies on IL-1beta production, activity of caspase-1 and NF-kB, apoptosis, and general inflammatory markers (ROS-production, integrin expression), blood was drawn from the patient at different time-points after drug administration, and from five healthy age- and gender-matched subjects. Results: Mutation analysis of the patient’s genes encoding NALP3, ASC and TUCAN revealed variations in the NLRP3
(Q705K) and CARD-8 (C10X) genes. The allele frequencies of these single nucleotide polymorphisms (SNPs) in the population were 6.5% and 34%. The elevated activity of caspase-1 and the high levels of IL-1beta measured in samples from the patient were returned to normal levels after treatment with anakinra, an IL-1 receptor antagonist (IL-1Ra). Delayed apoptosis and elevated ROS and integrin expression observed in patient were independent of IL-1Ra administration, while NF-kB activity was similar to that of controls irrespective of IL-1Ra. Conclusions: Our results indicate that the patient’s symptoms are due to elevated levels of IL-1beta, since treatment with anakinra effectively abolished the symptoms. The compound SNPs may explain the increased IL-1beta levels and inflammatory symptoms observed, but further studies to reveal a functional relationship are needed. The process of leukocyte apoptosis was affected, whereas expression of CD11b and ROS-production seemed not to be consequences of a dysregulated NALP3-inflammasome. The prevalence of the polymorphisms (4% of the population carry both SNPs) in the general population may suggest a genetic predisposition for common inflammatory disorders.

(abstract 43)

TWO NOVEL MISSENSE MUTATIONS IN CIAS1 GENE (M299V AND A227V) ASSOCIATED WITH SEVERE INFLAMMATION

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TWO NOVEL MISSENSE MUTATIONS IN CIAS1 GENE (M299V AND A227V) ASSOCIATED WITH SEVERE INFLAMMATION

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Background: CIAS1 (also known as cryopyrin or NALP3) forms a complex with proteins ASC and CARD8 called as inflammasome, which causes caspase-1 activation and cleavage of pro-Interleukin-1β to Interleukin-1β (IL-1β). Mutations in the CIAS1 gene give rise to a spectrum of clinical symptoms, which can be classified into systemic auto-inflammatory diseases. We have previously identified a Q705K amino acid change in exon 3 of CIAS1 gene in a Swedish patient with a severe inflammatory disease responding to IL-1 receptor antagonist treatment. We here report about two patients suffering from severe inflammation with antibiotic resistant fever. The aim was to search for mutations in the genes comprising the inflammasome that could explain the symptoms. Methods: DNA was isolated from whole blood. Exon 3 of the CIAS1 gene was amplified using specific primers and bi-directionally sequenced. One hundred healthy DNA samples were also amplified and sequenced. In addition, the levels of IL-1β in mononuclear cells were determined. Results: Patient 1 presented at 44 years of age with bouts of high fever, thoracic discomfort, and generalised muscle pain. Despite conventional anti-inflammatory treatment his symptoms persisted. 10 years later, IL-1β antagonist treatment caused a rapid improvement. Patient 2 developed a severe rheumatoid arthritis at 72 years of age and had very high leukocyte count (44 x 109/L), but improved upon treatment with etanercept and methotrexate. Patient 1 displayed a mutation in the CIAS1 gene leading to an amino acid change from methionine to valine at codon 299, whereas patient 2 displayed a CIAS1 mutation leading to an amino acid change from alanine to valine at codon 227. Mononuclear cells from patient 1 showed increased IL-1β compared to healthy controls, whereas this was not seen in patient 2. These two novel mutations could not be detected in DNA from one hundred control samples. Conclusion: The two novel mutations in the CIAS1 gene could be the possible cause of the inflammatory phenotype presented by these two patients.
Inflammatory bowel diseases
(abstract 189)

Effect of methotrexate and sulphasalazine in animal models of colitis.
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Inflammatory bowel disease (IBD) includes Crohn’s disease (CD) and ulcerative colitis (UC) and is an idiopathic disease probably involving an immune reaction of the body to its own intestinal tract. Methotrexate (MTX) and 5-Aminosalicylate (5-ASA) derivatives (Sulphasalazine) are therapeutic approaches used in treatment of IBD patients. Methotrexate (MTX) is considered an appropriate alternative in case of Azathioprine/6-Mercaptopurine failure while Sulphasalazine can be used orally or in topical formulation on the basis of disease location to treat mild active disease and as maintenance treatment in UC. For compound efficacy evaluation we used a model induced by chemical irritation, Trinitrobenzene sulfonic acid (TNBS) induced colitis colitis. This model is reported to resemble human CD in terms of histopathological features and cytokines profile. The aim of this work was to evaluate the effect of MTX and Sulphasalazine in the models of TNBS-ind. Colitis in mouse and rat, respectively. Methotrexate was administered orally at 0.1, 0.3 and 1 mg/kg to female Balb/c mice for 5 consecutive days from the day in which lesions were induced by TNBS-enema. Methotrexate significantly reduced mortality. Considering the entire experimental period, methotrexate significantly reduced body weight loss (by 38% at 1 mg/kg), clinical symptomatology, and post-mortem read-outs: colon weight (by 30% at 1 mg/kg) and macroscopic lesions (hyperemia, wall thickening and ulcerations). Sulphasalazine was administered intracolonically to female Sprague-Dawley rats at the doses of 10, 30 and 100 mg/kg starting 24 hours after disease induction and treatment was continued for 7 consecutive days. Sulphasalazine significantly reduced post-mortem read-outs: colonic lesions (ulcerations, wall thickening and adherences) and colon weight. At histology, treatment with Sulphasalazine induced reduction of crypt loss, erosions/ulcerations and edema. In light of these results we can conclude that treatment with MTX and sulphalazine blocks development and severity of colitis lesions in animal models of colitis confirming the predictivity of these models for screening new potential, pharmacologically active compounds to treat IBD. Additionally, feasibility and efficacy of local treatment in rats allows testing of potential formulations for local/controlled release treatment.

(abstract 210)

Inflammatory and apoptotic effects of NOD2 in patients with Crohn’s disease
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NOD-like receptors are intracellular receptors for bacterial peptidoglycans. NOD2/CARD15 is a member of NACHT-LRR (NLR) family of proteins. Mutations in the LRR region of the NOD2 gene are implicated in the pathogenesis of Crohn’s disease. Several studies have shown the stimulatory effects of NOD2 interaction with muramyl dipeptide (MDP) on cytokine production and inflammation. Although the role of NOD2 in the induction of cytokines and inflammatory reactions is well established, only scarce data are available regarding its role in apoptosis. NOD2 is structurally similar to apoptotic protease-activating factor-1 and overexpression of both NOD1 and
NOD2 was able to induce caspase-9-dependent apoptosis. We hypothesized that modulation of apoptosis by NOD2 could play an important role in the pathogenesis of Crohn’s disease. We stimulated peripheral blood mononuclear cells (PBMCs) of healthy volunteers, wild type Crohn’s disease patients (NOD2wt) and Crohn’s disease patients homozygous for the 3020insC NOD2 mutations (NOD2mut) with MDP. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) concentrations were determined by ELISA. When PBMCs of healthy volunteers were stimulated with MDP, they released significant amounts of TNF (454 ± 112 pg/ml) and IL-1 (298 ± 45 pg/ml), whereas cells of NOD2mut patients did not release cytokines upon MDP challenge. Isolated neutrophils and PBMCs were incubated with RPMI and 10% pooled serum. Cells were stimulated with MDP and anisomycin, a protease inhibitor that induces apoptosis, and apoptosis was measured using annexin-V-FITC and PI at different time points (0, 4, 12, 24 hours). Despite the significant lower pro-inflammatory cytokine production in NOD2mut patients, no differences were observed in percentage of lymphocytes and neutrophils in early apoptosis between healthy volunteers, NOD2wt and NOD2mut patients after incubation with RPMI, MDP and anisomycin separately. The NOD2 ligand MDP induces production of proinflammatory cytokines by mononuclear cells, but did not influence granulocyte or lymphocyte apoptosis. In line with this, mononuclear cells isolated from patients with Crohn’s disease bearing mutations in NOD2 displayed defective cytokine responses, but normal apoptosis. In conclusion, NOD2 engagement by MDP is mainly involved in cytokine activation and inflammatory reactions, with negligible effects on cell apoptosis.

(abstract 213)

The Magnitude of Microscopic Inflammatory Changes of Colonic Mucosa in Patients with Psoriatic Arthritis.

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Background: Patients with psoriatic arthritis may present microscopic inflammatory changes of colonic mucosa varying from typical inflammatory bowel disease to non-specific elements and unclassified forms of colitis. Objective: To identify the magnitude of microscopic inflammatory changes of colonic mucosa in patients with psoriatic arthritis. Methods: 32 patients with psoriatic arthritis (23 males and 9 females) underwent total colonoscopy and systematic colonic biopsies. Only 6 patients were symptomatic – 1 patient with rectal hemorrhages and 5 patients with diarrhea with no microscopic hemorrhage. Results: the typical aspect of ulcerative colitis (ulcerations, hemorrhages, erosions, granular mucosa, absence of normal mucosal vascular pattern) was found in 2 patients. Other 13 patients presented aspects such as: discontinuous lesions with a normal rectal mucosa, deep ulcers with normal vascular pattern of the mucosa, stenosis, or involvement of ileocecal valve and terminal ileum. Conclusions: In our study a significant number of patients (48.75% patients) with psoriatic arthritis presented clinical and endoscopic features of ulcerative colitis. The most are not specific and only the histological exam could define the right diagnosis of colonic lesions. Systematic biopsies offer the diagnosis of ulcerative colitis or Crohn disease, although in many cases the inflammatory changes are non-specific and infraclinical.

(abstract 129)

ULCERATIVE COLITIS DEVELOPED DURING THE COURSE OF RHEUMATOID ARTHRITIS TREATED WITH INFLIXIMAB. A CASE REPORT

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Background: Only a few cases of ulcerative colitis (UC) in patients with rheumatoid arthritis are cited in literature, some linked to serum IgA deficiency. There is no data regarding ulcerative colitis developed during anti-TNFα treatment of rheumatoid arthritis. Is well-known that infliximab may be considered a remission-inducing agent in patients with moderate to severe UC which is refractory to oral corticosteroids. There is insufficient evidence to advocate using infliximab as a first-line agent for UC patients with mild or moderate to severe disease. Case report: We report the case of a 43 years old female diagnosed with rheumatoid arthritis for 10 years, who presents for fever, abdominal pain and haematochezia. The rheumatic disease was treated subsequently with methotrexate (maximum dose of 20mg/wk), sulphasalazine (3g/day), metilprednisolone (16mg/day), leflunomide (20mg/day) in monotherapy or DMARD associations, with suboptimal or unsustained response – DAS scores constantly above 4.6. For 14 months infliximab was initiated - 3mg/kg at 0, 2, 6 and then every 8 weeks, associating methotrexate 20mg/wk, with an early sustained response - DAS28≤3.6. Patient relapses after the 15th infliximab infusion, with polyarthralgias and polyarthritis, increase in CRP, ESR and DAS 28>5.8. Fever (38.6 Celsius degrees), abdominal pain and haematochezia appeared after 10 days. Colonoscopy revealed edematous erosive mucosa and mild hemorrhage throughout rectum and sigmoid colon, without pathological findings in descending, transverse, ascendening colon and in terminal ileum. Histopathological findings consisted in cryptitis, caused by neutrophil infiltration and mild crypt abscesses. The patient was diagnosed with left-side UC and was successfully treated with metilprednisolone and mesalazine. Conclusion: UC appeared during rheumatoid arthritis is a rare event, exceptional during infliximab therapy. The peculiarity of the case consists in the debut moment which overlaps rheumatoid arthritis loss of response for infliximab.

**Innate immunity driving adaptive immunity**

(abstract 194)

**High levels of XIAP associated to a polymorphism in its gene are a risk factor for development of periodic fever.**


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Hereditary periodic fever syndromes are due to uncontrolled activation of the inflammatory response with excessive function of IL-1 or TNFα. They include Familial Mediterranean Fever (FMF), Hyper-IgD Syndrome (HIDS), TNFR-Associated Syndrome (TRAPS), and Cryopyrinopathies, due to mutations of the MEFV, MVK, TNFRSF1A, and CIAS1 genes respectively. However, the mutated gene is not known in other patients with idiopathic periodic fever (IPF). The X-linked inhibitor of apoptosis (XIAP) is involved in both caspase inhibition and NF-kB signalling, which are two processes influencing inflammatory cell function. We sequenced the XIAP gene (BIRC4, X-linked) in 39 IPF patients and 173 controls and detected a 1268A>C variation, causing a Q423P amino acid substitution, whose allelic frequency was significantly different in patients and controls (423Q allele:77% vs 62%, p=0.003; OR=2.05, 95% CI: 1.26-3.34). By contrast, frequencies displayed by 13 HIDS, 18 TRAPS, and 14 FMF patients were not significantly different from the controls. In primary lymphocytes and transfected cell lines, 423Q was associated with higher XIAP expression and lower caspase-9 activation than 423P. In macrophages, it was associated with lower basal secretion of IL-1 and TNFα, but higher LPS-
mediated induction of these cytokines. These data suggest that 423Q favors IPF development, possibly by influencing macrophage function.

(abstract 73)

**Acquired Progressive Spastic Diplegia as a Presenting Feature of SLE in Three Children**

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Background Mutations in the encoding genes for the Exonuclease TREX1 have recently been associated with Aicardi-Goutières Syndrome (AGS), SLE and familial chilblain lupus. Progressive Spastic Diplegia is a prominent clinical feature of AGS. Here we describe 3 children with acquired progressive spastic diplegia preceding the definite clinical and serological diagnosis of SLE. Case Reports Three children presented at a very young age with an acquired neurological deficit of unknown aetiology. A few years later, all 3 patients manifested cutaneous, articular or renal inflammatory disease; high titres of ANA/anti-dsDNA with complement activation confirmed the diagnosis of SLE. Clinical/laboratory data are summarized below. All patients were treated with corticosteroids and immunosuppression which controlled the systemic features of SLE. The therapy could not reverse the spastic diplegia although its progression seemed to be slowed down. TREX-1 gene mutation analysis is pending. Patient 1 was a Turkish boy who presented with Spastic Diplegia at 22 months. Imaging showed bilateral basal ganglia calcification. At 4 years vasculitic rash, arthralgia, myalgia and leucopenia. Lab results showed positive ANA, Anti-dsDNA, Anti-Cardiolipin Abs. Patient 1 also showed manifest growth retardation. X Ray findings were compatible with a spondyloenchondrodysplasia Patient 2 was a Caucasian girl who presented with Spastic Diplegia age 3 years and four months . Imaging showed SPECT uptake abnormalities. At 6 Years presented with a vasculitic rash and low platelets. Lab results showed positive ANA, Anti-dsDNA, Anti-Cardiolipin Abs. Patient 3 was Caucasian female who presented with Spastic Diplegia age 2 years. Imaging showed demyelinisation on MRI pateint then presented with Lupus nephritis age 3 years. Lab results showed positive ANA, Anti-dsDNA, Anti-Cardiolipin Abs. Discussion The finding of TREX-1 gene mutations in both AGS and SLE may suggest some pathogenetic overlap between both disorders. The present patients, manifesting phenotypic features of both entities seem to endorse this hypothesis. Although at first sight, these findings may suggest the existence of a monogenic form of SLE, the presence of significant spondyloenchondrodysplasia in one of them clearly demonstrates the intricate complexity of these autoimmune-inflammatory disorders

(abstract 134)

**Can mutations in the FMF gene protect or alleviate systemic lupus erythematosus burden?**
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Introduction: FMF is an autoinflammatory polyserositis associated with mutations in the Mediterranean fever gene, MEFV. The carrier rate of three common mutations in Israeli FMF patients is very high in the general population. SLE is an autoimmune disease affecting multiple
organs and is associated with a diverse spectrum of auto-antibodies and T cells that recognize self antigens. A lower than expected concurrence of FMF and SLE, as well as mild severity of FMF in co-morbid patients, was found in preliminary reports, suggesting that the patho-physiologies of SLE and FMF may counterbalance, and that MEFV mutations may reduce the risk to develop SLE. Aim: To determine if MEFV mutations reduce the risk to develop SLE or alleviate disease severity.

Methods: 90 SLE patients were screened for the M694V, V726A and E148Q mutations and their disease severity was assessed by the SLICC damage index. Results: The SLE cohort included 75 female patients and 15 males. 35 (36%) were of Ashkenazi origin, 52 (60%) non-Ashkenazi and 3 (4%) were mixed. 12 patients (7.8%, 1:7) carried an MEFV mutation, 6 had the M694V mutation, 3 the V726A mutation and 3 the E148Q mutation, and one patient had two mutations V726A/E148Q. While the mutation rate was calculated similar to that expected in a virtual, origin matched cohort based on historical controls, the mutations were over represented in male patients (5/15 males, 33%, vs. 8/75 females, 11%, OR 4.2, 95% CI1.14-15.4, p=0.04). A trend toward a higher rate of the M694V mutation in the North-African Jewish patients (5/30 chromosomes) compared to historical controls was detected (16/240 chromosomes, OR 2.8, 95% CI0/94-8.3, p=0.067). Moreover, there was a trend toward a younger age at SLE onset in the carriers of the M694V mutation when compared to that of non-carriers (26.7 vs. 37.3 yrs respectively). There were no differences in the average SLICC-DI score, the spectrum of affected organs or antibody spectrum between carrier and no-carrier patients. Intriguingly, although not statistically significant, the mutation carriers did not suffer from renal damage. Conclusions: Contrary to prior expectations, one FMF associated mutation does not appear to confer protection from SLE but rather to increase the risk of morbidity in males and North-African Jewish subjects. These results as well as the sparing from kidney damage await confirmation by a second cohort. Supported by the Israeli SLE research and registration (SLERI) fund.

(abstract 37)

The International Society for Systemic Auto-Inflammatory Diseases (ISSAID) website
http://fmf.igh.cnrs.fr/ISSAID

MILHAVET F. (1), SARRAUSTE de MENTHIERE C. (1), TOUITOU I. (2)*, The ISSAID provisional steering committee o. (0)

1) Institut de génétique humaine, CNRS-UPR1142, Montpellier, France; 2) Unité médicale des maladies auto-inflammatoires, CHU, Montpellier, France; 3) on behalf of the provisional steering committee of the International Society for Systemic Auto-Inflammatory Diseases (ISSAID): I Akseitjejivich (USA), J Frenkel (Netherlands), M Gattorno (Italy), R Gershoni-Baruch (Israel), A Gül (Turkey), D Kastner (USA), I Koné-Paut (France), A Livneh (Israel), R Manna (Italy), L Mcdermott (UK), M Medlej-Hashim (Lebanon), S Ozen (Turkey), T Sarkisian (Armenia), M Tunca (Turkey), J Yagüe Ribes (Spain). The ISSAID society was born in November 2005, during the Fourth FMF and Beyond International Congress on Systemic Autoinflammatory Diseases (Bethesda, USA) upon the suggestion of Dr. DL Kastner. The ISSAID logo was drawn by Drs. A. Gul and D. Cattan. We constructed a portal website to gather resources designed specifically for auto-inflammatory diseases (AID) and to facilitate contacts between physicians, biologists and basic researchers. The ISSAID website offers the following amenities: Information on AID’s diseases and actors - A tentative definition and a non-exhaustive classification of AID, recapitulating all acronyms, extended names, synonyms, and phenotypes associated with the genes and their proteins. - A list of experts and health professionals involved in care and study of patients with AID. Their information can be sorted by country and specialty. Links to connected resources: - Locus maps and OMIM of all genes and diseases. - Pubmed abstracts from studies of auto-inflammatory genes and diseases. - Existing mutation and patient
registries. The corresponding clinical forms and informed consents are also posted. A special form is proposed for easy submission of information on novel registries. - Past and forthcoming ISSAID meetings. Where available, links to the corresponding meeting abstracts, workshops, and published reports are posted. - Other relevant societies (PRES, PRINTO …). This service is open to suggestions. New applications. - Already available: A tool for quality control schemes for the molecular diagnosis of hereditary auto-inflammatory disorders (Moderator, I Touitou). Activity of registered laboratories can be seen at a glance. - Under construction: A forum, displaying several rubrics (clinical diagnosis, complex cases, patient care, treatment, research, and quality of life), enables professionals to share thoughts, feelings and experiences on auto-inflammatory diseases. The moderators are to be defined. - Under construction: Image libraries. One shows characteristic physical features from patients suffering from AID (Moderator, I Koné-Paut). Another library is devoted to microarrays (Moderator, to be defined). We hope this new website will help disseminate information, promote advances in the search for the causes and cure, and improve the quality of life of patients affected by AID. Any suggestion is warmly welcome.

(abstract 38)

**Patient registries for autoinflammatory diseases: The evidence for a common central database**

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1) Unité médicale des maladies auto-inflammatoires, Montpellier, France; 2) Service de Pédiatrie, Versailles, France; 3) Service de Pédiatrie générale, Bicêtre, France; 4) Clinical databases for patients, especially those affected by rare diseases, represent a valuable tool to estimate disease incidence and prevalence, delineate disease criteria and/or natural history, identify phenotype-genotype correlation, and evaluate outcome. Hereditary autoinflammatory diseases (AID) are conditions caused by mutations in genes involved in the innate immunity. These genes, also thought to act as susceptibility factors or modifiers in AID multifactorial diseases, encode modulators of inflammatory signalling pathways and apoptosis. Interleukin-1, a potent pyrogenic proinflammatory cytokine, is the common key factor dysregulated in AID. Accordingly, fever and several types of serositis represent the main clinical features of these disorders, making differential diagnosis sometimes challenging. We surveyed 6 registries for autoinflammatory diseases. The Hyper-IgD syndrome (HIDS) registry (HIDSnet) created by Dr A Simon at http://www.hids.net/, the Familial Mediterranean Fever (FMF) registry created by the International Study Group for Phenotype-Genotype correlation in FMF (MetaFMF), at http://fmf.igh.cnrs.fr/metaFMF/index.html, and the Periodic Fever Adenitis Pharyngitis and Aphthosis (PFAPA) registry created by Dr Hofer at http://www.pfapa.net/ are available online. The Behçet’s disease registry by Pr I Koné-Paut, the TRAPS registry (EuroTRAPS) by Dr M Gattorno, and the Blau Syndrome registry by Prs C Rose and C Wouters are planned to be posted on the Internet shortly. To address the extent of overlap between these registries, we reviewed all questions present in the corresponding questionnaires. Items were defined as questions with the same meaning (for example serositis in the joints and arthritis). We listed 130 items, that could be grouped into demographic, clinical, paraclinical and genetic categories. The number of item per disease ranged from 32 (Blau syndrome) to 69 (PFAPA syndrome). 5/8 of the demographic items were common to all 6 questionnaires. 73%, 44%, and 24% of the items were present in at least 2, 3 and 4 registries, respectively. We also reviewed the literature for complex cases. We identified 32 patients with more than one mutated gene and/or fulfilling clinical criteria for more than one disease. The very high overlap between the 6 registries analysed herein, and the increasing number of unclassified patients advocates for a common database for all autoinflammatory diseases. This would consist in a core module including epidemiological and genetic data where available (stable over-life). This module would be connected to various specific disease forms according to the initial
diagnosis. Several items of each form would be common to more than one disease. Each patient
data would be validated by the disease editor corresponding to the initial diagnosis (the registry
creators aforementioned). Follow-up and specific projects could be integrated over-time. The
advantages of a centralized database are enormous. Colleagues are very solicited and could be kept
motivated with a single URL, a single questionnaire, a single submission even if the diagnosis of
the patient has to be refined. Solid statistics need homogeneous data to be run, longitudinally (many
items in a single disease) or transversally (a single item across different diseases). The prevalence of
each AID could be compared accurately. Time and money would be saved.

Mechanisms of IL-1 secretion
(abstract 104)

Impaired isoprenoid biosynthesis induces caspase-1 activation in a Rac1/PI3kinase/PKB
dependent fashion: implications for the Hyper IgD syndrome.

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Background: Mevalonate kinase deficiency (MKD) is an autosomal recessive disorder characterized
by recurring episodes of fever and inflammation. Peripheral blood mononuclear cells from MKD
patients secrete high levels of IL-1β when stimulated with lipopolysaccharide due to the presence of
hyperactive caspase-1. This readily secreted IL-1β is thought to be a primary cause of the recurring
inflammation. However, the molecular mechanism of mevalonate kinase deficiency-induced
caspase-1 activation remains unclear. Methods: To investigate this, we incubated monocytic cells
(THP-1) with simvastatin to artificially impair the isoprenoid biosynthesis pathway, mimicking
MKD, after which cells are were stimulated with LPS. Results: Simvastatin-treated THP-1 cells
stimulated with LPS demonstrated enhanced release of IL-1β. LPS enhanced transcription of IL-
1beta, which was shown to be partially dependent on p38 MAP kinase-mediated activation of NF-
κB. Simvastatin-mediated effects were shown to be mediated by phosphatidylinositol 3 kinase
(PI3K) and protein kinase B (PKB/c-Akt). Inhibition of PI3K strongly reduced IL-1β secretion,
whereas introducing constitutively active PKB enhanced it. In addition, simvastatin-induced IL-1β
secretion required the small GTPase Rac1. Simvastatin treatment increased GTP-bound Rac1 levels
and inhibition of Rac1 reduced simvastatin-mediated IL-1β secretion. Rac1 functioned upstream of
PKB, since Rac1 inhibition abolished simvastatin-induced phosphorylation of PKB. Simvastatin-
mediated activation of the Rac1/PI3K/PKB pathway enhanced IL-1β secretion through activation of
caspase-1, since inhibition of both Rac1 and PI3K blocked the release of active caspase-1 subunits.
The importance of Rac1 in MKD was confirmed when a specific Rac1 inhibitor was shown to
inhibit spontaneous IL-1beta release by MKD PBMC. Conclusion: Rac1, PI3K and PKB are
involved in simvastatin-induced secretion of IL-1beta through regulation of caspase-1 activity.
Hence, Rac1 is a potential new therapeutic target in MKD.

(abstract 62)

ATP released following activation of various pathogen-sensing receptors autocrinally induces
IL-1beta and IL-18 secretion by monocytes

IL-1 beta is a multifunctional cytokine and a major soluble mediator of inflammation. Despite its extracellular localization and function, IL-1 beta lacks a secretory signal peptide and does not follow the classical ER-Golgi pathway of secretion. IL-1 beta is synthesized in the cytosol upon activation by inflammatory stimuli as a 35-kDa precursor (proIL-1 beta), and then proteolytically processed to the mature active form of 17-kDa by caspase-1/ IL-1 beta converting enzyme (ICE). The processing pathway is arranged in two steps. First, Toll-like receptor ligands, such as lipopolysaccharide (LPS) induce gene expression and synthesis of the inactive IL-1 beta precursor. Then a second stimulus is necessary to induce IL-1 beta processing and secretion. Among the second stimuli, exogenous ATP that strongly enhances the proteolytic maturation and secretion of IL-1 beta is the best characterized. A crucial role in IL-1 beta processing is played by the inflammasome, a multiprotein complex responsible for the activation of caspase-1, which, in turn, converts proIL-1 beta to the mature IL-1 beta. As the cleavage of the inactive proIL-1 beta is immediately followed by the release of the mature cytokine, processing and secretion appear to be linked. The complexity of the process is further increased by the observation that inflammasomes harbouring diverse molecular components exist. Also IL-18, a pleiotropic cytokine involved in the early events of the defensive innate immune reaction lacks a secretory signal peptide and like IL-1 beta requires cleavage by caspase-1 to be secreted in its active form. A defective control of their release may cause serious inflammatory diseases. Here we show that in primary human monocytes, several microbial components (pathogen associated molecular patterns,PAMPs) as well as molecules released by injured tissue, called danger associated molecular patterns (DAMPs) acting on different pathogen-sensing receptors (PPRs) are all competent to induce synthesis, maturation and secretion of IL-1 beta through a process that requires extracellular release of endogenous ATP, K+ efflux and activation of phospholipase A2. Moreover, all molecules inducing IL-1 beta also trigger processing and secretion of IL-18, indicating that, like for IL-1 beta, different signalling pathways converge on caspase-1 activation and IL-18 secretion. However, the amount of IL-18 secreted was very low compared to IL-1 beta. Antagonists of the surface ATP purinergic receptor P2X7, or treatment with the ATPase apyrase, prevent IL-1 beta and IL-18 maturation and secretion triggered by the different stimuli on healthy monocytes. Differently, IL-1 beta maturation and secretion induced by the ionophore nigericin, that elicits K+ efflux without activation of pathogen-sensing receptors, is not inhibited by blockers of P2X7 receptors. Remarkably, P2X7 inhibitors did not affect PAMPs-induced IL-1 beta secretion by monocytes from CINCA patients carrying a mutated NALP3, confirming our previous observation that inflammasome in CINCA patients does not require exogenous ATP for activation. These data indicate that in human monocytes from healthy individuals the autocrine stimulation of P2X7 receptors by the externalized ATP is required for IL-1 beta and IL-18 processing and release. Thus, stimuli acting on different pathogen-sensing receptors converge on a common pathway where secretion of ATP is the first step in the cascade of events leading to inflammasome activation and IL-1 beta and IL-18 secretion.

(abstract 197)

Different pattern of synthesis and secretion of IL-1β in patients with CIAS-1 mutations and in patients with systemic onset juvenile idiopathic arthritis (SoJIA) responding to IL-1 blockade

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Aim: to compare the in vitro synthesis, processing and secretion of IL-1β in pts carrying CIAS-1 mutations and in SoJIA pts, in an effort to understand the mechanism modulating IL-1β secretion in the different pathologies and the possible association with the response to antiIL-1 treatment. Pts and methods: Monocytes from 6 CINCA and 9 SoJIA pts selected for treatment with Anakinra were activated with 1μg/ml of LPS for 3 hrs, at baseline and after 7 days from the beginning of the treatment. For comparison, monocytes from 24 healthy donors were studied. Intracellular pro-IL-1β and secreted IL-1β were analysed by Western blotting and ELISA before and after 15 min exposure to exogenous ATP that accelerates IL-1b secretion. Results: Unstimulated monocytes from controls express very low if any pro-IL-1b. Differently, CINCA and SoJIA pts exhibited variable levels of intracellular pro-IL-1β in the absence of stimulation. In healthy subjects LPS-induced IL-1b secretion was variable but consistently ≤5ng/ml and it was markedly increased by exposure to exogenous ATP (up to 20ng/ml). Monocytes from CINCA pts secreted abnormally elevated amounts of IL-1b after LPS stimulation (up to 40ng/ml) that were not increased by ATP. Conversely, monocytes from SoJIA pts did not secrete more IL-1b than controls in response to LPS, but similarly to CINCA pts presented a low response to ATP. While the unresponsiveness to ATP in CINCA is conceivably related to CIAS-1 mutations, the reason of the low response to ATP in SoJIA pts is unknown. All CINCA patients displayed a dramatic clinical response to Anakinra that correlated with a strong downmodulation of IL-1b secretion. Conversely, 4 SoJIA patients exhibited a sustained response to Anakinra, but 5 did not, with no correlation with the pattern of in vitro IL-1b synthesis and secretion at baseline. No significant modification of the IL-1b secretion pattern was induced in SoJIA patients by the treatment, independently of the clinical response. Conclusion: Despite a similar clinical response to anti-IL1 treatment, the pattern of IL-1b secretion of monocytes from Anakinra-responder SoJIA pts significantly differ from that observed in pts CIAS-1 mutations. This study suggests a different hierarchy in the pathogenic mechanisms leading to the inflammatory response in different diseases responsive to anti-IL-1 treatment. *S.C. and D.L. equally contributed to the work.

Mevalonate-kinase deficency
(abstract 133)

Follow-up, clinical features, and quality of life in 103 patients with HyperImmunoglobulin D syndrome
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Introduction: The Hyper Immunoglobulin D and periodic fever syndrome (HIDS), one of the auto-inflammatory syndromes, is caused by mutations in the gene coding for Mevalonate Kinase (MVK). A patient registry database was founded in 1994 by the international HIDS study group. The aim of our current study was to assess the genetic, laboratory and clinical features as well as complications and course of disease in patients with mutation-positive HIDS. In addition we studied the quality of life and course of life in a selection of patients. Patients and methods: Follow-up data was acquired by a questionnaire that was sent to all submitting physicians. In addition, the course of life and
quality of life (QoL) was assessed in Dutch patients >16 years using validated QoL instruments. Results: Follow-up data was obtained from 103 patients (81.6% of total patients in HIDS registry) from 16 different countries. The median age of first attack was 6 months (range 0-120). Mean diagnostic delay was 14.8 years; this did not decrease in recent years. Most frequent symptoms that accompanied attacks of fever include lymphadenopathy (87.3%), abdominal pain (85.3%), arthralgia (83.3%), vomiting (71.6%), diarrhoea (72.3%), skin lesions (68.8%), and aphthous ulcers (52%). Amyloidosis is an infrequent complication (2.9%). The median highest serum IgD level was 409 U/ml. IgD levels were normal in 24% of patients. The four most prevalent mutations (V377I, I268T, H20P/N, P167L) account for 71.5% of mutations found. There was no association between age of onset, number of attacks, or clinical features and presence of a specific mutation. Frequency of attacks decreases with age: in the first decade of life 43.5% of patients have more than 12 attacks per year, in the second decade of life 23.9%, and 17.8% in patients >20 years. Many drugs have been tried in HIDS. Some patients respond to high dose prednisone (25% good response). Anakinra and etanercept can also be effective (good response 36% and 25%). HIDS impaired several aspects of QoL. Social functioning, general health perception, and vitality are significantly lower than controls, as is autonomy -and social development. Conclusion: HIDS patients have an early onset of disease, with often a large delay in diagnosis. The attacks gradually decrease during life, but persist in many. HIDS significantly impairs the quality of life.

(abstract 191)

Phenotypic variation of diseases related to Mevalonate Kinase (MVK) gene: from Hyper IgD Syndrome (HIDS) to Mevalonic Aciduria (MA)


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Background. HIDS and MA are caused by MVK gene mutations. This gene is located on the long arm of chromosome 12 and codes for MVK. HIDS and MA represent different conditions of the same disease where the former is the more benign. We describe two patients with clinical characteristics that are intermediate between the two classical disease phenotypes. Case reports. G.P., a female, has had recurrent fever since the age of 7 months, associated with lymphadenopathy, severe microcytic anemia and hepatosplenomegaly. Diagnosis at 14 months and genetic study showed a double heterozygosis: V377I, the most frequent mutation related to HIDS, and 606insG, a severe mutation leading to frame shift and premature stop, whose clinical significance is not known. In contrast to classic HIDS, she also has high urinary level of mevalonic acid outside febrile periods. She developed a progressive psychomotor delay and a severe growth deficiency. She was treated with steroids, cyclosporine and etanercept. Steroids led to a mild improvement, while etanercept was withdrawn after one month because of EBV infection. G.P. is now 5 years old and over the last four months has developed a severe pulmonary disease with multiple infiltrations and interstitial disease, whose nature is still to be defined. A.V., a male, was diagnosed with MA at 4 months of age. He is homozygous for the T60A mutation. He has developed typical characteristics such as multiple dimorphisms, growth failure, recurrent fever associated to hepatosplenomegaly, lymphadenopathy, arthralgia, oedema, rash and high urinary level of mevalonic acid independently of fever. At the age of 3 years he has not developed any neurological symptoms, such as ataxia and mental retardation. At the age of 18 months he developed a severe polyarthritis, involving small and large joints, with persistent thrombocytosis not previously reported in MA patients. Treatment with anakinra has been effective for fever, but not for the progressive arthritis. Conclusions. MVK mutations induce heterogeneous diseases. The genotype-phenotype relationship of MVK mutation is still unclear and the wide clinical heterogeneity makes it difficult to define the prognosis.
INFLAMMATION IS NOT DUE TO A PROTEIN PRENYLATION DEFECT IN MEVALONATE KINASE DEFICIENCY.

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Mevalonate kinase deficiency (MKD) is an autosomal recessive inborn disorder of cholesterol biosynthesis, due to mutations in the MVK gene coding for mevalonate kinase (MK), the second enzyme of the mevalonate pathway for the biosynthesis of cholesterol and non sterol isoprenes. Defective synthesis of isoprenoids has been associated with the inflammatory phenotype, but the molecular mechanisms involved are still poorly understood. It was demonstrated that the disease is not due to the accumulation of mevalonate, but rather to the lack of mevalonate-derived isoprenoids. This hypothesis is in agreement with clinical and biological data showing that drugs like statins and aminobisphosphonates, which also produce some defect in mevalonate metabolism, could lead to inflammatory reactions both “in vitro” and “in vivo”. In order to investigate the role of the mevalonate-derived compounds in the pathogenesis of MKD, and in particular in its inflammatory aspects, we set up a cellular model of chemically induced mevalonate pathway inhibition. Human monocytes isolated from health donors and murine RAW-264 cell line were treated with aminobisphosphonate alendronate, as mevalonate pathway inhibitor, and LPS, as second pro-inflammatory boost. According to other authors, LPS enhanced the inflammatory effect of the aminobisphosphonate in both cellular models. Inhibitors of protein prenylation, such as manumycin which acts on farnesyl-transferase, were added to these models to study their influence on inflammatory phenotype in the hypothesis that this inhibition - lowering definitely intracellular levels of prenylated proteins - could enhanced the aminobisphosphonate-induced inflammatory response, as previously suggested. Our results did not confirm this idea, whereas farnesyl-transferase inhibitors and other similar compounds prevented, almost partially, the activation of human monocytes and RAW-264 cells in combination with alendronate and LPS. A novel hypothesis emerged: the lack of isoprenoids in MKD - due to poor or absent enzymatic activity of mevalonate kinase – do not alter prenylated protein picture, but lower other mevalonate-derived products that could display regulatory properties in inflammation, such as the recently discovered presqualene-diphosphate (PSDP). Further researches are needed to better understand the molecules and the pathways involved in the development of inflammation in MKD.

An inherited hyper IgD autoinflammatory syndrome apparently unrelated to mevalonate kinase mutation: successful treatment with allogeneic bone marrow transplantation


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We describe 3 sisters born to Pakistani first cousin parents. The index case presented in the first year of life with recurrent fever, erythema nodosum-like rash, severe oromucocutaneous ulceration, nasal septal collapse, and failure to thrive. No infectious or autoimmune aetiology was identified, but serum IgD persistently exceeded 900 IU/ml (normal < 100). Comprehensive screening of the genes associated with HIDS, FMF, TRAPS and CAPS was normal and mevalonic aciduria was absent during fever attacks. Latterly we also documented evidence of ongoing endothelial injury by isolation and enumeration of circulating endothelial cells (CECs) on several occasions. She failed to respond to various therapies including colchicine and cyclophosphamide, infliximab, rituximab, and anakinra. Her symptoms were partially suppressed by high dose corticosteroids but there continued to be objective evidence of severe inflammation, including an intense acute phase response with serum amyloid A protein (SAA) concentrations exceeding 600 mg/L (healthy range < 5). In a bid to control her persistent severe refractory autoinflammatory disease, she underwent allogeneic bone marrow transplantation (BMT) at the age of 12 years. Within one month there was complete resolution of her symptoms, normalisation of her inflammatory markers as well as a fall in CECs. She engrafted fully with no complications. She is well 18 months post BMT on low-dose cyclosporine. Her younger female sibling aged 11 years has similar but milder symptoms, again associated with serum IgD persistently above 1000 IU/ml; her symptoms have been controlled partially with anakinra at 3 mg/kg/day, although her SAA remains elevated at >100 mg/L. A third sister presented at the age of 2 months with a severe clinical phenotype similar to the index case but with the addition of sterile bony lytic lesions in the cervical spine that have required surgical stabilisation. This infant died at 12 months from bronchopneumonia. Two further siblings are completely well. Conclusion: This Pakistani family appears to have a recessively inherited severe autoinflammatory syndrome associated with hyperimmunoglobulin D, but in which MVK may not implicated. As in HIDS, the disease was eradicated by BMT. Future genetic studies may elucidate the molecular basis of this novel HIDS-like disorder.

(abstract 72)

Defective isoprenylation in mevalonate kinase deficiency
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Mevalonate kinase deficiency (MKD), including Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA), is caused by mutations in the MVK gene, resulting in depressed activity of the encoded enzyme mevalonate kinase (MK). MK is an enzyme in the isoprenoid biosynthesis pathway and, although the reduced MK activity in MKD in principle affects the biosynthesis of all isoprenoids, it appears to have in particular an effect on non-sterol isoprenoid biosynthesis. Because small GTPases highly depend on isoprenylation (i.e. geranylgeranylation) for their proper signaling function, we studied the effect of MK deficiency on the isoprenylation and activation of the two small GTPases RhoA and Rac1. We used cultured primary skin fibroblasts from a healthy individual and an MKD patient, in which the isoprenoid biosynthesis pathway can be readily manipulated by varying the culturing conditions. Cells were cultured in the presence of simvastatin, an inhibitor of HMG-CoA reductase, or GGTL-298, an inhibitor of geranylgeranyl transferase, enzymes located upstream and downstream, respectively, of the enzyme defect in MKD. Incubation with simvastatin showed a differential effect on the inhibition of the geranylgeranylation of RhoA and Rac1 in both control and MKD cells with the latter cells being more sensitive. In addition, the treatment resulted in an increased activation of RhoA and Rac1. Incubation with GGTL-298 resulted in similar effects in both control and MKD cells, including decreased geranylgeranylation and increased activation of RhoA and Rac1. The effect of the
disturbed activation of these GTPases may provide important insights into the pathophysiology underlying the inflammatory episodes in patients with MKD.

(abstract 74)

**Isoprenoid detection by tandem mass spectrometry**


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Background: Isoprenoids constitute an important class of biomolecules that participate in many different cellular processes. Most detection methods for intermediates of isoprenoid biosynthesis so far have only focused on the detection of one or two specific compounds following radioactive or fluorescent labeling. We here report the development of a rapid non-radioactive and sensitive procedure for the simultaneous detection and quantification of the 8 main intermediates of the isoprenoid biosynthesis pathway. Methods: Intermediates of the isoprenoid biosynthesis pathway were analyzed by liquid chromatography-tandem mass spectrometry in the selected-reaction monitoring mode using a silica-based C18 HPLC column. For quantification stable-isotope-labeled compounds were used as internal standards. HepG2 cells were used to validate the method. Results: Mevalonate, phosphomevalonate and the 6 subsequent isoprenoid-pyrophosphates could be detected with detection limits ranging from 0.03 to 0.5 µmol/L. The intra- and interassay variations for HepG2 cell homogenates supplemented with isoprenoid intermediates were 3.9-12.5% and 4.4-12.1%, respectively. Under normal culturing conditions, isoprenoid intermediates in HepG2 cells are below detection limits, however, incubation of the cells with pamidronate, an inhibitor of farnesyl pyrophosphate synthase, results in increased levels of MVA, IPP/DMAPP and GPP. Conclusion: We developed a sensitive and specific LC-MS/MS-based method for the detection and quantification of nearly all intermediates of the isoprenoid biosynthesis pathway. This method will be suitable to measure profiles of isoprenoid intermediates in cells with compromised isoprenoid biosynthesis, and the specificity of potential inhibitors of the pathway.

(abstract 162)

**SPECIFIC INCREASE OF CASPASE-1 ACTIVITY AND SECRETION OF IL-1 FAMILY CYTOKINES: A PUTATIVE LINK BETWEEN MEVALONATE KINASE DEFICIENCY AND INFLAMMATION**


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Sylvain Normand and Benoit Massonnet contributed equally to this work. The “Hyper-Immunoglobulinemia D and periodic fever syndrome” (HIDS) as well as the Mevalonic Aciduria (MA) are autosomal recessive disorders characterized by recurrent episodes of fever, systemic inflammation and, at least for HIDS, high serum IgD levels. They are caused by mutations in the
gene encoding Mevalonate Kinase (MK), an enzyme of the cholesterol synthesis pathway, leading to its inactivation. The link between inactivation of the MK and inflammation remains to be elucidated. Using simvastatin, an inhibitor of the 3-hydroxy-3-methylglutaryl CoA reductase, the enzyme upstream of MK, we assessed in vitro the effect of an impairment of the cholesterol pathway on cytokine production. Simvastatin critically inhibited the production of IL-4, IL-10, IL-2 and IFN-gamma but had no effect on TNF-alpha production by activated T lymphocytes. In contrast, simvastatin dramatically and specifically enhanced the production of the cytokines of IL-1 family (IL-1alpha, IL-1beta and IL-18) by LPS-activated PBMCs and THP-1 monocytes, while IL-10, IL-6 and TNF-alpha productions were unchanged. According with these results, we showed that activated dermal fibroblasts from MKD patients secrete more IL-1beta than control cells, and this production is not further enhanced by simvastatin. Whereas quantitative real-time RT-PCR and Western Blot revealed that pro-IL-1beta expression was not modified by simvastatin, we showed that simvastatin enhanced caspase-1 activity. Geranylgeranyl, a metabolite downstream of MK, is involved in geranylgeranylation of G proteins. The geranylgeranyl-transferase inhibitor GGTL-298 also enhanced IL-1alpha, IL-1beta and IL-18 production, suggesting that geranylgeranylation is involved in this process. Pull-down assays showed that GTP-loading activity of Rac-1, a small G protein requiring geranylgeranylation, and implicated in caspase-1 activation, was modulated by simvastatin. Taken together, these data strongly suggest that the inhibition of MK induces the secretion of IL-1 family cytokines by activating caspase-1.

(abstract 192)

SPECTRUM OF MVK GENE MUTATIONS IN A SPANISH COHORT OF PATIENTS WITH MEVALONATE KINASE DEFICIENCY.


Mevalonate-Kinase deficiency (MKD) represents a clinical continuum spectrum ranging between the severest form mevalonic aciduria (MA) to the mildest Hyper-IgD and periodic fever syndrome (HIDS). MKD is recessively inherited and it is associated with mutations on the MVK gene, which maps at 12q24. Patients & Methods: 15 MKD patients from 13 unrelated families were included in the study. 2 patients were from Centro European ancestry, 2 from Gipsy families and the remaining 11 were from Spanish ancestry. Clinical and laboratory data were collected through a specific questionnaire. Genomic DNA was extracted from peripheral blood leukocytes and mutational analysis of autoinflammatory diseases-associated genes was performed by PCR amplification and bi-directional sequencing. Results: 12 out of 13 families were afflicted by HIDS, while the remaining one suffered from an intermediate MKD phenotype. MKD gene mutational analysis revealed 10 different mutations among the 13 unrelated MKD families studied. The genotypes identified were: homozygous V377I in the 2 Gipsy families, compound heterozygous in 9 families and single heterozygous in the remaining 2 families. Nine of the mutations were point nucleotide changes resulting in missense amino acid exchanges, two of them novel (L246P and R241C), while the other one was an intronic mutation (IVS3-3C>G). No nonsense mutations neither deletions nor insertions were detected. Concerning MVK mutations detected, there was an overrepresentation of the V377I mutation (11 out of 24 mutated chromosomes; 45.83%) and the I268T mutation (5 out of
Conclusions: Novel and recurrent mutations in MVK gene have been detected among Spanish MKD patients. We must highlight the presence of two homozygous V377I families from Gipsy ethnicity, which enables us to state that other than Centro European ethnicities could be afflicted by MKD. Although we have detected mutations in different exons of the MVK gene, 2/3 of all mutations are located in two different amino acid residues (268 and 377). From a practical point of view, this fact could facilitate a fast genetic screening for these two specific mutations in patients with a possible diagnosis of MKD. Finally, two patients of our cohort carried a single heterozygous genotype, which will require additional studies of MVK gene expression in order to elucidate the physiopathological basis of their disease.

(abstract 32)

**NATURAL ISOPRENOIDS ARE ABLE TO RESCUE INFLAMMATION IN A MOUSE MODEL OF MEVALONATE KINASE DEFICIENCY**


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Mevalonate kinase deficiency (MKD) is an autosomal recessive inborn disorder of cholesterol biosynthesis, due to mutations in the MVK gene coding for mevalonate kinase (MK), the second enzyme of the mevalonate pathway for the biosynthesis of cholesterol and non sterol isoprenes. Different degrees of disease severity were observed, being linked with the residual activity of MK, ranging from autoinflammatory hyper immunoglobulinemia D and periodic fever syndrome (HIDS, OMIM #260920), with a 1-8% residual MK activity, to mevalonic aciduria (MA, OMIM #610377) in which MK activity is below the level of detection. Patients with the HIDS phenotype typically present only recurrent episodes of fever and associated inflammatory symptoms, whereas patients with MA show, in addition to these episodes, developmental delay, dysmorphic features, ataxia, cerebellar atrophy, psychomotor retardation, and may die in early childhood. HIDS patients usually are treated with anti-inflammatory drugs and in particular corticosteroids; thalidomide is also employed but its effect is limited. In most severe cases, patients may benefit from treatment with biologic agents such as etanercept and anakinra. No treatment has been proven effective in curing the neurological symptoms in severe cases of MKD. Although the genetic defect has been known for a decade, the molecular mechanisms underlying the inflammatory phenotype are still unclear, and thus an etiologic treatment for MKD is still unavailable. The disease is not due to the accumulation of mevalonate, but rather to the lack of mevalonate-derived isoprenoids. This hypothesis is in agreement with clinical and biological data showing that drugs like statins and aminobisphosphonates, which also produce some defect in mevalonate metabolism blocking geranylgeranyl-pyrophosphatase, could lead to inflammatory reactions both “in vitro” and “in vivo”. Moreover, it was suggested that “in vitro” inflammation could be reversed by the addition of some isoprenoids, such as geranylgeraniol and farnesyl pyrophosphate. Starting from these observations, we treated Balb/c mice with the aminobisphosphonate alendronate, to induce a baseline inflammatory phenotype similar to MK deficiency. Bacterial muramylidipeptide (MDP) was added as second boost to trigger an acute condition. This behaviour could reproduce, at least in some aspects, the condition of MKD patients, where mild stimuli, such as a vaccination, are able to induce a typical inflammatory episode. As expected, alendronate lead to a strong inflammatory response in mice, which was further amplified by MDP. Recently, the effect of some natural isoprenoid compounds was tested in vitro on peripheral blood cells of HIDS patients, and a partial
rescue of inflammatory phenotype was obtained. We decided to test the anti-inflammatory effect of these compounds in our MKD-like mice, hypothesizing a possible future use in the human condition. Exogenous isoprenoid intermediates, geraniol, farnesol and geranylgeraniol, were administered in alendronate-MDP-treated mice with different results. Geraniol was able to reduce inflammation in alendronate-MDP-treated mice, counteracting the aminobisphosphonate effect. These data were partially reproduced using the other two isoprenoids. Geranylgeraniol, although less effective than geraniol, reduced inflammation. Farnesol did not give all the expected results. Our data strengthened previous observations about an important role of isoprenoids in inflammation, even if they do not explain how isoprenoids mediate this event. These compounds play an important role in different biological function, including protein farnesilation. Furthermore, the inhibition of farnesil-transferase was evaluated in this model to demonstrate that protein farnesilation is really involved in MKD pathogenesis. In conclusion, we hypothesize that the inflammatory phenotype observed in MKD and in aminobisphosphonate-treated mice is due to the lack of mevalonate-derived isoprenoids. While further research will be necessary to identify which of these molecules is mainly involved in the observed anti-inflammatory activity, our data support the idea of developing and testing isoprenoid-based treatment for MKD in humans.

(abstract 116)

**MVK Mutations in Unusual Populations**


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Hyperimmunoglobulinemia D periodic fever syndrome (HIDS) is a recessively inherited disease caused by mutations in the gene for mevalonate kinase (MVK). It is typically diagnosed in Northern European populations although cases have also been reported in Japan and India. Only one case has previously been reported in the Arabic population. We describe here two further cases of HIDS in Kuwaiti Arabs and a further probable case in a Pakistani man referred with unexplained AA amyloidosis. Case 1 was a 22 year old Kuwaiti woman referred with a history of unexplained fevers from childhood. Her disease had worsened in the previous 3 years, comprising 2 week long attacks of fever, diarrhoea, rash and knee arthralgia with cervical and auxilary lymphadenopathy that occurred monthly. Her older sister had apparently had a similar illness and died aged 25 of renal failure, which in context may have been amyloidosis. Gene studies identified MVK V377I along with a novel deletion in exon 3 L29fs(c.86-delT), and she was also found to be heterozygous for the common pyrin E148Q variant. Her father was heterozygous for V377I and her mother and two surviving siblings carried the novel deletion mutation. Case 2 was a 5 year old Kuwaiti boy who had presented in infancy with severe diarrhoea, pan colitis, perianal fistulae, fevers, and mouth ulcers. He had an elevated serum IgD concentration of 600 mg/L. Sequencing of MVK showed him to be heterozygous for V377I and S272F. Case 3 was a Pakistani man who presented aged 48 with myalgia and nephrotic syndrome. He had a marked acute phase response and renal biopsy demonstrated AA amyloidosis. Extensive investigations failed to characterize the nature of his underlying inflammatory disease but speculative sequencing of his periodic fever genes demonstrated that he was heterozygous for a novel MVK mutation D386N as well as the MVK polymorphism S52N. We are further considering the possibility that he may have ‘phenotype 2 HIDS’. The prevalence and clinical phenotype of inherited periodic fever syndromes have been little studied in Arabic and Asian populations, and indeed in many other populations in the World. These cases, which possibly extend the phenotypic spectrum of HIDS, emphasize the need to consider the possibility of HIDS among patients with inflammatory disorders of undetermined aetiology in atypical populations.
A clinical prediction rule to exclude the hyperimmunoglobulin-D syndrome in patients with recurrent fever

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Introduction – The hyperimmunglobulin D syndrome (HIDS) is an autosomal recessive autoinflammatory disease caused by mutations in the mevalonate kinase gene (MVK). Our objective was to define a clinical criterion able to exclude HIDS without genetic testing in some patients. Methods – Clinical data were extracted from the request formularies sent to a French reference laboratory with blood samples for HIDS genetic testing. Patients with mevalonic aciduria or with a family member already tested positive were excluded. A recursive partitioning algorithm was applied to find the most discriminatory composite clinical criterion satisfied by all patients with two mutated MVK alleles. The criterion was validated in an independent cohort of patients tested in a Dutch reference laboratory. Clinical data were extracted from the HIDS database (www.hids.net). Patients were excluded if a family member was already tested positive. Results – The French derivation cohort included 149 patients with genetic testing, among whom 35 were positive. Attacks in positive patients had the following characteristics (medians for quantitative variables and percentage for binary variables): onset at 3 years old, attacks lasting 4 days with fever in 100%, lymphadenopathy 73%, abdominal pain 82%, vomiting 44%, diarrhoea 60%, joint pain 86%, skin lesions 59%. The composite criterion [onset < 5 years old OR (attacks with arthralgia/arthritis AND attacks < 14 days)] was the most discriminatory. It had a sensitivity (Se) = 100% [95% confidence interval: 90-100], a specificity (Sp) = 37% [28-46] and a negative likelihood ratio (NLR) = [0-0,27] in the derivation cohort. The Dutch validation cohort included 93 patients with genetic testing, among whom 28 were positive. Attacks in positive patients had the following characteristics: onset at 2 years old, attacks lasting 5 days with lymphadenopathy in 89%, abdominal pain 85%, vomiting 81%, diarrhoea 69%, joint pain 88%, skin lesions 63%. The composite criterion had a Se = 100% [88-100], a Sp = 28% [17-40] and a NLR = [0-0,44] in this cohort. Conclusion – In both cohorts, a negative composite criterion was sufficient to rule out HIDS. If genetic testing had been limited to patients whose attacks began before the age of 5, or are associated with joint pain and last less than 14 days, the total number of tests would have decreased by 30% in the derivation cohort and 20% in the validation cohort, without missing a single positive patient.

New therapies

Safety and efficacy of Infliximab and Rituximab in patients with refractory Behcet disease


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Background: Behcet disease (BD), a systemic vasculitis, has a wide variety of clinical
manifestations resulting from ubiquitous small-vessels inflammation. A defect in immune regulation has been proposed, and one or several infectious agents may trigger the immune system. In patients with BD, CD4+ T lymphocytes are found in the inflammatory infiltrates; and Th1 response predominates (producing IL-1, IL-2, IL-6, IL-12, INF gamma and TNF). BD treatment includes colchicines, corticosteroids, and/or cyclophosphamide. In refractory disease, methotrexate or mycophenolate mofetil are used as well as anti-TNF agents. Objective: To study safety and efficacy of biological therapies in patients with refractory BD. Methods: We studied 9 patients with BD refractory to corticosteroids and conventional immunosuppressive agents. Results: Nine patients, 5 female and 4 male, mean age was 34.3 years (17-53), and mean time disease evolution of 10.67 years (6-13) at the end of the study. Their clinical manifestations included: genital and oral ulcerations (100%); neurological manifestations (55.5%); mucocutaneous (33.3%); uveitis (33.3%); artritis (55.5%); motor diarrhea (33.3%), Raynaud phenomena (22.2%); thrombosis and pathergy phenomena (22.2%). These patients were previously and unsuccessfully treated with: high-dose corticosteroid (>20mg/day of prednisone) and colchicine (100%); cyclosporine (55.5%); azathiohpine (44.4%); cyclophosphamide (33.3%); chloroquine (11.1%); methotrexate (66.6%); and salazopirine (11.1%). All the patients discontinued using these treatments because of severe side effects or inefficacy. We decided to prescribe them infliximab 5 mg/kg/infusion as Rheumatoid arthritis protocol. Nine patients received infliximab during a mean time of 1.25 years (SD: 2.05), 5 patients improved their symptoms and the remaining 4 patients had to stop infliximab infusion because of allergic reaction (1) and inefficacy (3: repetitive neurological manifestations, mucocutaneous lesions and artritis). The 4 non-responder patients were switched to rituximab therapy. Patients on rituximab are free of symptoms after a mean duration of 10.6 months (SD: 8.9), and continued only with low-dose of corticosteroid. Conclusions: Infliximab, a well tolerated and safe treatment, is a therapeutic alternative in patients with refractory BD. Those patients, who may not respond to infliximab, should get benefit from an alternative and safe option as rituximab therapy may offer.

(abstract 160)

Long-lasting response to ACZ885 (a new human IgG1 anti-IL-1β monoclonal antibody) in patients with Muckle-Wells Syndrome (MWS)


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MWS is a rare autoinflammatory disease caused by mutations in the CIAS1 gene. The current treatment is anakinra, a soluble IL-1 Ra; however, frequent and high-dose injections are not well tolerated. Herein, we report a single-centre interim analysis of an open-label study to investigate safety and efficacy of ACZ885. Patients with documented CIAS1 mutation and active disease were recruited. Patients received one ACZ885 s.c. injection (adults 150 mg/children 2 mg/kg) followed by an observation period and re-dosing upon relapse. Complete response was defined by physician’s global assessment of disease activity (PGADA) and assessment of skin disease ≤2 on a 5-point scale (1=absent, 2=minimal, 3=mild, 4=moderate, 5=severe) and normal serum values (below 10 mg/L) of C-reactive protein (CRP) and/or serum amyloid A (SAA). ACZ885 5 mg/kg was administered i.v. to patients with incomplete response within 7 days. Patients overall rating of disease severity was recorded on a 5-point scale (0–4). Of 12 pts (median age: 27.6 [4.3–47.4]; 4 children below 14 yrs), 9 pts had a medical history of treatment with anakinra up to 8 mg/Kg. All 12 pts treated with ACZ885 achieved complete and rapid clinical and serological response. Data presented below show the median of 12 pts at baseline, 8 and 38 days after treatment, 9 pts at Day
68 and 3 pts at Day 98 after treatment. PGADA was 4 at baseline and rapidly decreased to 1 at Day 8 and 38 and increased to 3 and 4 at Day 68 and 98. Patients rated disease severity as 2, 0, 1, 1 and 2 at baseline, Day 8, 38, 68 and 98, respectively. CRP decreased to normal values during the study. SAA (mg/L) decreased from baseline (16.05) to normal values at Day 8, 38, and 68, and increased to 17 at Day 98. 2 children and 1 adult received i.v. injection. The median time to re-dosing was 92 days (9 pts) after the first and 66 days (6 pts) after the second treatment cycle. 2 pts remained relapse free for 106 days. ACZ885 was well tolerated. AEs were upper respiratory tract infections (7 pts) and elevated pancreatic amylase and lipase (2 pts). 1 pt experienced an SAE (vertigo). In conclusion, ACZ885 achieved a complete and long-lasting response in 12 pts with MWS. Long treatment-free intervals of up to 106 days is a great advantage in patients who tolerate daily injections poorly. The first predictor of disease progression was clinical deterioration (PGDA assessment), while the rise in inflammatory markers was observed at a later timepoint.

(abstract 171)

Treatment of cryopyrin associated periodic fever syndrome with a fully human anti-IL-1beta monoclonal antibody (ACZ885): results from a subcutaneous administration study.


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Background NLRP3/cryopyrin plays a vital regulatory role in caspase 1 mediated processing of pro IL-1, and mutations in the gene for this protein cause the autoinflammatory disease cryopyrin associated periodic fever syndrome (CAPS). The pivotal role of IL-1 in the pathogenesis of CAPS has been confirmed by complete remission following treatment with short acting recombinant IL-1Ra (anakinra). Objectives: To assess the clinical efficacy, safety, pharmacokinetics and pharmacodynamics of a fully human anti-IL-1 monoclonal antibody (ACZ885) given by subcutaneous (s.c.) administration in CAPS. Methods: Eight adult patients with CAPS associated with NLRP3 gene mutations were recruited and gave informed consent. Entry criteria were elevated CRP and SAA concentration and moderate to severe symptoms of CAPS. All patients were treated with ACZ885 150 mg s.c. and clinical and laboratory outcomes were measured. A further dose was administered at each relapse. Clinical remission was defined as: absence of fever, rash, conjunctivitis and joint or muscle pain; CRP and SAA within the normal range of < 10 mg/L; and normal leukocyte count. Relapse or incomplete remission was defined as: return of at least two symptoms associated with CRP and/or SAA values >30 mg/L. Results: 5 women and 3 men (median age 35 years) received s.c. injections of ACZ885 for a median of 18.5 months. The drug was uniformly well tolerated and resulted in improvement of clinical symptoms within 1 day and complete clinical remission within 7 days. Each clinical remission lasted a median of 115 days (IQ range 91-127 days). The CRP and SAA fell to healthy values within one week of dosing. Modelling using data on ACZ885 pharmacokinetics and clinical symptoms indicated ACZ885 had a plasma half life of 29 days and that the critical drug concentration where there was a 50:50 probability of clinical relapse was 1.1 mcg/ml. Simulations from the model predicted that regular dosing of 150 mg every 8 weeks should maintain sufficient drug concentration to sustain disease control in patients over 40 kg. Conclusion: In 8 patients treated for up to 22 months, blockade of IL-1 with the monoclonal antibody ACZ885 was extremely well tolerated and produced complete clinical and biochemical remission of CAPS disease activity in all cases. Phase 3 placebo controlled studies are underway.
Effective Treatment with IV Pamidronate in Chronic Recurrent Multifocal Osteomyelitis (CRMO)-Resolution of Pain, Normalization of Radiologic Abnormalities, and Improvement of Elevated Urine-N-Telopeptide


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Objectives: To report clinical, magnetic resonance imaging (MRI), and bone turnover response to intravenous pamidronate (IVP), in pediatric patients with chronic recurrent multifocal osteomyelitis (CRMO). Methods: A prospective open label study was conducted of all CRMO patients (pts) treated with IVP between 2003 and 2007. 10 patients (5M:5F) received IVP. Mean age at CRMO diagnosis was 11.4 (range 4.5-16.3) years. Mean duration of symptoms pre-IVP was 23.9 (range 2-36) months. Involved sites included spine, femur, tibia, clavicle, sacroiliac joints (2,3,2,2,1,1 patients, respectively). All pts had failed NSAIDs and reported 10/10 on visual analog scale (VAS) for pain. Plain x-rays, bone-scan, MRI, and histology were compatible for CRMO for all pts. All pts had T1 signal hypointensity and T2 signal hyperintensity of affected bones on pre-IVP MRI, consistent with inflammation. Pamidronate was administered as intravenous cycles (IVP), 1mg/kg/day. All pts received an initial 3-day cycle, and subsequently either 1-day IVP monthly, or 3-day IVP every 3 months, with maximum dose < 11.5mg/kg/year. VAS for pain, and urine N-telopeptide/urine creatinine (uNTX/uCr) ratio (a marker for bone resorption), were measured at baseline and at monthly intervals during IVP treatment. MRI(s) of affected sites were obtained at baseline, every 2 months, and at suspected CRMO recurrence. The primary endpoint was pain response, the secondary endpoint MRI signal resolution. Results: VAS decreased from 10/10 to 0-3/10 by end of first IVP for all pts. MRI findings of bone marrow edema resolved by mean of 5.2 (range 2-10) months and IVP was discontinued. The mean number of IVP treatments was 5.2 (range 2-10); mean dose was 6.2 (range 2.5-9.5) mg/kg/year. Mean pre-IVP uNTX/uCr was 722 nmol/mmol/creatinine; with 63.3% reduction after first IVP (range 16-88%). Mean follow-up after initiation of IVP was 24.9 (range 12-54) months. 4 pts had MRI confirmed CRMO relapse at 12-18 months after IVP. All 4 pts responded clinically and by MRI to 1-day IVP re-treatment. UNTX/uCr increased by mean of 184% with flare. Conclusions: 1. IV Pamidronate resulted in rapid and sustained pain relief in these patients with severe CRMO. Only 1-day retreatment was required for flares. 2. MRI signal resolved more gradually than pain. 3. UNTX/uCr was elevated with active CRMO, decreased with IVP, and increased with CRMO flare. 4. IVP is an effective 2nd line agent for severe CRMO.

Placebo-controlled Pilot Study of Rilonacept (IL-1 Trap), A Long Acting IL-1 Inhibitor, In Refractory Chronic Active Gouty Arthritis


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Preclinical studies and a clinical case series suggest that blockade of the NLRP-3 (cryopyrin) inflammasome IL-1 pathway may offer a new treatment strategy for gout. Rilonacept, a soluble receptor-Fc fusion protein that blocks IL-1 showed rapid and sustained benefits in a Phase 3 study of subjects with cryopyrin-associated periodic syndrome (CAPS) arising from NLRP-3 mutations. This pilot study explored the potential utility of rilonacept in chronic active gout. Objective: To assess the safety profile and change in disease activity in subjects with chronic, active gouty arthritis after treatment with rilonacept. Methods: This was a multi-center, non-randomized, single-blind, placebo-controlled, monosequence crossover study. The study population included subjects with a diagnosis of ≥6 months of chronic gouty arthritis, ≥1 active joint for ≥4 weeks, and a self-reported pain visual analogue scale (VAS) of ≥3. A run-in period of 2 weekly subcutaneous (SC) injections of placebo (PBO) was followed by 6 weekly injections of rilonacept. Gout activity was assessed by Subject Pain VAS, Subject and Physician Global VAS, joint count, and hs-CRP. Results: Ten subjects (8M/2F) with a mean age of 62 years (50-78), mean disease duration of 13 years (6-26), and Day Subject Pain VAS of 5.1/5.0 (mean/median) were enrolled. There were no reported deaths or SAEs, and drug-related AEs were most often associated with mild-to-moderate ISRs. Mean/median changes in Subject Pain VAS rating from Day to Wk 2 with PBO treatment were 0.25/-0.25 (ns), respectively, and -3.2/-2.25 (p=0.02) with rilonacept treatment from Wk 2 to Wk 8. During this period, seven of 10 subjects on rilonacept showed at least 50% improvement in Subject Pain VAS (p<0.0001) and six of 10 subjects showed at least 75% improvement (p=0.0001), while no subjects showed improvement in this parameter while on PBO. Hs-CRP median decreased 59% (p=0.004) by Wk 8 after rilonscept therapy. At Wk 14 (6 weeks after last dose of rilonacept) a trend towards baseline hs-CRP levels was observed. Conclusion: Rilonacept was generally well tolerated. Substantial decrease in both clinical activity and hs-CRP was seen after blinded switch from treatment with PBO to rilonacept. These results support the hypothesis that IL-1 blockade may offer an important new therapeutic option in a subset of gout patients with long-standing arthritis that can not be managed with other treatments. Further studies are planned.

(abstract 208)

mIL-1 Trap Reduces Pain and Inflammation in Animal Models of Gout
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Gout is a common and very painful arthritic disease with increasing prevalence in the United States. Monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals induce interleukin-1 (IL-1) release through the inflammasome, and this release is thought to contribute to the pain and inflammation associated with gout and pseudogout. However, consequences of IL-1 blockade have not been explored with regard to clinically relevant joint pain and inflammation readouts in gout animal models. In this study, we employ IL-1R1 knockout mice and mIL-1 Trap, a high-affinity receptor-Fc fusion protein blocker of mouse IL-1, to assess the involvement of this cytokine in the pain and inflammation observed in mouse models of gout. We developed a new murine joint pain and inflammation model of gout in which endotoxin-free MSU crystals were injected intra-articularly into the ankle joint followed by measurement of thermal hyperalgesia and weight bearing distribution of the affected hind paw and ankle inflammation over a 4 days period. The effects of genetic (IL-1R1-null mice) and pharmacological (mIL-1 Trap) blockade of IL-1 signaling was studied in this model as well as in crystal-induced peritonitis and subcutaneous air pouch mouse models of gout and pseudogout inflammation. We show that blocking IL-1 leads to reduced neutrophil influx in both the MSU and CPPD crystal-induced peritonitis and subcutaneous
air pouch models. mIL-1 Trap’s efficacy in preventing neutrophil infiltration in the MSU crystal-induced peritonitis model was equivalent to that achieved with the highest tolerated dose of the gout medication colchicine. In the ankle joint pain model, inhibiting IL-1 lead to significant reductions in thermal hyperalgesia, weight bearing redistribution, ankle inflammation, and SAA levels. mIL-1 Trap was also effective in relieving established pain and ankle swelling when administered 1 day after ankle MSU injection. mIL-1 Trap was as effective in the joint model as the highest tolerated dose of colchicine. Our data demonstrate that the mIL-1 Trap is able to decrease both pain and inflammation in mouse gout models. These studies suggest that IL-1 Trap (rilonacept) may be a promising therapeutic candidate for preventing and treating acute gout attacks in human disease. Rilonacept may provide a new mechanism of action treatment option for patients in which currently available therapeutics are not well-tolerated or not sufficiently efficacious.

(abstract 138)

Rilonacept in Patients with Cryopyrin-Associated Periodic Syndromes (CAPS): The Durability of Response over 48 weeks


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Background: IL-1B inhibition with rilonacept has been shown to rapidly improve the clinical and laboratory signs and symptoms associated with Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS). Short term (6 wk) treatment of FCAS/MWS patients (Pts) with rilonacept 160 mg weekly demonstrated marked (85%) decreases in the 21 day mean key symptom score (KSS) (0-10 scale of rash, fatigue, feeling of fever/chills, joint pain and eye redness/pain) and normalized serum amyloid A (SAA) and C-Reactive Protein (CRP) compared to placebo. The study described here assessed the efficacy of rilonacept over 48 weeks. Methods: Pts completing an initial 24-week blinded treatment period (44/47) enrolled in a 24-week open-label rilonacept treatment extension (OLE) in which all received rilonacept 160 mg sc weekly. Symptoms were assessed daily (pt report), CRP and SAA were assessed at day and wk 1, 3, 6, 12, 18 and 24. Results: Of the 47 pts from the initial 24-week blinded study, 43 completed through the subsequent 24-week OLE. Results are ordered by the following groups: rilonacept-DB-week 6-(n=23), Placebo-DB-week-6 (n=24) and OLE-week-48-(n=44). Baseline mean KSS 3.1, 2.4, 2.8 Mean reduction in KSS from baseline -2.6 (84%), -0.3 (13%), -2.2(79%) Baseline mean number of disease flare days 8.6, 6.2, 7.6 Mean reduction in disease flare days from baseline -8.4(98%), -1.0 (16%), -6.9 (91%) Baseline mean SAA 60.4, 109.9, 86.6 Mean reduction in SAA from baseline -56.6(94%), -0.1(0.1%), -71.9 (83%) Baseline mean CRP 22.5, 29.7, 26.8 Mean reduction in CRP from baseline-20.1 (92%), -1.4 (8%), -20.9 (78%) All symptom-based parameters were based on 21-day observation period. all rilonacept groups were statistical significant from placebo; p< 0.03 In all analyses, the improvement in signs and symptoms of FCAS/MWS in pts treated with rilonacept was similar, and sustained for the duration of treatment, up to 48 weeks. The most common adverse events were injection site reactions and upper respiratory tract infections similar to those observed in the initial blinded studies. Conclusions: The marked improvements in clinical and laboratory signs and symptoms of FCAS/MWS seen with rilonacept treatment are maintained during long-term therapy.
Anakinra in a patient with idiopathic cold urticaria and bulbar dysarthria

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Idiopathic cold urticaria is an acquired disorder characterized by development of an urticarial rash and/or angioedema after cold exposure. Usually, a generalised acute phase response is absent. A 58 year old woman reported generalized urticarial rash with oropharyngeal angioedema after exposure to cold, from early childhood. There were no other symptoms and no acute phase response. The diagnosis of idiopathic cold urticaria was made at the age of 26. Despite treatment with antihistamines, she could not tolerate outside temperature < 10 °C, air-conditioned rooms and cold food and drinks. Exposure in a cold room (4 °C) for 5 min was enough to induce symptoms. Family history was negative and no mutations were detected in the CIAS1 gene. Shortly after referral to us, the patient developed slowly progressive dysarthria and problems with swallowing, which both worsened by cold exposure. Working diagnosis of neurological symptoms was lateral sclerosis. The severe, debilitating, cold urticaria prompted a trial of anakinra in this patient. After one injection of anakinra, exposure in a cold room for 15 min was tolerated without development of urticaria. Continuous treatment of anakinra once daily led to complete resolution of urticarial rash and angioedema after cold exposure and impressive improvement of quality of life. Also, the patient reported a normalisation of swallowing and subjective improvement of speech. Idiopathic cold urticaria is an important differential diagnosis of the cryopyrin-associated periodic syndromes. This case demonstrates that this disorder also responds to inhibition of interleukin-1. In patients with severe, debilitating symptoms, treatment with anakinra may be warranted. The concurrence in this patient of neurological symptoms of bulbar origin with a partial response to anakinra is intriguing.

Experience of anakinra in a UK specialist fever clinic


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Background: Recombinant IL-1 receptor antagonist (anakinra) is extraordinarily efficacious in CAPS. We report our experience of it in CAPS and its wider use as a speculative treatment in a fever clinic. Methods: 38 patients have received anakinra:18 had CAPS; 10 had chronic ‘autoinflammation’ of unknown cause; 2 had TRAPS; 1 had HIDS; 1 had FMF; 2 had vasculitis; 1 had Schnizler’s; 1 had recurrent pericarditis and 2 had severe urate arthropathy. AA amyloidosis was present in 10 cases (4 CAPS, 1 TRAPS; 4 unknown cause, 1 urate arthropathy) of whom 5 were nephrotic, 1 had advanced renal impairment, 2 were on haemodialysis, and 2 had received renal transplants. 30 patients received 100mg s.c. injections daily, 6 patients with CAPS received 25-50 mg, and patients on haemodialysis received 100mg 3 times weekly post-dialysis. Inflammatory activity was assessed by symptom scores and monthly estimates of serum amyloid A protein (SAA) and C-reactive protein (CRP). Results: Anakinra was well tolerated. Injection site rashes occurred in 3 cases, 1 developed transient neutropaenia and in 1 patient treatment was suspended due to infection. Median age at starting treatment was 34 years (range 11.2 to 63) and median duration of treatment is 20 months (range 1 week to 59 months). Median CRP pre treatment was 30 mg/L and SAA was 90 mg/L. There was a rapid complete clinical and biochemical response (median SAA/CRP < 5mg/L) in 26 cases (all 18 CAPS patients, 4 with inflammation of unknown cause, the patients with FMF, TRAPS, pericarditis and one with urate arthropathy). In a further 8
patients there was a good partial response in symptoms along with > 75% fall in SAA and CRP. 1 patient with Takayasu’s arteritis had a modest response, one patient with urate arthropathy was non compliant, and 2 patients (with Schnizler’s and PAN) did not respond. There has been regression of amyloid in 8 of the patients with AA amyloidosis, the 2 other patients having only received treatment for 3 months. Conclusion: Anakinra was well tolerated in this diverse group of patients, and there was evidence of significant benefit in 90% of cases. Clinical response was usually evident within 24 hours. Smaller doses appear effective in CAPS and 3 times weekly treatment was well tolerated and efficacious in patients on dialysis. Response was poorest in the 2 patients who on subsequent investigations were categorized as having vasculitis.

(abstract 172)
Efficacy of Etanercept in the Treatment of a Patient with Behçet’s Disease
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Behçet’s Disease is a systemic inflammatory disorder characterised by recurrent attacks of uveitis, genitai and oral ulcers, skin lesions, arthritis, or other manifestations affecting the blood vessels, the gastrointestinal tract, the respiratory and central nervous systems. The treatment is chosen on the basis of the type and severity of manifestations. It includes colchicine, benzhatin-penicillin, paraocular and systemic corticosteroids, immunosuppressants, thalidomide, cytotoxic agents. Recently biological agents have emerged as possible therapeutic alternatives in patients resistant to conventional therapy. In particular TNF inhibitors are playing an important role in the Behçet therapy. Etanercept is a soluble dimeric receptor and has greater bond affinity with TNF than monometric receptors. We describe the case of a 33 year old patient affected by Behçet’s Disease, resistant to conventional disease modifying antiheumatic drugs (DMARDs) and with a long history of cortisone dependency, treated for one year with etanercept (25 mg twice a week). Before starting biological therapy, the patient had pseudofolliculitis on his back and four limbs, arthritis, myodesopsia and oral ulcers. Laboratory tests showed an increase in the phase acute reactants (ESR of 52 mm/h and CRP of 15 mg/dl). He was taking 50 mg/day of oral prednisone. After the first week of treatment, we observed a significant reduction in the cutaneous manifestations and arthritis, and a disappearance of the myodesopsia and the ulcers. After one month, the patient was asymptomatic and the acute phase reactants had normalised. Over the following months the steroid was gradually tapered to 5 mg/day. Currently, after one year of follow up, the patient is still taking entanercept (25 mg twice a week) and 5 mg/day of prednisone. He is asymptomatic with normal laboratory parameters and suffers from no side effects.

(abstract 177)
Periodic fever syndrome with aphthous stomatitis, pharyngitis and cervical adenopathy treated with ketotifen- a case report
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The pathogenesis of PFAPA syndrome is unknown. According to Stojanov et al. cytokine profile in PFAPA syndrome suggests T-helper 1 (Th1) mediated inflammatory process resulting in continuous inflammation and reduced T-helper 2 (Th2) anti-inflammatory response. Interferon γ
might be responsible for suppressing the production of IL-4 and IL-10 (1). Hung et al. showed suppressive effects of ketotifen on the expression of Th1 and Th2 related chemokines of human monocytes. Th1 related chemokines can be induced by interferon γ (2). We report a case of a four years old girl with PFAPA syndrome. Attacks of fever started in the first year of life and recur every 2-4 weeks. They were accompanied by enlarged and tender tonsils, elevated inflammatory parameters and sometimes with aphthous ulcers and abdominal pains. The genetic testing for Familial Mediterranean fever (FMF), Hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) and Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) was negative. No immunodeficiency or autoimmune disease was proven. She responded well to oral corticosteroid treatment but intervals between attacks became shorter. Given the recent finding of possible suppressive effect of ketotifen on Th1 and Th2-related chemokines of monocytes and favourable safety profile of this medication, the patient was prescribed ketotifen 1 mg twice per day for 8 months. During this period we observed significant prolongation of interval between fever attacks. She had only 3 attacks in 8 months, comparing to attacks every 4-6 weeks before therapy with ketotifen. During the period of treatment with ketotifen she was without febrile attacks for 3 months, the longest period ever. After cessation of therapy with ketotifen attacks recur with previous frequency. Conclusion: Our case report suggests possible beneficial effect of ketotifen on the frequency of fever attacks in patients with PFAPA syndrome. Ketotifen may be considered as an alternative medication in patients with PFAPA syndrome and recurrent fever attacks.


PAPA Syndrome

(abstract 69)

Successful treatment of refractory PAPA syndrome with infliximab
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Introduction PAPA syndrome (PS) is rare autosomal dominant autoinflammatory disorder characterized by pyogenic aseptic arthritis, pyoderma granulenosum, and cystic acne. This disorder is caused by mutations in the CD2 binding protein-1 (CD2BP1) gene on the long arm of chromosome 15. PAPA syndrome has no specific treatment, although corticosteroids are frequently used with a partial response. In this report, we describe the effect of infliximab in a patient with PAPA syndrome refractory to other drugs. Case report Our patient is a 50-year-old female diagnosed of PAPA syndrome. Her past medical history was significant for acne lesions since adolescence; pyoderma gangrenosum since 3 years ago (anatomopathological findings in the skin biopsy were compatible) with several flares affecting lower limbs; and more recently she has experienced persistent arthritis of the right ankle, right wrist and metacarpophalangeal joint of left hand. Her labs were all normal except for serum hypogammaglulinemia and thrombocytopenia; with no history of hemorrhagic episodes). We also performed the following studies: a bone marrow biopsy; antinuclear and antiphospholipid antibodies; liver and renal function tests; and a thoracic-abdominal CT scan; without any pathological finding. She had previous received several unsuccessfully therapies and included the following: corticosteroids; intravenous gammaglobulin; and methotrexate. Recent trials have shown a beneficial effect of infliximab in patients with pyoderma granulenosum associated with inflammatory bowel disease, with an overall response rate
of 80% to 90%. Based on these previous reports, we decided to administrate intravenous infusion of infliximab (3 mg/kg) on week 2, 6 and 12. After 2 weeks of infliximab, she achieved a complete remission of her symptoms (skin lesions and arthritis) and serum immunoglobulins and platelet count reached normal levels. Discussion PAPA skin and articular lesions are characterized by polymorphonuclear leukocyte invasion of joints and skin, producing a destructive arthritis and skin lesions. The pathogenesis of this disorder is unknown, although consistent with one of the proposed pathogenesis, excess IL-1 levels have been noted, and increased levels may contribute to TNF production, raising the possibility of use these two cytokines as therapeutic target. Infliximab, a chimeric human-murine immunoglobulin monoclonal antibody, binds specifically to tumor necrosis factor-alpha (TNF-alfa) and neutralizes the biological activity of TNF-alpha; by binding to the soluble and transmembrane forms of TNF-alpha. How blocking of TNF works to treat pyoderma grangrenosum is not known and it seems to be also effective in patients with PAPA. Recruitment of neutrophils into sites of inflammation requires two major events: attachment and migration. Tumor necrosis factor and IL-1 induce the expression of adhesion molecules on endothelial cells, which are required for the attachment of lymphocytes and of neutrophils. Induction of TNF alone is not sufficient for neutrophil recruitment, but play a role in the leukocyte migration and may be in the PAPA syndrome pathogenesis. We conclude that anti-TNF alpha therapy may have an important therapeutic role in control PAPA syndrome flares.

Periodic fever pharyngitis adenitis (PFAPA)

(abstract 195)

Low specificity of current diagnostic criteria for PFAPA syndrome. Time to change?


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Objective: PFAPA syndrome is the most common cause of idiopathic periodic fever in children. This entity of unknown aetiology presents clinical manifestations largely overlapping with rare diseases, caused by mutations of genes involved in the regulation of the inflammatory response, namely Familial Mediterranean Fever (FMF), tumor necrosis factor (TNF) receptor-associated periodic fever syndrome (TRAPS) and Mevalonate kinase deficiency (MKD). Aim of this study was to evaluate the specificity of the current diagnostic criteria for PFAPA and to identify, in patients satisfying PFAPA criteria, clinical parameters associated with an high probability of carrying mutations in one of the genes associated with hereditary autoinflammatory syndromes.

Patients & Methods: 307 consecutive patients with a clinical history of periodic fever were screened for mutations of MVK, TNFRSF1A and MEFV genes and detailed clinical information was collected. Univariate and multivariate analyses have been performed in patients fulfilling PFAPA criteria in order to identify those clinical manifestations able to distinguish genetically positive versus negative patients. Results: 133 out of 307 patients satisfied PFAPA criteria. 33 carried relevant mutations on the screened genes (27 MKD, 3 TRAPS, 3 FMF), 28 were heterozygous for MEFV mutations, 7 carried R92Q mutation of TNFRSF1A gene. Rash (OR=2.975, p=0.009),
abdominal pain (OR=3.261, p=0.005) and vomiting (OR=2.445, p=0.3) were the variables most correlated to the positivity at the genetic test. Conclusions: Current PFAPA diagnostic criteria display a low specificity. Children fulfilling PFAPA criteria and presenting gastrointestinal manifestations and/or skin rash should be screened for genes associated to monogenic periodic fevers. Consistent modifications of ongoing clinical criteria are proposed.

(abstract 60)

PFAPA (Periodic fever, aphtous stomatitis, pharyngitis and cervical adenitis) SYNDROME REGISTRY: ANALYSIS OF A COHORT OF 214 PATIENTS.


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PFAPA syndrome was first described in 1987. The diagnosis is based on criteria, including unspecific symptoms and the exclusion of other periodic fever syndromes. Increased knowledge of the clinical and laboratory features of PFAPA may be useful to precise the diagnostic criteria. With the purpose to investigate the clinical spectre, the clinical course and long-term follow-up of this entity, we established a web-based multicentric registry as an international collaboration within the working party “periodic fevers” of PReS. Patients, with PFAPA syndrome according to the previously published criteria, were included by filling-up a questionnaire with clinical data, laboratory values and treatment. From November 06 to November 07, we included 214 patients with PFAPA from 14 centres and 8 countries: (122 males and 92 females; median age at onset 1.9 year; median age at diagnosis 4.0 years). The most prevalent clinical manifestation of the inclusion criteria was pharyngitis (94%) followed by cervical adenitis (83%) and aphtous stomatitis (59%), and 48% of the patients presented all 3 clinical features. 170 patients presented additional symptoms (gastrointestinal symptoms 131, arthralgias and/or myalgias 86, arthritis 4, genital aphtosis 5, skin rash 36, neurological symptoms 8 and splenomegaly 5). In 79 patients (37%) a genetic testing was done for periodic fever syndromes (FMF 49, TRAPS 52, HIDS 46, CAPS 7) and was negative, except for 8 cases (polymorphisms: 3, carrier for MEFV mutation: 5) without known clinical significance. Improvement or remission was observed in 99/105 patients with steroids, in 28/35 patients with tonsillectomy and in 5/15 patients with cimetidine. We describe the largest cohort of PFAPA patients presented so far. We confirm that PFAPA syndrome may present with varied clinical manifestations and that the definition of the disease needs to be improved. Based on a detailed analysis of the data of this cohort, a new definition of PFAPA with better-defined criteria will be proposed and discussed in an international consensus conference. As follow-up to this retrospective study, we will follow prospectively a cohort of PFAPA patients to evaluate the long-term outcome.

(abstract 61)
PFAPA syndrome: is there a familial predominance?

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Background: PFAPA syndrome is a recurrent febrile disease first described in 1987 by Marshall and characterized by periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis. Since this first description no clear etiology has been found. In opposite to other auto-inflammatory diseases, no genetic origin was underlined and no familial tendency was reported until now. To better understand this disease, we created a European web-based multicentric registry with the participation of 8 countries and 14 rheumatologic centres. Aim: to investigate the eventual familial tendency to present PFAPA or an other chronic inflammatory disease. Patients and methods: in 2 of the centres participating to the registry (Lausanne-Geneva, Switzerland and Bordeaux, France), we questioned all patients or their parents during a phone call interview to complete the family history. We used the same questionnaire for a control group taken from a general pediatric consultation. In the questionnaire, we asked for positive family history of recurrent fevers, PFAPA, and rheumatologic diseases (chronic inflammatory). Results: Eigthy-four patients with PFAPA were recruited: 45 in Lausanne-Geneva and 39 in Bordeaux and were compared to 34 control children. Family history for recurrent fever was positive in 19/45 (42%, CI95: 28-56) and 18/39 (46%, CI95: 30-62) PFAPA patients from Lausanne-Geneva and Bordeaux respectively, and always negative in the control group. 6/45 (13%, CI95: 3-23) and 3/39 (8%, CI95: 0-17) PFAPA patients had a family member with PFAPA, but none in the control group. The family history for rheumatologic diseases (chronic inflammatory) was more frequently positive in the Swiss PFAPA group (14/45), than the French PFAPA group (5/39) and the controls (2/34). Conclusion: These data show that history of recurrent fever and PFAPA is found more often in patients with PFAPA than in the general pediatric population. They suggest a familial susceptibility and a potential genetic origin for the PFAPA syndrome. This opens a wider spectrum for future research.

(abstract 105)

Unraveling the Pathogenesis of Periodic Fever, Aphthous Stomatitis, Pharyngitis and Cervical Adenitis (PFAPA) Syndrome: An Immunological Approach


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OBJECTIVE: Based on previous reports of leukocytosis during febrile episodes in patients with the syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA), we aimed to investigate if clinical features of PFAPA reflect an aberration of subpopulations of leukocytes. METHODS: Children with a potential diagnosis of PFAPA were screened for inclusion criteria of periodic fever and at least one of the associated clinical features. Blood samples were collected both during a “flare” (within 48 hours of onset of fever) and during an asymptomatic interval (“non-flare”) only in the absence of any recent immunomodulating medications. Paired analysis was performed of patients’ complete blood cell counts, inflammatory markers, and leukocyte immunophenotyping data. RESULTS: Of 43 patients with clinical PFAPA, genetic testing revealed variants in 8 children (19%) in the MVK (2), MEFV (3), TNFRSF1A (2), or ELA2 (1) genes. Paired samples (flare and non-flare) were collected from 15 of the remaining 35 patients with PFAPA. These 15 patients of whom we obtained paired samples had a median age of disease
onset of 16 mo (range 3-90 mo), a median duration of attacks of 4.5 d (2.5-11 d), and a median frequency of episodes of every 28 d (10-49 d). Febrile attacks of the 15 patients were associated with 100% cervical lymphadenopathy, 87% sore throat, 80% inflamed tonsils, 67% oral ulcers, 100% loss of appetite, 67% myalgias, and 67% abdominal pain. Ten of 15 patients (67%), who were treated with steroids during acute episodes, experienced aborted fevers. Following steroids, 6 of these 10 children (60%) experienced subsequently shortened intervals between episodes. Laboratory analyses revealed a relative leukocytosis with neutrophilia and monocytosis, as well as an elevated CRP during flares in comparison to non-flare intervals, representing statistically significant differences. A relative decrease of absolute numbers and percentages of T lymphocytes affected both CD4+ and CD8+ T cells. CONCLUSIONS: In a clinically well-defined cohort of PFAPA patients in whom hereditary periodic fever syndromes have been excluded genetically, attacks of fever manifest as a distinct cellular and clinical entity. The relative predominance not only of neutrophils but also of monocytes in relation to lymphocytes during attacks may potentially implicate an immune dysregulation in the pathogenesis of this disease.

(abstract 126)

Characteristics of a PFAPA cohort in a single European centre.

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Background: PFAPA syndrome is characterized by periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis. Children are healthy between fever attacks. First manifestation is usually before 5 years of age. The ethiopathogenesis is unknown. There are few diagnostic criteria which help to make the clinical diagnosis. Laboratory parameters are non - specific. The diagnosis is still made per exclusionem. We present an overview of the PFAPA cohort in our centre. Methods: Retrospective analysis of the case notes. Results: Over the years 2004 – 2007 we diagnosed 32 children (17 boys/15 girls) with PFAPA out of 87 patients referred to the fever clinic (36.8%). Median age at onset was 4 months (4–56), median interval between attacks was 4 weeks (2–12) and fever duration 3.5 days (1.5–7). Fever was generally above 39°C. It was associated with pharyngitis in 23 cases (71.90%), exsudative in 15 (46.88%), cervical adenitis in 24 (75%) and aphthous stomatitis in 11 (34.40%). Other symptoms included vomiting (4 cases; 12.50%), abdominal pain (7 cases; 21.90%), diarrhea (2 cases; 6.25%), arthralgia (7 cases; 21.90%), arthritis (2 cases; 6.25%), headache (4 cases; 12.5%), conjunctivitis (2 cases; 6.25%), rash (1 case; 3.13%). All children had elevated inflammatory parameters during an attack (median values: CRP 64.5, ESR 32/h) with subsequent normalization. The single Prednisone dose of around 1 mg/kg administered at the onset of an episode helped to reduce symptoms in 16/18 children. Such a repeated administration was never required for longer than 5 months. In 2 cases tonsillectomy led to the resolution of symptoms. After the median follow-up of 8.7 months (3.1–35.5) 4/32 patients (12.5%) have been in the full remission. Conclusion: PFAPA syndrome appears to be a relatively common cause of recurrent fever in early childhood. We have learnt to better differentiate it from hereditary fevers and other conditions by a battery of investigations and thorough history. Since genetic testing is not available in our country we request it only in limited, highly suspicious cases. We believe that early diagnosis and appropriate management can prevent unnecessary investigations and repeated antibiotic therapy as well as help to alleviate associated family stress. The rational diagnostic algorithm is currently being tested in our centre and will be incorporated in the local clinical recommendations until scientific evidence raises from European collaborative effort.
Recurrent Pericarditis

(abstract 4)

Pericarditis and familial Mediterranean fever
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Familial Mediterranean fever (FMF) is an inherited autoinflammatory hereditary disease characterised by the recurrent short limited serositical febrile attacks and by high incidence of amyloidosis in non adequately treated cases. The disease has high prevalence in Armenia (2%). The pericardium being also a serosal membrane is very rare the target of inflammation during attacks of FMF, however by mens of paraclinical methods one often can find acute inflammation of pericardium during FMF attacks, and flabby current affection of pericardium out of attacks (Lebacq E. et al., 1964, Adoue D. et al., 1984, Tauber T., Zimand S., Kotzer E., 1995). This affection one can reveal by echocardiography as thickening of pericardium (Dabestani A. et al., 1982), or as pericardial effusion, and also by ECG changes (Ayvazyan A.A., 1982, Nucera G. et al., 2002, Tutar E. et al., 2002). In thoracic course of FMF the development of pericarditis was proved in 1/3 of the patients (Vinogradova O.M. et al., 1989). We also have established that pericardium in FMF patients is thicker in patient with abdominal form of disease (2.6 +/- 0.11 mm vs 1.0 +/- 0.046 mm, together with H.Sisakyan et al.). In one case we observed constrictive pericarditis and pleurisy without amyloidosis. In one case we observed exudative recurrent pericarditis and pleurisy during attacks of FMF. In two cases exudative were only pericarditis. In another cases we observed only sick recurrent pericarditis during FMF attacks (8 cases). In one case we revealed recurrent pericarditis many years before the manifestation of main attacks of FMF. In one similar case we have established the diagnosis of FMF only by means of mutational analysis of MEFV gene (together with T.Sargsyan et al.). Two cases of manifestation of FMF with recurrent pericarditis some months before serositical attacks of FMF have reported also by Tauber T. (1995). Zimand S. and coauthors reported also one case, presenting initially with pericarditis and life threatening pericardial tamponade, developed clinical episodes characteristic of FMF few months later (1994). In one case with features of pericarditis during FMF attacks we revealed the elevation of the marker of cardiac lesion – troponine I. The all above noted cases must to warn physicians. In Armenia in all cases of pericarditis is prudent to investigate mutations of MEFV gene. In some genetically not proved cases we can also use diagnosis ex juvantibus (6 months regular treatment with colchicine followed 6 months control period, Livneh A., 2000).

(abstract 98)

Recurrent pericarditis in hyper IgD syndrome.


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Hyper-IgD and periodic fever syndrome (HIDS) is an autosomal recessive autoinflammatory syndrome caused by mutations in the mevalonate kinase gene (MVK). It is characterized by self limited recurrent attacks of fever, lymphadenopathy, abdominal pain, arthralgia and skin rash. Attacks begin in infancy and last approximately 3-7 days. Treatment of HIDS patients is mainly supportive and very difficult. Favorable experiences with etanercept have been reported. We observed a 12-year-old boy affected by HIDS who was successfully treated with etanercept but developed a recurrent pericarditis. The boy, born of Italian healthy parents, was diagnosed as having HIDS at the age of ten years by demonstration of an homozygous missense mutation on
exone 11 of MVK gene. He was also screened for MEFV and TNFRSF1A gene mutations resulting negative. A therapy with etanercept at the dosage of 0.4 mg/kg twice weekly was started. Attacks disappeared and no side effects were registered. Two years later, the patient presented fever, precordialgia and orthopnea. Pleural and pericardial effusions were demonstrated. He was started on prednisone 2 mg/kg/day with good clinical and laboratory response. Etanercept was interrupted. Four months later, a relapse occurred. Colchicine 1 mg/day and ibuprofen were associated to prednisone. Later, after prednisone tapering, the patient was symptom free. At present, one year after etanercept interruption, the boy presented new attacks of fever and polyarthralgia. No pericardial effusion was present. Recurrent pericarditis is a well-know but rare disorder in childhood. The etiology of the initial pericarditis often remains unclear. To our knowledge this is the first case of pericarditis associated with HIDS syndrome in a pediatric patient. In this patient, etanercept was successful in aborting the attacks of HIDS. It is not clear if a possible link between TNF blockade and the pathogenesis of pericarditis exists, although the relapse occurring after etanercept suspension reduces this possibility. Further investigation of this therapy is necessary.

(abstract 130)

**EFFICACY OF COLCHICINE THERAPY IN PREVENTING RECURRENT PERICARDITIS IN SJÖGREN SYNDROME**

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Background: Pericarditis due to primary Sjögren syndrome (pSjs) has been rarely reported and is usually asymptomatic. The relapse of pericarditis is accepted as an autoimmune process. Colchicine as first-choice therapy in order to prevent recurrent pericarditis in Sjögren syndrome decreases the recurrence rate. Objective: The aim of the study was to evaluate the efficacy of colchicine therapy in order to prevent recurrent pericarditis in patients with pSjs. Methods: We report our experience with a series of 10 patients with pSjs (fulfilled the Criteria of the American-European Consensus–2001),7 females and 3 males, mean age 50±15.8 years, whom presented with mild pericardial effusions at 5.8±3.2 months from first attack. Recurrent pericarditis was documented by chest pain and minimum one of the following signs: pericardial friction rub, fever, echocardiographic evidence of pericardial effusion, specific electrocardiographic changes. All patients received colchicine – 1mg in the first day, then a maintenance dose of 0.5mg daily for 12 months. Clinical, echo and electrocardiographic follow-up were performed at 72 hours, 10 days, 1, 3, 6 and 12 months. Remission was considered when patients were asymptomatic, with no echo and electrocardiographic specific signs. Results: During 120 patient-months of follow-up 2 recurrences were recorded – with a symptom-free period of 6 and 10 months respectively; no cardiac tamponade, constrictive pericarditis and no severe adverse events were documented. Increasing the dosage for colchicine to 1 mg/day associated with glucocorticoids efficiently controlled these 2 cases. Conclusion: Colchicine is safe and efficient as first choice treatment in preventing the recurrent pericarditis in patients with primary Sjögren syndrome. In refractary cases dose increasement and glucocorticoid association determine long-term remissions.

**Schnitzler Syndrome**

(abstract 99)

Interleukin 1 receptor antagonist (Anakinra) - induced changes in immune parameters in a
Patient with Schnitzler Syndrome

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The patient of Caucasian origin was referred to our clinic in 2006 complaining of recurrent high fevers with shivering fits, hives all over his body and arthralgias. The symptoms had relatively clear onset in 2002 when he was 37 years old. The association of clinical presentation with laboratory findings of significantly increased markers of inflammation during attacks, high levels of IgM, and IgM kappa monoclonal gammapathy led to the diagnosis of Schnitzler syndrome. Despite normal levels of IL-1 secreted by stimulated patient’s PBMC, treatment with IL-1R blocking agent (Anakinra) was introduced. The treatment led to prompt and marked clinical response. We performed detailed investigation of immune parameters, before and after the initial treatment period (7 days). Due to still unexplained pathogenesis of IgM gammapathy, the B cells were particularly examined and classified into their distinct populations. The fastest and most prominent response was the decrease of leukocyte count and normalisation of initial neutrophilia. The total number of B cells only slightly increased in both relative (from 13 to 18%) and absolute values (0.42 to 0.51x10^9/l) during the initial treatment period. Within the B cell population IgM+IgD+CD27+ (IgM memory, marginal zone B lymphocytes) cells markedly increased (from 4.6 to 43%), while IgD-IgM- (switched memory) B cells reciprocally decreased. Serum IgM levels slightly increased and stayed stable. Monoclonal gammapathy was persistent despite the treatment. Serum IgD levels were always low. There were no changes in T lymphocytes populations, autoantibody screening was repeatedly negative. We conclude that Anakinra may elicit an excellent clinical response in a patient with Schnitzler syndrome, however, its overall mode of action is not completely clear. As the pathogenesis of Schnitzler syndrome is unknown we suspect that the response seen in the B cell population may play a role in this process. Further investigation into effects of Anakinra is needed.

Systemic-Onset Juvenile Idiopathic Arthritis

(abstract 169)

The pattern of response to anti IL-1 treatment distinguish two subset of patients with systemic onset juvenile idiopathic arthritis (SoJIA).


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Aim: to assess the clinical response to IL-1 blockade in patients with SoJIA treated with the IL-1 receptor antagonist (Anakinra). Patients and Methods: twenty-two SoJIA patients (11 F, 11 M) were selected for the treatment with IL-1 receptor antagonist (Anakinra). All patients required prolonged cortico-steroid therapy, mostly in association with one or more second line agents. Response to treatment was evaluated according to the behaviour of a number of clinical (fever, rash, number of active joints) and laboratory (ERS, CRP, WBC, Hb) parameters during the follow-up. Complete response was defined as the absence of systemic and joint manifestations and complete normalization of acute phase reactants at follow up. Other patients were considered as incomplete or non responders. Results: our study shows that SoJIA can be divided into two subsets according to the type of response to Anakinra. A subset (complete responders), accounting for the 40 % of patients, had a dramatic and persistent response to IL-1 blockade that allowed the rapid discontinuation of any other treatment. In the other group of patients (incomplete or non responders), the treatment, although effective on systemic manifestations, was unable to control
arthritis and inflammation and was either withdrawn or continued in association with second line agents or steroids. At baseline, complete responders displayed different pattern of joint involvement with a significant lower number of active joints (median 3.5, range 1-10) in respect to incomplete and non responders (median 7, range 3-55, p=0.02). Conversely, no significant differences were observed in the systemic features (i.e. presence of fever, rash, hepatosplenomegalgy, serositis). Among laboratory parameters at baseline, no differences were observed for acute phase reactants (CRP, ESR, fibrinogen, ferritin) or haemoglobin levels between the two groups. Conversely, complete responders had a significant higher number of circulating neutrophils (median 19.3x103/mm3, range 6.1-30.9) as compared with incomplete and non-responders (median 9.1 x103/mm3, range 7.3-19.7) (p=0.02) Conclusions: we have shown that two subsets of SoJIA patients with distinct clinical features can be identified according to their response to IL-1 blockade. Our study provides for the first time a parameter to unravel the heterogeneity of SoJIA.

(abstract 145)

MEFV Mutations In Systemic Juvenile Idiopathic Arthritis

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Systemic form of juvenile idiopathic arthritis (JIA) is regarded as an autoinflammatory disease. Certain genetic polymorphisms in genes coding inflammatory proteins have been associated with the disease. On the other hand mutations of the MEFV gene cause a monogenic autoinflammatory disease, Familial Mediterranean Fever (FMF). In a previous study in adult rheumatoid arthritis 3 out of the 25 British patients who developed secondary amyloidosis had a mutation/polymorphism in the MEFV gene. The aim of this study was to analyse whether mutations in the MEFV gene had an association with systemic JIA. MEFV mutations were screened in a total of 32 systemic JIA patients. All had been classified as systemic JIA according to the Durban JIA criteria. None had disease characteristics that met the Tel Hashomer criteria for the diagnosis of FMF. The results showed : 2 carrier for M694V and two patients who were homozygote for MEFV mutations. Both of these patients were among the most severe patients in the group. One had an excellent response to etanercept whereas the other was resistant to anti-TNF and other conventional treatments and had only a partial response to thalidomide. Although the number of severe mutations were increased in this small group of patients with systemic JIA the difference with the Turkish population did not reach statistical significance, but the disease causing mutation (M694V) was significantly high in the patients with systemic JIA(p=0.02). However, the severe disease course in the aforementioned patients suggest that MEFV mutations may be a modifying genetic factor in systemic JIA.

(abstract 166)

High membrane expression of CD163 by bone marrow cells is not a specific marker of Macrophage Activation Syndrome (MAS)
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Background. Macrophage activation syndrome (MAS) is a life-threatening complication seen
predominantly in children with Systemic onset Juvenile Idiopathic Arthritis (SoJIA), often difficult to recognize because specific diagnostic criteria for MAS have not yet been devised. MAS is characterized by an overwhelming inflammatory reaction driven by excessive expansion of T cells and hemophagocytic macrophages. Preliminary studies suggest that high serum levels of sIL-2R α and soluble CD163 (sCD163) might be useful as diagnostic markers for MAS. Purpose. To assess the expression of CD163, hemoglobin-haptoglobin scavenger receptor, and of CD68 in bone marrow cells of patients with MAS and especially in those with SoJIA who developed MAS.

Methods. Thirteen bone marrow biopsies (BMB), seven from patients with MAS secondary to SoJIA and six with virus-induced Hemophagocytic Lymphohistiocytosis (HLH) and fifteen control BMB (from patients under solid tumor staging) were included in the study. Immunohistochemical staining was performed using monoclonal antibodies directed against CD163 (clone 10D6, dilution 1:100; Novocastra, Newcastle upon Tyne, England) and CD68 (clone PG-M1,dilution 1:200; DAKO, Carpinteria, CA).

Results. CD163 immunoreactivity was characterized by a strong, granular cytoplasmic or cytoplasmic and membrane staining pattern; the macrophage marker CD68 showed a granular cytoplasmic pattern. Increased number of macrophages were observed in the bone marrow of all MAS and HLH samples. Expression of CD163 in macrophages was brighter than that of CD68. The expression of CD163 was otherwise similar in MAS associated with SoJIA and in virus induced HLH. Conclusions. Our data demonstrate that CD163 in BMB is restricted to the monocyte/macrophage lineage but is not specific for MAS. Furthermore CD163 is similarly expressed by macrophages from biopsies of both SoJIA and HLH patients. This antibody is in fact a marker of activated macrophages but cannot differentiate patients with MAS from patients with other diseases of the macrophage lineage.

(abstract 7)

**Bcl I glucocorticoide receptor gene polymorphism and bone mineralization in systemic onset of juvenile idiopathic arthritis.**

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Systemic onset of juvenile idiopathic arthritis (soJIA) is a very severe form of juvenile arthritis, treated with high dose of glucocorticoids. Our previous studies received depending arthritis course and inflammatory activity on Bcl I glucocorticoide receptor genotypes and alleles polymorphism. Girls, suffered from different form of JIA, who are carriers G allele have more severe arthritis course and inflammatory activity degree, osteopenia and low bone metabolism, and prognosis. The aim of our study was whether Bcl I glucocorticoide receptor gene polymorphism associated with Systemic onset of juvenile idiopathic arthritis and bone mineralization in these patients. In our study we include 122 JIA children, from 1,5 to 17 years old, 43 boys (35,2%) and 79 girls (64,8%). The mean age was 11,3±4,36 years. Systemic onset of juvenile idiopathic arthritis was diagnosed in 10 children (8,2%), 8 girls (80,0%) and 2 (20,0%) boys. One girl had macrophage activation syndrome in JIA onset and next episode in 1 year after onset. All children were treated with high doses of glucocorticoids. Compared group was represented by other 112 nonsystemic onset JIA children, 71 girls (63,4%) and 41 boys (36,6%). Bcl I glucocorticoide receptor gene polymorphism detected by polymerase chain reaction with restriction assay. Uppercase letters represent absence, and lowercase letters represent presence, of gene restriction site. Bone mineral density (BMD), measured in SD (T score) was detected by dual-energy X-ray absorptiometry (Hologic QDR-4500C) in lumbar spine (L1-L4) with national referent database (L. A. Schepleyagina, 2005). Osteopenia was defined than BMD (Z score) was lower as at least 1 SD. Serum levels of intact osteocalcine, β-Cross Labs, parathyroid hormone, Ca, Ca++, phosphate, common alkaline phosphatase were tested in all patients. For statistical analysis of the allelic
frequency distribution in this polymorphism the two groups were compared using chi-squared test and Fisher’s exact test. Also t-test we used. We hadn’t revealed significant differences in genotypes and alleles distribution between children with soJIA and others children with JIA course: CC – 30,0% and 42,0%, GC – 40,05 and 45,5%, GG – 40,0% and 12,5%, relatively, but GG genotype frequency was higher in soJIA then other JIA children. The girl, suffered from soJIA with macrophage activation syndrome has GC genotype. Osteopenia has detected in 25,0% in soJIA group and in 21,7% in compared group (p=0,42), but we have revealed significant differences in BMD: Z score = -0,624±0,72 SD in first and Z score = -0,138±1,02 SD in second group (p=0,05). There were no significant differences between soJIA and nonsystemic onset JIA group in calcium-phosphate metabolism: Ca = 2,31±0,19 and 2,38±0,14 mmol/l (p=0,14), Ca++ = 1,07±0,12 and 1,06±0,11 mmol/l (p=0,44), P = 1,58±0,14 and 1,58±0,2 mmol/l (p=0,43), total alkaline phosphatase activity 320,1±107,9 and 350,8±123,3 U/l (p=0,48), but we detected differences in levels of bone metabolic markers, such as octeocalcine = 70,97±27,97 ng/ml and 106,95±54,26 (p=0,01), β-Cross Labs = 0,86±0,27 and 1,18±0,45 ng/ml (p=0,017), parathyroid hormone = 1,41±0,67 and 2,26±1,17 pcmol/l (p=0,01), relatively. Conclusion: children with systemic onset of JIA had more frequently GG genotype, significantly lower bone mineral density and significantly lower levels of bone metabolic markers, such as octeocalcine, β-Cross Labs and parathyroid hormone. High inflammatory activity, low physical activity, high doses of glucocorticosteroids and high GG genotype frequency may be reason of low bone metabolism activity.

MACROPHAGE ACTIVATION SYDROME WITH SYSTEMIC ONSET JUVENILE IDIOPATHIC ARTHRITIS IN CHINESE CHILDREN
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MACROPHAGE ACTIVATION SYDROME WITH SYSTEMIC ONSET JUVENILE IDIOPATHIC ARTHRITIS IN CHINESE CHILDREN Background: Macrophage activation syndrome (MAS) is a life-threatening complication of systemic onset juvenile idiopathic arthritis (SOJIA), which is characterized by fever, hepatosplenomegaly, lymphoadenopathy, pancytopenia, liver disfunction, pulmonary damage and CNS dysfunction. The diagnostic hallmark of the syndrome is found in the bone marrow aspiration, which reveals widespread signs of macrophage hemophagocytosis. The clinical and pathologic manifestations of MAS are thought to result from the activation and proliferation of T-lymphocytes and well-differentiated macrophages, which leads to a release of inflammatory cytokines. Objectives: To analyze the clinical features, treatment, and outcome of MAS with SOJIA. Design, setting and method: Retrospective review of cases of MAS from a prospectively collected database of children with SOJIA in Beijing Children’s Hospital from the year of 2003 to 2007. Results: Forty two patients (34 boys, 8 girls) were diagnosed MAS with SOJIA from 159 SOJIA cases. Mean age was 7 years, the duration prior to MAS, 11 months. High fever, hepatosplenomegaly, pancytopenia, liver dysfunction were in all cases (100%). Bleeding on skin, mucous membrane and gastrointestinal tract were in 12 (28.6%). Nineteen (45%) has had CNS dysfunction. Ten (24%) were with ARDS. Two patients suffered from renal damage. The lab. tests revealed elevated live enzymes and ferritin, decreased value of ESR, album, CBC and fibrinogen in all. Bone marrow examination supported the diagnosis with definite haemophagocytosis in 42 cases. Lymph node biopsy was done for one case and found out it was filled of activated macrophage. In the treatment, thirteen only received high dose steroids (four of thirteen died), twenty-one got high dose steroids plus cyclosporine (four died), five were steroids plus cyclosporine and etoposide (no died). The causes of death were ARDS and CNS involvement. Three of them gave up treating. Conclusions: MAS is a rare and potentially fatal complication of...
SOJIA. Most of our patients were male. Bone marrow studies support the diagnosis. CNS involvement and ARDS are poor prognostic signs. Early diagnosis and aggressive therapy is essential.

The inflammasome

(abstract 103)

Targeted Genes Sequencing and High-throughput Parallel Strategies for Mutation Screening in Patients with Autoinflammatory Diseases

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We have collected DNA samples from ~1,800 patients with periodic fevers (PF). Based on the clinical diagnosis, these patients were screened for mutations in one or more known PF genes and only ~30% were found to be mutation positive. Subsets of patients with a similar phenotype were screened for mutations in candidate genes that were selected based on their function in autoinflammatory pathways. Patients suspected to have cryopyrin-associated disease were screened for mutations in NALP1, 2, 4, 6, 10, and 12, CARD8, ICEBERG, IPAF, IL1-RN, PSEUDO-ICE, PYCARD, and EBBP. 3 patients with TRAPS-like symptoms were screened for mutations in 10 exons of TNFRSF1B, and in 20 exons of ARTS1. Patients diagnosed with PAPA or CRMO were screened for mutations in 15 exons of PSTPIP1, in 13 exons of PSTPIP2, and in 19 exons of PTPN12. This extensive targeted gene analysis yielded no disease-associated variants, and because of this lack of success, we have developed a custom 300kb resequencing chip with Affymetrix. Our INFLAMMOCHIP interrogates 91 genes that were chosen because of their role in inflammation and immunity. Among them are all the known PF genes and many inflammasome-related genes, TLRs, cytokines, caspases, and members of the TNF signaling pathway. After the genes were selected, oligonucleotides representing the coding sequences and including 5’ and 3’ regulatory regions were incorporated on Affymetrix GeneChip® CustomSeq® Resequencing Arrays. We designed PCR primers for a nearly 500 amplicons ranging in size from 200bp to 5,000bp. These primers were tested for amplification using various DNA polymerases, and a success rate of ~ 99% was eventually achieved. The PCR amplification requires ~30 ug of high quality DNA from a patient. We amplified and hybridized a preliminary set of 16 patient samples and are beginning the process of data analysis. The Affymetrix 300kb resequencing chip has not yet been tested for mutational screening in humans, and the system was originally designed to sequence the genomes of haploid prokaryotes. Given the complexity of human genomic sequence, we are charged with optimizing hybrization and the base-calling algorithm to increase the detection of heterozygous variants. We are now on the verge of evaluating the effectiveness of this tool in our search for new disease-associated variants, and this unique high-throughput method appears to be a viable option for mutation discovery given the lack of success with targeted sequencing.

(abstract 174)

A variant truncation form of ASC has a reduced pro-inflammatory profile.

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Unlike many genes of the human immune system, the apoptosis-associated speck-like protein with a CARD domain (ASC) has no recorded genetic polymorphisms. During a screen to identify genes that may confer di-generic inheritance of familial Mediterranean fever (Booty et al, Poster#) we discovered the first known genetic change in human ASC, and this results in a coding mutation that prematurely truncates the protein. The base change (G513A) results in a stop codon in place of amino acid 172 and this deletes two out of the six alpha helices that comprise the C-terminal CARD domain of ASC. We performed in vitro analysis of this variant form of ASC to determine its possible contribution to autoinflammatory disease. The mutated protein was overexpressed in mammalian cell lines, but only achieved very low expression levels compared to the wild type protein. It was heavily oligomerized, and remained so under denaturing and reducing conditions, but it retained its known ability to bind Caspase-1, an interaction that is mediated by the CARD domain of both proteins. When overexpressed together with Caspase-1 and ProIL-1β, ASC usually has a biphasic mode of action, at lower doses promoting the cleavage and secretion of mature IL-1β, and at high doses inhibiting this process. We found that the prematurely truncated form of this protein is no longer able to function in this capacity. Finally ASC is thought to function in the reduction of NF-kB signaling by virtue of an interaction with RIP2, and we have preliminary data to suggest that the mutant form of ASC has a dominant negative effect in this regard. Therefore our analysis does not suggest that this mutation of ASC would promote autoinflammatory disease, and in fact people who are homozygous for this change may be profoundly immunodeficient. To this end we are now sequencing patients with immunodeficiencies of unknown origin to see if they could be caused by mutations in ASC.

(abstract 200)

Mutations in the NALP3 inflammasome are associated with delayed apoptosis


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We have recently described a patient with a long history of inflammatory disease resulting from excessive IL-1β production, who was found to be a heterozygous carrier of two common polymorphisms in the genes encoding NLRP3 (NLRP3) and TUCAN (CARD-8); both of which are components of the NALP3 inflammasome. This compound polymorphism was found in 4% of the general Swedish population, and the prevalence suggests a genetic predisposition for common chronic inflammatory diseases. In the present study, the spontaneous and microbe-induced apoptosis was investigated in two patients carrying the NLRP3 (Q705K) and CARD-8 (C10X) polymorphisms. The apoptosis in neutrophils from both patients and matched healthy donors was investigated by Annexin V staining, and the mitochondrial damage was measured by tetramethylrhodamine ethyl ester (TMRE). The degree of spontaneous apoptosis in neutrophils was determined after 6 and 19 hours of incubation, whereas the microbe-induced apoptosis was investigated 6 hours after incubation with the bacteria (Salmonella or Staphylococcus aureus). The protein levels of Mcl-1 and the phosphorylation status of Akt and ERK were investigated by Annexin V staining, and the mitochondrial damage was measured by tetramethylrhodamine ethyl ester (TMRE). The degree of spontaneous apoptosis in neutrophils was determined after 6 and 19 hours of incubation, whereas the microbe-induced apoptosis was investigated 6 hours after incubation with the bacteria (Salmonella or Staphylococcus aureus). The protein levels of Mcl-1 and the phosphorylation status of Akt and ERK were investigated using Western blotting. Both patients had significantly delayed spontaneous as well as microbe-induced apoptosis compared to controls. The patients showed both increased phosphorylation and increased levels of Akt and ERK. Moreover the levels of the anti-apoptotic protein Mcl-1 were increased in the patients compared to the control subjects. Blocking the activation of Akt, accelerated the spontaneous apoptosis. We find that the combined NLRP3 and CARD-8 polymorphisms are
associated with delayed spontaneous as well as microbe-induced apoptosis. This can be explained by the finding that the patients show increased levels/phosphorylation of Akt, ERK and Mcl-1, resulting in an anti-apoptotic profile. In addition, increased IL-1beta levels have been shown to delay spontaneous apoptosis in neutrophils, raising the question if the mutations per se or their impact on the cytokine production is responsible for the delayed apoptosis. S. aureus assembles the NALP3 inflammasome, whereas Salmonella activate the Ipaf inflammasome. Therefore, increased knowledge regarding the interplay between different inflammasomes, as well as between inflammasomes and apoptosomes, is required to understand the regulatory mechanisms behind the apoptotic process during immunological homeostasis and inflammatory condition.

TNF-associated periodic syndrome

(abstract 182)

Abnormal TNFR1 cell surface expression and NF-KappaB activation in TNFR1-associated periodic fever syndrome (TRAPS)


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Objective: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory condition caused by mutations in the TNFRSF1A gene. The cellular mechanisms by which mutations in this gene trigger inflammation are currently unclear. As NF-kappaB is the major intracellular signaling component inducing secretion of pro-inflammatory cytokines, we sought to determine whether differences in the clinical phenotype of patients with TRAPS may be due to variable effects of TNFRSF1A mutations on TNFR1 expression, localization, or NF-κB activity. Methods: Peripheral blood mononuclear cells were obtained from patients with informed consent, and cellular nuclear and cytosolic fractions generated by subcellular fractionation. IkappaB alpha and NF-kappaB localization was determined by Western blotting of the resultant fractions. NF-kappaB subunit activity was determined by ELISA analysis and confirmed by EMSA. Subcellular localization of TNFR1 was determined by immunofluorescence confocal microscopy, or by immunoblotting following affinity-isolation of plasma membrane by subcellular fractionation. Results: Cells from patients with the fully penetrant C73R mutation have marked activation of the pro-inflammatory p65 subunit of NF-kappaB. By contrast, cells from patients with the low penetrant R92Q mutation displayed high levels of DNA-binding by the p50 subunit, an interaction previously linked to repression of inflammation. Interestingly, while C73R cells have no TNFR1 shedding defect there was nonetheless an unusually high concentration of functional TNFR1 at the plasma membrane. Conclusion: This study shows for the first time that the R92Q mutation associated with low penetrance TRAPS likely results from p50-p50 NF-kappaB homodimer repression. The P46L mutation however does not alter p50 signalling, but instead
renders TNFR1 non-functional. By contrast, the fully penetrant C73R mutation results in persistent elevated localization of functional TNFR1 at the cell surface, and is associated with increased TNF induction of the pro-inflammatory intracellular NF-kappaB pathway. Thus variation in NF-κB activity in PBMCs of patients with different TNFR1 genotypes provides an explanation for the observed variation in clinical phenotype.

(abstract 155)

Identification and analysis of gene-expression signatures in peripheral blood leukocytes of patients with TRAPS.

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Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is a rare autoinflammatory syndrome characterized by self-limiting episodes of recurrent fevers, abdominal pain and systemic inflammation. We undertook microarray analysis in an effort to characterize the molecular mechanisms involved in regulating the inflammatory response in these patients. Peripheral blood mononuclear cells were obtained from 29 patients with structural (high penetrance) TRAPS-associated mutations and 34 age and sex matched controls. cRNA was hybridized to Affymetrix U133A 2.0 microarrays and data processed using MAS5 algorithm. P-values were corrected for multiple testing using the false discovery rate (FDR). Genes were validated for the degree of expression by quantitative real-time PCR. Ingenuity Pathway Analysis (IPA) software was used to assess the relevance of the differentially expressed genes (DEG) to known biological pathways. Of the 29 TRAPS patients included, seven had clinical symptoms and biochemical evidence consistent with active disease. A further 7 patients had an elevated C-reactive protein or erythrocyte sedimentation rate consistent with subclinical inflammation. Sixteen patients were treated with etanercept, of these 2 patients were maintained on transplant immunosuppression and 1 required anakinra therapy. At an FDR of 10% and fold change (FC) of ≥1.4, 255 transcripts encoding 187 unique genes were identified. The most significantly DEGs were hemoglobin delta (FC 3.4) and SNF1-like kinase (-1.78). IPA analysis generated multiple networks of genes, with the most highly ranked centered on nuclear factor kappa B. Analysis of symptomatic patients identified a larger number of DEGs with high fold change values including 15 with a FC >5, enrichment of erythroid (delta-aminolevulinate synthase (FC 50)) and antigen presenting genes were notable. DEGs in common with symptomatic cryopyrinopathy (CAPS) patients included alpha-synuclein (5.3), selenium binding protein 1 (9) and carbonic anhydrase I (11.1). Validation of genes of interest using real-time PCR techniques is ongoing. This study identified a number of DEGs in patients with TRAPS, with particularly marked changes noted in symptomatic patients. Similarities with the DEGs identified in CAPS patients and a relative lack of DEGs downstream of TNF may support the ‘ligand independent’ theory of disease pathogenesis, and implicates a role for IL-1 dependent genes in TRAPS.

(abstract 149)

Consequences of the TRAPS-associated R92Q TNFRSF1A mutation.

1) Autoinflammation & Clinical Immunology Research Group (ACIRG)
Tumour necrosis factor receptor associated periodic syndrome (TRAPS) is a hereditary autoinflammatory disease that involves autosomal-dominant missense mutations in the gene encoding the 55 kDa tumour necrosis factor receptor superfamily 1A (TNFRSF1A), the main cell surface receptor for TNF-alpha. TRAPS is characterized by unexplained episodes of fever, skin rashes, myalgia, abdominal pain, and amyloidosis in severe cases. Several mechanisms of disease pathogenesis have been proposed, including our hypothesis that the mutations in the ectodomain of TNFRSF1A cause structural changes leading to ligand-independent aggregation and signalling. R92Q is a low penetrance TNFRSF1A mutation associated with TRAPS with relatively mild symptoms. It is considered also to be susceptibility factor in other diseases, including Behcet’s disease and cardiovascular disease, and it is one of the few TRAPS-related TNFRSF1A mutations that have been identified in the general population. We have previously shown that, in contrast to other TRAPS-associated TNFRSF1A mutants, R92Q TNFRSF1A is much more like WT TNFRSF1A in terms of cell surface expression and TNF-alpha binding capacity in transfection models. Thus, we hypothesised that R92Q TNFRSF1A may behave as a pathophysiologic factor in a different way to other TNFRSF1A mutants. Our aim was to identify functional characteristics of R92Q TNFRSF1A that distinguish it from WT TNFRSF1A. Using stably transfected HEK-293 cells, we compared full-length WT and R92Q variants of TNFRSF1A in relation to their functional affinity for TNF-alpha, cellular trafficking and cellular effects. Using enzyme-linked immunosorbent assay (ELISA) with the mild chaotropic agent diethylamine, we found that R92Q TNFRSF1A had a higher functional affinity for TNF-alpha than WT TNFRSF1A. In the cellular trafficking studies, we tested the internalisation of the both TNFRSF1A variants in response to TNF-alpha over different time intervals using anti-TNF-alpha primary antibody and goat anti-mouse immunoglobulin -FITC secondary antibody, and the results suggested that the internalisation of TNF alpha occurs earlier with R92Q than with WT TNFRSF1A. Moreover, R92Q TNFRSF1A expression directed the transfected HEK-293 cells more towards apoptosis compared to the WT receptor. We conclude that these differences between R92Q and WT TNFRSF1A may contribute to the association of the R92Q TNFRSF1A mutation with both TRAPS and polygenic inflammatory diseases.

(abstract 156)

An impaired internalization of TNF-TNF receptor is associated with the defect of TNF-induced apoptosis observed in TRAPS patients


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Aim. A defect of shedding of TNFRI and TNF-induced apoptosis has been showed in circulating monocytes and neutrophils from TRAPS patients. Moreover studies on transfected have also shown a defect in the trafficking and signal transduction of the mutated protein. Aim of this study was analyze the possible intracellular mechanisms related to the defect of TNF-induced apoptosis associated to TNFRSF1A mutations using either cells derived from TRAPS patients both and 293T cells transfected with the correspondent mutant forms of TNFRI. Patients and methods: Monocytes from 8 TRAPS patients and 15 healthy controls and 293T transfected cells were stimulated with and without 30 ng/ml recombinant TNF-α for 0,30,60 minutes. After incubation with phycoerythrin(PE)-conjugated anti-TNFRI monoclonal antibody, cells were analyzed by flow
cytometry. 293T cells were transiently transfected with 500 ng of pcDNA3.1/CT-GFP-TOPO carrying in frame cDNA of wild type TNFRI and of its following mutant forms: C29Y, C55Y R92Q, C43R and c.586-612del27 (D’Osualdo et al A&R 2006). The Y207A mutation of the internalization domain was used as internal control. Transfections have been performed using Fugene6 Transfection Reagent and cells were harvested after 48h. Results: Monocytes from TRAPS patients displayed a partial defect of the intensity of fluorescence of surface TNFRI in respect to healthy control. An impaired internalization after TNF- stimulation was also observed in TRAPS patients. No evident defect of binding of TNF- was observed in TRAPS patients when compared to healthy controls. 293T cells transfected with wild-type TNFR1 and the R92Q displayed an equivalent expression and internalization of TNFRI receptor, whereas no TNFRI could be detected in cells transfected with cysteine mutations. Cells transfected with c.586-612del27 normally expressed TNFR1 displayed the same impaired internalization observed in 293T cells transfected with a mutant form of TNFR1 carrying an amino acid substitution affecting the internalization consensus motif (Y207A). Conclusion: An impaired internalization of mutated TNFRI after binding with TNF- may explain the defect of TNF-induced apoptosis observed in TRAPS patients with a severe phenotype and might play a relevant role in the pathogenesis of the disease. Discrepancies among data obtained from ex vivo and in vitro transfected cells will be discussed.

(abstract 10)

Heterogeneous expression of a single mutation within the TNF 1A gene within a family

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Heterogeneous expression of a single mutation within the TNF 1A gene within a family

Introduction TRAPS – tumour necrosis factor receptor –associated fever syndrome is a rare, dominantly inherited autoinflammatory disorder, characterised by recurrent episodes of non-infective prolonged fever and elevated laboratory indices of inflammation. TRAPS is associated with multiple mutations with in the tumour necrosis factor receptor superfamily 1A (TNFRSF1A) gene on chromosome 12p13 (1). The condition has been reported in several ethnic groups but predominantly in Northern Europeans (2). Here we report a child with classical clinical phenotype of TRAPS associated with mutation in the tumour necrosis factor receptor TNFRS1A gene, specifically C55Y. There was considerable delay in establishing the diagnosis, highlighting the potential challenge in managing patients with inherited fever syndromes. This case is of particular importance as the patient has a Caucasian father and mother of Afro-Caribbean descent. Her mother also carries the same mutation in the TNFRS1A gene and despite evidence of marked episodic inflammatory activity on serial testing, has remarkably asymptomatic disease. Case history A 15-year-old girl was referred to a regional paediatric rheumatology centre with a history of multiple febrile illnesses requiring hospitalisation since 4 years of age and a putative diagnosis of recurrent Rheumatic fever. Each admission was typically associated with fever, malaise, polymorphous rash, generalised arthralgia and myalgia. With each acute episode there were consistently elevated laboratory markers of inflammation and various other signs and symptoms. On each occasion the patient made a complete clinical recovery, with normalisation of laboratory markers of inflammation between episodes. The duration of attack was more than 7 days on most occasions; the duration of attacks can be quite variable (3) and previous studies (4) have shown that this in itself cannot be used as a diagnostic argument for TRAPS. The ASO titres were raised on at least 4 of the several admissions, which now in retrospect were only ‘red herring’ for a diagnosis of recurrent rheumatic fever though it is possible that the infections triggered an exaggerated inflammatory response. The diagnosis was reviewed at several occasions and a multiple diagnosis
were considered including Kawasaki disease, recurrent streptococcal infection, recurrent Rheumatic fever before she was finally thought to have Periodic fever and a diagnosis of TRAPS was made at 15 years of age. Discussion Previous studies have shown that TRAPS remains a under diagnosed cause of recurrent inflammatory syndrome (5) and this case again highlights the importance of increasing the awareness of autoinflammatory syndromes among health professionals. The number of mutations causing TRAPS is rapidly expanding. While in 2002, only 22 mutations were reported in patients with TRAPS, the list has rapidly extended to 83 mutations currently, though only 44 of them have the classical TRAPS phenotype (6). This is not surprising considering the genetic and clinical heterogeneity of this disease. This may result in varying clinical presentation in those who have the TRAPS mutation and on the other hand, some patients have clinical illness suggestive of TRAPS but no identifiable mutation (7, 8, 9). In our present case, though mother had exactly the same mutation as the index case, she remained largely asymptomatic throughout her childhood and was only diagnosed with TRAPS when family screening was done about a year back, further illustrating the heterogeneity in the clinical presentation and severity of the disease. Interestingly, since her diagnosis, she has had 2 attacks within a span of 6 months, responding well to short course of steroids. The TRAPS mutation is found among broad range of ethnic groups though it is predominantly found in families of Irish and/or Scottish descent (10). However it is extremely rare in Afro-Caribbean origin and though there is one case documented to have the TRAPS mutation on INFEVERS website (6) having mixed African American origin, the clinical phenotype is unknown. To the best of our knowledge, this is the first family of afro-Caribbean ancestry that has the TNFRSF1A mutation and clinical phenotype of TRAPS mutation. The mutation in the TNFRSF1A C55Y was caused by a missense substitution in exon 2 is a novel one and has not been reported before. Many of the patients who have substitutions in the cysteine residues present with a typical clinical picture of TRAPS (11, 12), though in clinical practice it may not always be easy to diagnose as is illustrated by the fact that it took us several years to reach at the correct diagnosis.

(abstract 216)

TNFRSF1A Gene Intron 4 has a Full Open Reading Frame and is Expressed as a Splice Variant

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TNFRSF1A gene encodes p55 tumor necrosis factor (TNF) receptor, and several mutations in the exon 4 and exon 5, which encodes the extracellular domains of the receptor, were found to be associated with an autoinflammation disorder [TNF-receptor associated periodic syndrome (TRAPS)]. We herein report that the intron between exon 4 and 5 has a full open reading frame and is expressed as a splice variant. Methods. We analysed the genome databases for sequence comparison. For the SNP frequencies, we genotyped 103 healthy Turkish controls using PCR-RFLP method with Mnl1 restriction enzyme. For the expression of full length and splice variants of TNFRSF1 gene, we used a real-time PCR method (LightCycler, Roche Diagnostics) with exon 4 and exon 5 primers and with exon 4 and intron 4 primers. Results. We found that all intron 4 sequence can encode a 72-aminoacid fragment. This sequence can be expressed in an alternative splice variant which includes exons 1-4 and intron 4. The expression of the splice variant was checked in the total mRNA isolated from 5 healthy controls, and a considerable amount of expression was detected by real-time PCR. We then identified that a C>T intronic SNP (rs1800692) can cause an aminoacid change (S220F) in the splice variant. The frequency of C allele was found to be 57%, and the frequency of T allele 43% in 206 chromosomes from Turkish healthy controls. The C/C genotype frequency was 37%, C/T genotype frequency was 41% and T/T genotype frequency was 22%. Conclusions. Association of exon 4 and exon 5 mutations with an
autoinflammatory disorder (TRAPS) supports the critical role of this region in p55 TNFR protein. Therefore, the splice variant of p55 TNFR with the expressed intron 4 but no exon 5 needs to be analysed further for the functional importance.

(abstract 22)

TNF receptor-associated periodic syndrome (TRAPS) in Japan: Prevalence and Characteristics of Japanese Patients with TRAPS


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Background: TNF receptor-associated periodic syndrome (TRAPS) is an autosomal dominant inherited disease characterized by prolonged episodes of periodic fever and localized inflammation. TRAPS patients are known to be rare in Asia. We previously reported a Japanese TRAPS patient associated with systemic lupus erythematosus (SLE) with a novel TNF receptor superfamily 1A gene (TNFRSF1A) mutation (T61I) [Rheumatology 43:1292, 2004].

Objectives: 1) To know the prevalence and characteristics of Japanese Patients with TRAPS, we surveyed TRAPS patients with TNFRSF1A mutation in Japan. 2) To know the role of the TNFRSF1A receptor in autoimmunity, we examined whether the T61I mutation was commonly associated with autoimmune diseases.

Methods: 1) We examined patients with unknown fever derived from 15 main hospitals in Japan and we surveyed TRAPS patients with TNFRSF1A mutation according to literature and the Japanese meetings. Fifty-four patients with unknown fever were consulted for us. We examined serum levels of cytokines (TNFa, sTNFRSF1A, sTNFRSF1B, and IL-6), TNFRSF1A mutations (exon 2, 3, 4, 6, 7), and clinical charts to make a diagnosis of TRAPS. 2) As the T61I mutation destroys an Hph I restriction site, exon 3 of the TNFRSF1A gene was analyzed in Japanese patients with autoimmune diseases, as well as healthy Japanese controls monitored by sensitivity to the Hph I nuclease. Moreover, we reviewed charts of the patients with T61I mutation. Results: 1) Until now 20 TRAPS patients from 6 pedigree including 5 different mutations (C30R, C30Y, T61I, C70S, C70G) had been reported in Japan. Chest pain and abdominal pain were rare clinical symptoms in the Japanese TRAPS patients. There were seven sporadic cases of TRAPS without TNFRSF1A mutation in our epidemiological study. 2) We demonstrated that the T61I mutation was detected in 5 of 98 SLE patients (5.1%), 1 of 102 RA patients (1.0%), of 78 other autoimmune diseases including Behçet disease and primary sjögren’s syndrome (0%) and in 10 of 320 healthy individuals (3.1%). No significant differences were detected between autoimmune diseases and healthy controls in the percentage of patients with the T61I mutation. 3) We recognized unique clinical symptoms (recurrent fever, lipodystrophy, hypertrophic osteoperiostosis, skin eruptions, calcification of the basal ganglia, etc) in two cases similar to Nakajo syndrome which was reported by Dr. Atsushi Nakajo in 1939. This autosomal recessive inherited syndrome might be a new autoinflammatory syndrome. Conclusion: There are 20 TRAPS patients from 6 pedigree including 5 different mutations (C30R, C30Y, T61I, C70S, C70G) in Japan now. The T61I mutation could be related to a generalized proinflammatory effect in autoimmune diseases, analogous to the R92Q mutation, rather than an additive effect for susceptibility to autoimmune diseases.

(abstract 158)

The R92Q Variant: Phenotype as a Reflection of Genotype in Tumor Necrosis Factor
Receptor-Associated Periodic Syndrome (TRAPS)

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OBJECTIVE: To describe the unique clinical features of pediatric patients with the R92Q substitution in the Tumor Necrosis Factor Receptor Superfamily 1A (TNFRSF1A) gene relative to patients with other TRAPS mutations and patients with Periodic Fever, Aphthous Stomatitis, Pharyngitis and Cervical Adenitis (PFAPA). METHODS: This study involved the analysis of data gathered from pediatric patients seen at NIH between 2002 and 2007 with periodic fevers and known substitutions in the TNFRSF1A gene. TNFRSF1A substitutions were categorized into 3 groups: the structural mutations (cysteine substitutions, T50M, and H22Y), the R92Q variant, and the P46L substitution. A subset of PFAPA patients was included as a comparison group. This PFAPA cohort consisted of patients with at least 2 defining clinical features of PFAPA (aphthous stomatitis, pharyngitis, and/or cervical adenitis) who had genetic testing that excluded other hereditary periodic fever syndromes including testing for the R92Q variant. RESULTS: Genotype-phenotype correlations revealed 100% fever as well as a median duration of attacks in the R92Q cohort that was similar to PFAPA and shorter than attacks of patients with the P46L substitution and the structural TRAPS mutations. In comparison to structural mutations of TRAPS, the R92Q population demonstrated a lower percentage of ocular symptoms, abdominal pain, rash, leukocytosis, and elevated inflammatory markers. In contrast, R92Q patients were more commonly affected with oral ulcers, lymphadenopathy, and sore throat in relation to structural mutations in TRAPS. The percentage affected in the R92Q group with lymphadenopathy, abdominal pain, sore throat and oral ulcers was similar to the percentage affected in PFAPA. However, other clinical characteristics such as arthralgia, pleurisy, ocular symptoms and rash were higher in patients with the R92Q variant in contrast to PFAPA patients. CONCLUSIONS: Pediatric patients with the R92Q variant comprise a distinct clinical entity when compared to structural mutations of the TNFRSF1A gene, the P46L variant, and PFAPA. The severity of associated symptoms that are hallmarks of TRAPS are decreased in the R92Q cohort relative to patients with structural mutations. Patients bearing the R92Q variant share the clinical criteria of PFAPA while exhibiting other distinguishing clinical features--emphasizing the spectrum of genotype and phenotype in TRAPS.

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Anakinra Treatment in 2 patients with C33Y mutation TRAPS

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TNF Receptor Associated Periodic Syndrome (TRAPS) is an autosomal dominant periodic fever characterised by fevers, abdominal pain, localised myalgia and skin rashes. A proportion of patients respond to Etanercept but treatment remains difficult in some. There have been recent reports of Anakinra use in TRAPS (Simon, A et al, Hawkins, P–personnel communication). We tried Anakinra in 2 C33Y mutation patients with life-long severe disease who had failed on Etanercept. Patient 1 This 55 year old male suffered constant symptoms from TRAPS particularly leg and chest pains. Apart from steroids, none of the treatments he had received had provided relief. Etanercept had produced an initial response but without long term benefit (Drewe, E). Following commencement of Anakinra 100mg S/C daily his CRP and white cell count fell rapidly but he had only limited relief from his symptoms. His dose was increased to 150mg daily but his symptoms remained unchanged. However, after 8 weeks he developed neutropenia and Anakinra was withdrawn when his neutrophils fell to 1.2 x 109/l. Pre-anakinra Day 1 Day 2 Day 8 6 weeks 8
weeks CRP 21-135 16 6 < 5 < 5 11 neutrophils 5.6 2.4 3.2 6.7 2.7 1.6 Patient 2 This 58 year old patient had developed AA amyloidosis as a result of TRAPS and had received a cadaveric transplanted kidney. He had also developed hypogammaglobulinaemia and was on regular Immunoglobulin replacement therapy following episodes of sepsis. He had been treated with Etanercept for 4.5 years with good effect. Following deterioration in his general condition and renal function he developed pancytopenia. Withdrawal/reduction of his renal medications had no effect on the pancytopenia but on cessation of the Etanercept the pancytopenia resolved within 3 days. The renal function remained poor and dialysis was recommenced. He started Anakinra (100mg alternate days in view of his renal function). Initial findings suggest suppression of acute phase response but limited control of his clinical symptoms. Further information will be available and presented in the poster. Conclusion Anakinra appears to suppress the acute phase response in 2 C33Y TRAPS patients but clinical symptoms are not fully controlled. Simon A, et al, 2004, 117; 208-210. Am J Med Drewe, E Powell, R J McDermott, E M 2007 Dec;46(12):1865-6. Rheumatology (Oxford).

(abstract 139)

PERSISTENT EFFICACY OF ANAKINRA IN TRAPS PATIENTS. A PILOT STUDY


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Aim To analyze the efficacy and safety of the treatment with IL-1 receptor antagonist (Anakinra) in TRAPS patients requiring high cumulative doses of steroids. Patients and methods: Four children (mean age 9.1 years, range 4-13) and a 33- year old man with TRAPS were enrolled in the study. Three children with cysteine mutations (C52Y, C55Y, C43R) had prolonged and frequent fever attacks. One child with R92Q mutation and the adult patient with C43R mutation displayed a more chronic disease course, with fluctuating, nearly continuous symptoms and persistent elevation of acute phase reactants (including serum amyloid A). Patients were treated with 1.5 mg/kg/day of Anakinra. Results: All TRAPS patients displayed a prompt response to Anakinra with disappearance of symptoms and normalization of acute phase reactants, including serum amyloid A. In all pediatric patients Anakinra was withdrawn after 15 days of treatment. After a few days (mean 5,6 range 3-8) they had a disease relapse with a new dramatic response to the re-introduction of the Anakinra. Results: All TRAPS patients displayed a prompt response to Anakinra with disappearance of symptoms and normalization of acute phase reactants, including serum amyloid A. In all pediatric patients Anakinra was withdrawn after 15 days of treatment. After a few days (mean 5,6 range 3-8) they had a disease relapse with a new dramatic response to the re-introduction of the Anakinra. During the following period of observation (mean 11.4 months, range 4-20 months) all patients did not experience fever episodes or other disease-related clinical manifestations. Acute phase reactants remained in the normal range. No major adverse reactions or severe infections were observed. Conclusions. Continuous treatment with Anakinra is able to effectively control both clinical and laboratory manifestations in TRAPS patients and to prevent disease relapses.

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Aberrant intracellular signalling in leukocytes from patients with tumour necrosis factor receptor-associated periodic syndrome

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Objective: Tumour necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) is a systemic autoinflammatory disorder caused by mutations in the gene coding for type 1 TNF receptor (TNFRSF1A). The pathomechanism of this disorder remains incompletely understood. Since activation of the transcription factor NF-κB is critical for the TNF-induced proinflammatory response, we chose to examine the phosphorylation levels of NF-κB and the mitogen activated protein kinase p38 in TRAPS patients with three different TNFRSF1A mutations (C88Y, F112I and C73R).

Patients and methods: Resting and TNF-stimulated phosphorylation levels of NF-κB p65 and p38 in fresh monocytes, lymphocytes and neutrophils from 10 TRAPS patients, asymptomatic at the time of drawing of the blood sample, and 10 healthy control persons were determined using phosphospecific monoclonal antibodies in whole blood flow cytometry. Area under the curve (AUC) values for generation of phosphorylated NF-κB and p38 in response to different concentrations of TNF (AUCdose) and those to different time periods of stimulation (AUCtime) were calculated using the trapezoidal rule. Results: Patients with TRAPS had significantly lower TNF-induced NF-κB phosphorylation levels in monocytes (AUCtime, p=0.005; AUCdose, p=0.034, Mann-Whitney U test) and lymphocytes (AUCtime, p=0.001; AUCdose, p=0.001) than healthy control persons. Compared with healthy control persons there were significantly lower TNF-induced p38 phosphorylation levels in monocytes (AUCtime, p=0.001) and neutrophils (AUCtime, p=0.007) in patients with TRAPS. Conclusion: The TNF-induced phosphorylation patterns of NF-κB and p38 were generally lower in patients with TRAPS than in healthy control persons. This finding of aberrant intracellular signalling lends support to the hypothesis that, in spite of the hyperinflammatory phenotype, TRAPS results from a deficiency of the innate immune system.

(abstract 18)

Characterisation of an inflammatory gene expression profile in endothelial cells transfected with TNFRSF1A mutants associated with Tumour Necrosis Factor Associated Periodic Syndrome (TRAPS)


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Mutations in the TNFRSF1A (TNF receptor 1) gene cause the autosomal dominant, auto-inflammatory tumour necrosis factor receptor associated periodic syndrome (TRAPS). TRAPS is characterised by recurring attacks of fever, abdominal pain, arthralgia, myalgia, conjunctivitis, migratory skin lesions and systemic amyloidosis. In addition, a range of inflammatory markers is up-regulated in TRAPS, either during clinical attacks or constitutively: these include CRP, ESR, IL-6 and IL-8. It is still not understood which inflammatory markers are primarily expressed as a consequence of the effects of mutant TNFR1, and which are a secondary consequence of the overall inflammatory status of the patients. We hypothesised that different mutations in TNFR1 lead to distinct intracellular consequences due to the extent of intracellular signalling initiated. Our aim was to identify genes and pathways, which are differentially modulated as a result of mutant receptor expression in a cell line directly relevant to the inflammation on TRAPS. We undertook
transcriptomic analysis to identify which markers are differentially modulated as a consequence of mutant receptor expression and analysed the gene expression profile of SK-Hep-1 human endothelial cells stably transfected with full-length constructs of wild type (WT) or TRAPS-associated mutant TNFR1. We also investigated whether the alterations differed between different mutants, and whether or not signalling by the TNFR1 death domain was involved. Compared to WT, cells expressing mutant TNFR1 showed up-regulation of multiple pro-inflammatory genes: IL-8, PTX3, GM-CSF, G-CSF, CXCL1, CCL5 and CCL2. This was confirmed in the SK-Hep-1 cells at transcript levels (RT-PCR) and at protein level (ELISA). Interestingly, different profiles of gene expression were induced by different TNFR1 mutants, indicating mutation-specific effects. Also, the expression of most genes was induced by a death domain-dependent mechanism since they were not induced by expression of TNFR1 mutants with an inactivated death domain. In summary, the differential expression profiles of WT vs. TRAPS-associated mutant TNFR1 endothelial cells has identified a number of potentially key genes known to play a role in inflammation and innate immunity that may play a significant role in the pathogenesis of the disease. We propose that the up-regulation of the cytokines G-CSF, GM-CSF, CCL2, CCL5 and PTX3 in the mutant transfected cells lead to a general imbalance in the inflammatory response with a deregulation in the cytokine/chemokine network which may contribute to the chronic inflammation observed in TRAPS patients.

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**No Regression of Renal Amyloid Mass Despite the Remission of Nephrotic Syndrome in a Patient with Tumour Necrosis Factor Receptor–Associated Periodic Syndrome Following Etanercept Therapy**


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In recent years, there have been several reports suggesting the favorable role of tumour necrosis factor (TNF-α) blocking agents in the treatment of TRAPS as well as in other inflammatory disorders complicated with AA amyloidosis. We report herein a case of TRAPS complicated with renal amyloidosis in which treatment with etanercept was associated with remission of the nephrotic syndrome and attacks of TRAPS but no regression of amyloid mass on the second renal biopsy which repeated after 2 years of treatment. Indeed, amyloid deposition was noted to be more pronounced on the second renal biopsy, particularly at tubular basement membranes. Two months following the renal biopsy, he admitted to the hospital with an inflammatory attack and relapse of nephrotic syndrome. Upon questioning he reported 3 weeks of discontinuation of etanercept before the onset of the attack. Within 3 months of reinstitution etanercept his proteinuria again regressed to 0.3 g/24h. The patient is currently remains on etanercept 25 mg twice weekly without further deterioration in his renal function. While the clinical progress and reversal of nephrotic syndrome in our patient are in accordance with several previous reports, the exact quantification of amyloid deposition 2 years after the institution of anti-TNF treatment is noteworthy and demands additional comment. To our knowledge this is only the second case of nephrotic syndrome due to AA amyloidosis treated with anti-TNF agents in which a second kidney biopsy was performed. We suggest that mechanisms other than amyloid regression might have contributed to the observed improvement in nephrotic syndrome. One explanation could be that the amyloidogenic precursor (serum amyloid A) is the pathogen and not the amyloid mass per se. In this regard, anti-TNF agents might not only reduce the synthesis of amyloid precursors but also heal cellular dysfunction by attenuating the interaction between SAA and their receptors. Another rationale might be the direct effect of TNF-α on the pathogenesis of nephrotic syndrome. Furthermore, our observation of
markedly rapid relapse of nephrotic syndrome after the discontinuation of etanercept underlines the importance of continuous and life-long treatment in order to prevent progression to end-stage renal disease in such patients.