



Third International Conference on Familial Mediterranean Fever and Other Hereditary Inflammatory Disorders

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This meeting was held in Montpellier, France on 23–27 September 2002 and was organized by Isabelle Touitou of Montpellier University. The opening ceremony took place at the Faculty of Medicine of Montpellier University, one of the oldest medical schools in Europe. Altogether, 190 physicians participated in the meeting, during which 180 abstracts were presented. The spectrum of the paper presentations encompassed all known episodic febrile auto-inflammatory disorders, including FMF (familial Mediterranean fever), HIDS (hyper-immunoglobulin D periodic fever syndrome), TRAPS (tumor necrosis factor receptor-associated periodic fever syndrome), CINCA (chronic infantile neurologic and articular syndrome), FCAS (familial cold auto-inflammatory syndrome), and MWS (Muckle-Wells syndrome). Due to space limitations and the absence of most disease entities other than FMF in Israel, we will focus only on presentations related to FMF.

Familial Mediterranean fever is an autosomal recessive disorder that affects mostly Sephardic Jews, Armenians, Turks and Arabs. The disease presents as short febrile attacks (24–72 hours), peritonitis, pleuritis, arthritis, and erysipelas-like erythema. Amyloidosis occurs in untreated cases. Colchicine, 1–2 mg daily, ameliorates or decreases the frequency of the febrile attacks and prevents amyloidosis. At this meeting, M. Pras (Israel) estimated that the number of FMF patients in the world today is close to 120,000.

Genetics of FMF

In 1997 the FMF gene, *MEFV*, was cloned from chromosome 16p using positional cloning. The gene comprises 10 exons and encodes a 781 amino acid-long protein. Close to 40 mutations have been identified, most of them clustered in exon 10. Genotype-phenotype correlation, thoroughly studied over the past few years, suggested that mutations located within the mutational hotspots in codons 680 and 694 are associated with severe disease, early onset, high frequency of attacks, the necessity of a high dose of colchicine to control attacks, and frequent occurrence of amyloidosis in untreated patients. In contrast, the E148Q mutation was found to be less penetrant and associated with mild symptoms, if any at all. R. Topaloglu (Turkey) described a cohort of FMF patients homozygous to the E148Q mutation, who had mild disease, usually with febrile abdominal attacks. A striking feature of these patients was

vomiting during the abdominal attacks. Rare mutations, found only recently and reported at this meeting, include the V487M and R501G (P. Picco, Italy), S108R and E474K (M. Medeej-Hashim, Lebanon), E163A (J.I. Arostengi, Spain), and H478Y (J. Yague, Spain).

E.L. Shanti (Jordan) stressed the role of modifier genes, such as the major HLA complex, in addition to the mutation, in creating the FMF phenotype. He also pointed out that the FMF mutations are ancient. The persistence of these mutations over the years, as well as their high frequency, may indicate an advantageous situation for the heterozygotes; however, this could not be demonstrated. S. Ozen (Turkey) suggested that *Mycobacterium tuberculosis* could have been the culprit against which carriers are better defended. Similarly, D. Cattan (France), who observed a lower mortality from tuberculosis among North African Jews as compared to their Arab neighbors, also suggested that this difference is due to the specific advantage of FMF heterozygosity. Another association with the carrier status reported by Dr. Ozen is lower frequency of allergic disease (and increased Th1 inflammatory response). These findings, however, do not reflect the biologic advantage of heterozygotes. The elucidation of such an advantage may not be feasible. Indeed, J. Feingold (France) pointed to the difficulty in proving a heterozygote advantage, as only a small increase of less than 5% in heterozygote fitness is sufficient to sustain a high frequency of disease (FMF) alleles.

While chronic inflammation induced in carriers of FMF allele may confer protection against infection, it may also result in a deleterious outcome. Y. Shinar (Israel) presented data suggesting that *MEFV* mutations increase the risk of rapid deterioration in non-Ashkenazi Jewish patients with multiple sclerosis, and that patients carrying one common *MEFV* mutation may present with a more severe, seropositive rheumatoid arthritis.

Some new population studies and phenotype/genotype correlation studies were presented. These include cohorts from Lebanon, England, Australia, and Cyprus. M. Tunka (Turkey) provided data on mutation analysis, performed on 1,090 patients by the multicenter Turkish FMF study group, showing that the most frequent mutations were M694V (51.4%), M680I (14.4%) and V726A (8.6%). H. Chaabouni (Tunis) reported that the most common mutations in

the Tunisian population of FMF patients were M680I, M694V, M694I and E148Q. H. Ajrapetyan (Armenia) found that in 100 families of probands with FMF the disease transmission was autosomal recessive in 91.5% and pseudodominant in 8.5%. E. Nucera (Italy) noted that homozygotes at codon 694 and 680 and any type of compound heterozygotes at these codons are associated with a moderate phenotype. However, none of the known MEFV mutations were found in 65% of the Italian patients. Finally, R. Gershoni-Baruch (Israel) found that the M694V and the complex V726A-E148Q alleles, endemic in Moroccan Jews and Arab Druze, respectively, are associated with a severe form of disease, while the M680I mutation, detected exclusively in patients of Muslim Arab origin, contributes to a moderate form of disease.

Pathogenesis

C. Notarnicola (France) reported that in HeLa and SW480 cell lines transfected with serially deleted fragments of the 5'-flanking region of the MEFV gene, the promoter of MEFV lies between nucleotides -152 and +41 from the ATG translation initiation codon. Between nucleotides -550 and -152 there are one or more enhancer elements, causing a tenfold increase in transcription activity. A mutation (identified in an FMF patient) in the construct significantly decreased MEFV gene expression. Dr. Notarnicola also showed a decreased transcript of pyrin in FMF patients as compared to healthy controls. The message levels were intermediate in carriers. The lowest levels were demonstrated in M694V patients. S. Papin (France) demonstrated that tumor necrosis factor- α (TNF α), but not interferon (IFN) α or γ , or lipopolysaccharide (LPS), had an effect on pyrin promoter. Indeed, this promoter displays a binding site to known TNF-inducing transcription factors, such as NF- κ B and C/EBP. A. Diaz (USA) reported somewhat different findings. In his hands, stimulation of fibroblasts and polymorphonuclear cells (PMNs) by all cytokines – including LPS, interleukin-1 (IL-1), TNF or IFN – induces pyrin. Several isoforms of transcripts of pyrin, containing a single or double splicing event, were found. The most abundant, however, is the full-length transcript. Isoforms lacking exon 2 were the only products with nuclear localization. The other pyrin transcripts were localized to the cytoplasm.

Recently, apoptosis came to the forefront among FMF researchers. Much emphasis was put on the role of the pyrin protein in this process. The findings of several workers are still conflicting. A. Diaz (USA) showed that the pyrin domain, encoded by exon 1 of the pyrin protein, is a death domain-related structure that interacts with other proteins involved in inflammation and apoptosis. Thus, the pyrin protein may be a regulator of the apoptosis processes. Indeed, peritoneal macrophages from transgenic mice expressing wild-type human pyrin exhibit a phenotype characterized by enhanced expression of various pro-apoptotic genes, accelerated apoptosis rate and increased chemokine production.

S. Papin (France) demonstrated that pyrin, whether wild-type or mutated, co-localizes with the apoptotic protein ASC in cellular specks. Thus, the loss of pyrin function in FMF is not due to a failure in the level of its subcellular localization. P. Schaner (USA) suggested that in macrophages there are two types of cellular organelles: apoptosomes, in which only ASC and pyrin co-localize;

and inflammasomes, in which pyrin, ASC, actin, actin binding protein and caspase-1, a major apoptotic protein, co-localize. This finding is independent of pyrin mutations but may be different in other cell types, such as PMNs.

Finally, D. Kastner (USA) reported his findings in pyrin-knockout mice, which usually have only the N-terminal of pyrin, and findings in cell lines transfected with ASC, caspase and pyrin genes. He suggested that wild-type pyrin suppresses caspase-ASC apoptotic activity and decreases the shift from pro-IL-1 to IL-1. In contrast, mutated or truncated pyrin brings about high IL-1 levels and increased apoptosis.

Clinical findings

Several new clinical findings in FMF patients were presented. M. Pras (Israel) proposed a new and simple diagnostic criterion that is composed of two elements: recurrent short febrile inflammatory episodes, and favorable response to continuous colchicine treatment. M. Rosenbaum (Israel) reported dysautonomic cardiovascular reactivity, detected by head-up tilt test in patients with FMF. E. Nazaretyan (Armenia) claimed that untreated FMF patients have an increased rate of myocardial infarction, lending support to the role of inflammation in atherosclerosis. E. Tutor (Turkey) confirmed the benign course and lower frequency of FMF pericarditis, as observed in 2,500 FMF patients studied. R. Brik (Israel) found restrictive lung impairment in a small number of patients with the M694V mutation. A. Mor (Israel) presented a new severity scoring system, based solely on three criteria: frequency of attacks, number of sites/organs involved in a single attack, and number of sites/organs involved during the course of the disease. M. Lidar (Israel) presented the criteria distinguishing patients with mono-arthritis as the sole manifestation of FMF from patients with arthritis due to other arthritides, and A. Livneh (Israel) characterized colchicine non-responsiveness in FMF. Dr. Livneh's study found that colchicine treatment failure was associated with inadequate concentration of colchicine in lymphocytes.

U. Saatci (Turkey) described the spectrum of renal involvement in FMF, and found that 12.9% of FMF patients had amyloidosis and 0.8% had a non-amyloid glomerular disease. The final diagnosis relies on kidney biopsy. In his cohort he observed a large variety of glomerular diseases, including membranoproliferative, mesangial, focal glomerulosclerosis and membranous. In four patients both amyloidosis and glomerulonephritis coexisted. E. Ben Chetrit (Israel) reviewed some important questions related to the reproductive system in FMF, including female and male fertility, the safety of colchicine prophylaxis, the need for amniocentesis, and breast feeding.

A. Bakkaloglu (Turkey) confirmed an increased frequency of vasculitides in about 3,000 FMF patients. These included Henoch-Shönlein purpura (2.6%), polyarteritis nodosa (PAN) (0.8%), Behçet's disease (0.5%), acute rheumatic fever (4.9%), and inflammatory bowel disease (0.2%). Of note is Behçet's disease, which was significantly more common among patients with FMF than in the general population ($P < 0.001$). This finding, we hope, will terminate skepticism raised by other groups with regard to our original observation on the association between the two diseases.

G. Hatemi (Turkey) compared 8 patients with FMF-PAN to 20 patients with classical PAN. FMF-PAN was characterized by a more severe PAN, younger age at onset, more major organ involvement, joint complaints, and skin vasculitis.

D. Cervirgen (Turkey) noted the considerable ignorance that exists among patients regarding various aspects of their disease, particularly with respect to genetics, pregnancy, and treatment. N. Zaks (Israel) found that more than 10 years delay in FMF diagnosis is associated with female gender, immigration to Israel, and physician and patient unawareness, and not with a mild form of the disease, as intuitively assumed.

Amyloidosis

Serum amyloid A (SAA) is an HDL3-associated apolipoprotein and an acute-phase reactant, found to be increased in FMF attacks up to 1,000-fold the remission concentration. This protein is the precursor of the amyloid protein. P.N. Hawkins (England) argued that serial monitoring of SAA is required for management of FMF amyloidosis, since the only known prerequisite for developing AA amyloidosis is sustained and substantial elevation of plasma SAA concentration, and regression of amyloidosis is strongly associated with maintaining SAA levels below 10 mg/L. A. Duzova (Turkey) found that compared to erythrocyte sedimentation rate, C-reactive protein and fibrinogen, SAA is the most sensitive indicator of subclinical inflammation in FMF (it was highest in untreated patients and in patients with two affected FMF alleles). Patients on colchicine who had no attacks may still have persistent elevated SAA levels. Additional colchicine to 26 patients led to a dramatic decrease in SAA levels.

Several risk factors for development of amyloidosis were discerned or confirmed. Homozygosity to SAA α was described as an important predisposition to amyloidosis, by R. Gershoni-Baruch (Israel), A. Bakaloglu (Turkey), E. Yilmaz (Turkey) and M. Medley-Hashim (Lebanon). R. Gershoni-Baruch demonstrated that male gender and joint involvement added to the susceptibility to develop amyloidosis brought about by certain MEFV and SAA α genotypes. M. Papazyan (Armenia) counted early age at onset, male gender, frequent attacks, protracted arthritis, and homozygosity to the M694V mutation as risk factors to develop amyloidosis in a population not receiving colchicine.

Contrary to numerous publications relating M694V to increased risk of developing amyloidosis in all affected ethnic groups, F. Yalcinkaya (Turkey), on behalf of the Turkish FMF study group, reported that according to the genetic analysis of 1,090 patients,

amyloidosis could not be related to any one of the FMF mutations. Finally, H.J. Lachmann (England) described the course of amyloid kidney disease in FMF, showing regression by serum amyloid P scanning in a patient presenting without renal failure in whom inflammation is controlled by colchicine treatment.

Laboratory findings

Several laboratory findings are of interest: S. Yalcinskaya (Turkey) found that the anti-streptococcal response is exaggerated in children with FMF. J.C. Lecron (France) found increased immunoglobulin D levels in serum of FMF patients, with higher concentration in patients with two mutations, as compared to one or no detectable MEFV mutations. G.M. Mkrtchyan (Armenia) reported circulating immune complexes in FMF. The main antigens were C-reactive protein and hemoglobin chain. Finally, A.G. Panossian (Armenia) reported low plasma nitric oxide levels during FMF attacks. This finding is an exception for inflammatory disease and needs to be confirmed and explained.

Management

About 5% of FMF patients do not respond to treatment with colchicine 2 mg daily. M. Pras (Israel) recommended that the dose of colchicine be increased to 2.5 mg, and even to 3 mg as an option if the patient can tolerate it. In patients with chronic arthritis and spondyloarthritis of FMF, treatment with the anti-TNF α drugs etanercept and remicade may be useful. P.N. Hawkins (London) reported on one such patient.

During this meeting other treatment modalities were suggested for non-responders. E. Seyahi (Turkey) presented a case report describing the efficacy of thalidomide in a colchicine-resistant FMF patient. G. Amaryan (Armenia) reported that a herbal drug, Immuno-Guard, was safe and efficacious in the treatment of FMF patients. M. Lidar (Israel) suggested the addition of weekly intravenous colchicine treatment in FMF patients unresponsive to oral colchicine therapy. F. La Regina (Italy) found that eradication of *Helicobacter pylori*, prior to administration of colchicine, could be useful in improving colchicine tolerance. Finally, M. Calguneri (Turkey) reported the efficacy of IFN in seven colchicine-resistant FMF patients and found it to be helpful and safe.

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Capsule

Oxidative stress and early-onset Parkinson's disease

In Parkinson's disease, motor abnormalities develop as dopaminergic neurons of the nigrostriatal pathway in the brain die. Bonifati et al. identified mutations in a gene called *DJ-1* in an Italian and a Dutch family with an early-onset autosomal recessive form of Parkinson's disease. Although the function of

DJ-1, a ubiquitously expressed and highly conserved protein, is unknown, there is indirect evidence that it is involved in the cell's response to oxidative stress.

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