

Update on Auto-Inflammatory Diseases and Familial Mediterranean Fever

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Auto-inflammatory disease (AID) is a relatively new term that describes a group of disorders characterized by recurrent or persistent inflammation, appearing in the absence of infection or other apparent cause. Moreover, inflammation occurs without antigen-specific T cells or autoantibodies, a feature that distinguishes these disorders from the more widely known auto-immune conditions. Our understanding of the biologic basis of AID has advanced significantly in the past decade [1,2].

Monogenic defects of genes involved in innate immunity underlie the majority of auto-inflammatory diseases [3]. Several classification schemes have been proposed, according to either the underlying immunologic mechanism or clinical phenotype. One clinically defined subgroup comprises disorders formerly referred to as periodic or episodic fever syndromes. Among periodic fevers, the most common is familial Mediterranean fever (FMF); others include tumor necrosis factor receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), and cryopyrin-associated periodic syndrome (CAPS) [4,5]. Another subgroup of AID includes those with significant skin manifestations, such as Chronic Atypical Neutrophilic Dermatitis Lipodystrophy Elevated temperature (CANDLE) and interleukin-36 receptor antagonist deficiency (DITRA). Significant bone involvement characterizes disorders including deficiency of interleukin-1 receptor antagonist (DIRA), chronic recurrent multifocal osteomyelitis (CRMO), and Majeed syndrome. The latter is a disorder comprised of CRMO, congenital dyserythropoietic anemia and neutrophilic dermatosis, and caused by mutations in the *LPIN2* gene. A newly identified subgroup of granulomatous disorders includes Blau syndrome (early-onset sarcoidosis), caused by mutations in *NOD2/CARD15* gene, PAPA (pyogenic sterile arthritis pyoderma gangrenosum acne) syndrome, and early-onset inflammatory bowel disease (associated with mutated alleles in *IBD28*, *IL10RA*, *IBD25* and *IL10RB* genes) [6].

A newly identified subgroup includes inherited vasculopathies, including monogenic polyarteritis nodosa, a form of vasculopathy with highly varied clinical expression that is associated with recessive loss-of-function mutations of the *CECR* gene, encoding adenosine deaminase 2 [7].

The advent of next-generation sequencing techniques, including whole exome and whole genome sequencing, has greatly accelerated the rate of discovery and characterization of AID. Study of these rare defects of innate immunity has in turn contributed significantly to our understanding of the mechanisms of inflammation.

FMF

FMF is the most widespread and best-characterized monogenic AID, affecting mainly ethnic groups originating in or around the Mediterranean basin: Sephardic Jews, Armenians, Turks, and Arabs. This disease is characterized by irregular, self-limited febrile episodes of inflammation of serous membranes and marked elevation of acute-phase proteins. Amyloidosis, the most significant complication of FMF, is the major cause of serious long-term morbidity and mortality. The present review will focus on the clinical and genetic features of FMF.

GENETIC FACTORS

Nearly 20 years after the landmark discovery that FMF is caused by mutations in the Mediterranean Fever (*MEFV*) gene, our understanding of this auto-inflammatory disease continues to evolve [8]. *MEFV* is located on chromosome 16, and encodes a 781-amino acid protein termed pyrin, expressed mainly in polymorphonuclear leukocytes and macrophages [9]. This protein appears to play a pivotal role in the regulation of both inflammation and apoptosis, and mutated pyrin leads to full-blown inflammation characterized by excessive Il-1 secretion in FMF [10]. To date, more than 310 *MEFV* sequence variants have been reported to Infervers (<http://fmf.igh.cnrs.fr/ISSAID/infervers/>), an online registry of AID mutations [11]. Most are located in exon 10, including the most common *M694V*, *V726A*, *M680I* and *M694I* mutations. The wide clinical variability in FMF is partly explained by genetic heterogeneity. More severe disease is associated with the *M694V* as well as the *M680I* mutation and the complex allele *V726A-E148Q* [12]. The role of *E148Q*, found in exon 2 of *MEFV* gene, as a disease-causing mutation

is controversial [13,14]. Homozygous *E148Q* mutations are very rare in patients with symptomatic FMF, and this gene variant is very common in populations with low FMF prevalence. In a large cohort of Israeli children with FMF, molecular testing for common *MEFV* mutations identified two mutations in 60% of patients and no mutation in 10%. A significant proportion (30%) of patients with clinical FMF and a favorable response to colchicine had only one mutation [15]. Initially it was assumed that these patients harbor less common *MEFV* mutations on the second allele of the gene. However, sequencing of *MEFV* gene and the promoter, and multiple ligation-dependent probe amplification in search for deletions and duplications failed to identify mutations in these patients, confirming that FMF may be caused in these patients by a single mutation in the *MEFV* gene [16]. Conversely, most individuals with one mutation in *MEFV* are asymptomatic carriers of the disease, and carriers of two mutations may exhibit no overt signs of disease. Screening of a sample of the Israeli general population has shown that the number of individuals with unexpressed double-mutated *MEFV* exceeds the number of patients with overt FMF by several times [17]. Taken together, these data suggest an important role for environmental factors and/or modifier genes on the clinical expression of FMF.

The possible effect of several potential modifier genes on FMF phenotype has been studied. *MEFV* mutations were reported to affect the phenotype of Crohn's disease. Mutations in *NOD2/CARD15* gene, a major susceptibility gene for Crohn's disease, were detected at a comparable rate in a group of children with FMF and their controls from Israel. However, FMF patients carrying a *NOD2/CARD15* mutation had more severe disease and a higher rate of colchicine resistance as compared to patients without mutations [18]. The frequency of mutations in *TNFRSF1A*, a gene associated with the AID TRAPS, was studied in a cohort of FMF patients from Israel and found to be comparable with that of controls. Despite the fact that TRAPS and FMF share common biochemical pathways, the authors have found no evidence for an interaction between these two genes [19].

FMF is generally considered to be an autosomal recessive disease. However, Stoeffels and colleagues [20] described an autosomal dominant auto-inflammatory disease associated with *MEFV* mutations affecting amino acid 577 in three families, each of different ethnic background. Febrile attacks in these patients were prevented by colchicine, but differed from typical FMF in that attacks were prolonged, lasting up to weeks. The dominant inheritance pattern implies these patients had a gain-of-function mutation, suggesting that the mechanism of disease in these patients may also be different to that of classical FMF [20].

PHENOTYPE

FMF is characterized by febrile attacks accompanied variably by serositis-peritonitis, pleuritis, synovitis, or less commonly by erysipelas-like erythema or acute scrotum. The attacks are

typically irregular, short (less than 3 days) and resolve without treatment. Between these episodes, patients are usually asymptomatic.

In most patients, symptoms begin before the age of 10 years, and in a third of pediatric patients with FMF the manifestations first appear before 2 years of age. In an Israeli study the clinical manifestations in the majority of children with early as compared to late-onset disease were found to be comparable. However, diagnosis was delayed significantly in children with early-onset disease. The subgroup of patients diagnosed before age 2 years had the highest frequency of attacks, which typically consisted of fever as the sole manifestation, without serositis. Most of these very early-onset patients were homozygous for M694V mutations [21]. Yalcinkaya et al. [22] described FMF manifestations in a cohort of 83 children from Turkey with disease onset before age 3 years. These patients tended to have more severe symptoms, required relatively high doses of colchicine to control their disease, and were diagnosed after a significant delay. Although one might have expected that a positive family history of FMF would lead to early diagnosis in symptomatic children, in both these studies a positive family history did not result in a shorter interval between onset of symptoms and initiation of therapy. This may be due to the fact that early in life, FMF may present with attacks of fever alone without signs of serositis and thus is more difficult to recognize. Progression to more typical disease with symptoms typically begins several years later [23]. These findings emphasize the need for vigilance among physicians who provide clinical care for children in FMF-endemic areas; otherwise, diagnosis and treatment may be delayed significantly.

FMF attacks can occur following discontinuation of colchicine treatment or omission of doses. In addition, attacks may be triggered by infections, physical or emotional stress, or menstruation. Yenokyan and co-author [24] conducted an epidemiologic study of triggers in FMF in a cohort of 104 Armenian FMF patients to quantify the effect of external triggers on FMF attacks. A number of stressful events were associated with FMF attacks. The highest risk attacks occurred on the second day following exposure to a trigger. A negative, statistically significant relation between consumption of high fat-containing food items and the likelihood of developing FMF attacks was also observed. The authors proposed that a possible reason for this finding could be reduced appetite and low consumption of any food prior to FMF attacks. High levels of perceived stress were also associated with FMF attacks. Unexpectedly, in this study physical exertion and menstrual periods were not associated with FMF attacks.

In addition to genotype, it is well recognized that other factors, including ethnicity and geography, greatly influence FMF disease penetration. To gain further insight into the effects of environment on disease expression, Ozen and colleagues [25] used the Eurofever registry of AID to identify and compare

pediatric patients of Mediterranean origin living in an FMF-endemic country to children of the same ethnic origin currently living in Europe. They found disease to be less severe among the patients living in Europe, pointing to a clear effect of environment on disease expression [25].

Although FMF is considered a periodic disease, some forms of FMF morbidity may be persistent rather than episodic. These include protracted febrile myalgia, FMF-associated vasculitis, and persistently elevated inflammatory markers without apparent clinical signs. Other long-term complications include peritoneal adhesions, which may lead to intestinal obstructions and female infertility; and amyloidosis involving the gastrointestinal tract, liver and spleen. These complications occur mainly in untreated or colchicine-non-adherent patients, and are associated with certain ethnicities and countries of residence [26]. Amyloidosis of the kidney is the most serious long-term complication of FMF, potentially progressing to end-stage renal disease [27].

DIAGNOSIS

The diagnosis of FMF is based on clinical symptoms and supported by ethnic origin and family history [28]. As discussed above, some patients may present with incomplete or atypical attacks, especially small children and patients with mild disease. In such cases, diagnosis can be difficult, resulting in delayed initiation of treatment. Since the discovery of the *MEFV* gene, molecular genetic testing is used as a diagnostic adjunct in these cases. In patients with possible clinical disease, a finding of two mutations in *MEFV* gene confirms the diagnosis of FMF. In patients with atypical attacks and one mutation (heterozygotes) or no mutation, the genetic test is considered non-diagnostic, and a therapeutic trial of colchicine is given for 3–6 months to ascertain whether there is a decrease in the severity and frequency of attacks. Clinical rather than genetic criteria have remained the basis for diagnosis of FMF; the use of genetic testing for diagnosing asymptomatic patients or for screening of unaffected family members of patients with FMF is not currently recommended [29].

ADVANCES IN TREATMENT

Colchicine has been the mainstay of FMF treatment for over 40 years, completely preventing attacks in 60–65% of patients and inducing partial remission in a further 30–35%. In addition, regular use of colchicine eliminates the long-term risk of amyloidosis in FMF patients. In reporting their 1 year follow-up of 153 consecutive children with FMF, Padeh and co-authors [30] concluded that colchicine is safe for use in FMF, even in infancy. Approximately 15% of treated patients had mild and transient diarrhea, usually on initiation of treatment and improving later. Another side effect was a mild and transitory elevation in hepatic transaminases observed in 10% of children in association with an intercurrent illness. All patients reported

a good response to colchicine therapy, with less than two attacks during the year. Only four children required a dose reduction, and in none was discontinuation of treatment necessary [30].

Although colchicine is a safe and effective drug for most patients, unfortunately it is not a panacea for FMF. Despite good adherence to colchicine at maximally tolerated doses, 5–10% of patients report attacks [31]. Lidar et al. [32] used a telephone survey to assess FMF symptoms among adult patients homozygous for severe M694V mutation, as compared to patients with either M694V/V726A or V726A/V726A genotypes. Patients with M694V/M694V mutations reported using higher colchicine doses, more colchicine side effects, and a greater number of FMF attacks; 10% of these patients reported no amelioration of their symptoms at all while taking colchicine [32]. These results are similar to those reported in a study involving 200 children with FMF from Turkey. Thus, the M694V/M694V genotype identifies a subgroup of FMF patients carrying increased risk for colchicine resistance, who may require supplemental treatment to prevent frequent attacks.

Based on the concept of FMF as an AID, and its association with hyper-secretion of the pro-inflammatory cytokine interleukin (IL)-1b, IL-1 antagonists have been proposed as a possible treatment for colchicine-resistant FMF. IL-1 antagonists that have been used in clinical trials include canakinumab, rilonacept and anakinra [33]. Canakinumab is a high affinity fully human monoclonal anti-IL-1 β antibody, with a half-life of 4 weeks, that binds human IL-1 β and blocks its interaction with receptors, thus functionally neutralizing cytokine bioactivity without preventing IL-1 β binding to the natural inhibitor. An open-label pilot study investigated the efficacy of canakinumab in a small group of nine adult colchicine-resistant FMF patients. All patients achieved a 50% or more reduction in attack frequency, and only one patient had an FMF attack during the treatment period [34]. Rilonacept, an IL-1 decoy receptor, was used to treat 14 patients in a study with a randomized double-blind single-participant alternating treatment design. In this study, the number of FMF attacks during rilonacept treatment was reduced significantly as compared to the period during which patients received placebo. However, the length of attacks and the acute-phase reactant concentrations between attacks were not reduced significantly [35]. The efficacy of anakinra (11 patients) and canakinumab (11 patients) was reported in a Turkish single-center retrospective case series of children with colchicine-resistant FMF [36]. It should be emphasized that colchicine treatment should be continued in FMF patients receiving IL-1 antagonists, since the latter has not been shown to reduce the long-term risk of amyloidosis.

CONCLUSIONS

During the past few years there have been significant advances in our understanding of FMF and other auto-inflammatory diseases. These advances have allowed earlier and focused

therapeutic interventions. For FMF patients with uncontrolled disease or intolerant to colchicine, supplemental treatment using IL-1 antagonists may be an effective but expensive way to improve control of the disease.

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