

MEFV Mutation Carriers and Diseases Other than Familial Mediterranean Fever: Proved and Non-Proved Associations; Putative Biological Advantage

Daniel Cattan*

Praticien Hospitalier, Faculté de Médecine de Créteil, Centre Hospitalier, F 94195 Villeneuve-Saint-Georges, France

Abstract: Vasculitis is definitely associated with familial Mediterranean fever. This familial Mediterranean fever-associated vasculitis takes one of three forms: polyarteritis nodosa, with or without microscopic polyangiitis, and Henoch-Schonlein purpura. Behcet disease and inflammatory bowel diseases may also be associated with familial Mediterranean fever, though this is yet to be formally proven.

The selective biological advantage, if any, for carriers of simple heterozygotic mutations in the gene responsible for familial Mediterranean fever, *MEFV*, is not known. Indirect arguments are given for a better defense against certain groups of bacterial pathogens and amongst intra-cellular bacteria, *Mycobacterium tuberculosis*.

Keywords: Familial Mediterranean fever, *MEFV* gene, Vasculitis, Behçet disease, Inflammatory bowel diseases, Selective advantage, Jews, Tunis, Tuberculosis.

INTRODUCTION

Familial Mediterranean fever (FMF) is an auto-inflammatory disease mainly inherited as an autosomal recessive condition observed in Non-Ashkenazi Jews, Middle East Muslims, Turks and Armenians. It is characterized by recurrent attacks of fever with peritonitis, pleuritis, arthritis, and erysipela-like skin lesions. During these attacks an intense biological inflammatory syndrome, including high elevation of the erythrocyte sedimentation rate, the white blood cell count, and the levels of fibrinogen, C-reactive protein (CRP), and serum amyloid A component (SAA), is observed [1, 2, 3]. Cytokine activation [4, 5] and a TH1 polarization [6] of the inflammatory process have also been reported.

The familial Mediterranean fever gene (*MEFV*), on chromosome 16p, encodes a protein of 781 amino acids, marenostin or pyrin [7, 8], mainly expressed in mature granulocytes [9]. This protein probably has an important role in the down regulation of inflammation. About 70 mutations in the Mediterranean fever (*MEFV*) gene are established causes of the disease (INFEVERS; INternet periodic FEVERS, <http://fmf.igh.cnrs.fr/infervers>, register). Most of the mutations are located in exon 10 at the carboxy-terminal portion of the protein.

The most serious complication of FMF is amyloidosis, causing chronic renal failure. The efficiency of colchicine (1-2.5mg *pro die*) in the prevention of attacks and the manifestation of amyloidosis is remarkable; only 5 to 10% of patients do not respond to this treatment (See [10] for a review).

Studies of the connections of FMF with other diseases include: (i) investigating the associations between FMF and other diseases, and (ii) research into specific disease(s) or groups of diseases against which FMF patients and carriers of *MEFV* mutations could be genetically protected.

A chronic subclinical inflammatory syndrome has been evidenced in FMF patients between attacks. This biological syndrome includes an increased ESR [1, 2], and increases in the levels of CRP and SAA [3]. Moreover, in asymptomatic heterozygote subjects, considered as carriers, increased levels of CRP [3, 11, 12, 13], SAA [3, 11, 12], and the mRNAs for the cytokines TNF α , IL1 β , IL6, and IL8 [14] as well as a TH1 polarization of the inflammation process [6] have also been reported. This asymptomatic inflammatory syndrome can be observed even in carriers of low penetrance mutations, for instance E148Q [12, 15], and has been considered as probably responsible for manifestations of amyloidosis in asymptomatic FMF carriers [12, 15, 16] whether or not they suffer from other diseases predisposing to amyloidosis. Thus, FMF has a major subclinical component, and the frequency and severity of asymptomatic inflammation presumably underlies the considerable risk of AA amyloidosis rather than that which occurs during the occasional short attack [12]. Moreover this inflammatory syndrome is probably a facilitating factor in the manifestation (or the severity) of associated inflammatory diseases [17, 18, 19, 20, 21] and perhaps was a protective factor against frequent and lethal infectious diseases before the antibiotic era [20, 21], explaining the high prevalence of marenostin/pyrin mutations.

*Address correspondence to this author at the Praticien Hospitalier, Faculté de Médecine de Créteil, Centre Hospitalier, F 94195 Villeneuve-Saint-Georges, France; Tel: +33-611380196; E-mail: danielcattan@yahoo.fr

ASSOCIATIONS

I. Diseases Associated with Familial Mediterranean Fever

Three forms of vasculitis are definitely associated with FMF: polyarteritis nodosa (PAN), microscopic polyangiitis and Henoch-Schonlein purpura. Because the prevalence of FMF-associated vasculitis is at least as frequent as some of the rare manifestations of classical FMF itself, a number of authors argue that vasculitis is an integral part of FMF.

Diseases that have been proposed to be associated with FMF include Behcet disease (BD), inflammatory bowel disease (IBD), and juvenile chronic arthritis (JIA). To be given the status of a disease associated with FMF the following conditions should be fulfilled: (i) FMF must be proven either by genetic or by recognized clinical criteria. (ii) The diagnosis of the candidate FMF-associated disease must be made according to internationally recognized criteria. (iii) The prevalence of the candidate FMF-associated disease in both adults and children of the general population and/or in the ethnic group of interest must be well-known. (iv) Two control populations of the same ethnic group as the FMF study population must be investigated in parallel. One of these should consist of healthy subjects, while the second should consist of patients with another unrelated inflammatory disease, for example rheumatoid arthritis. (v) Inclusion of all FMF cases observed during the study period is necessary, eliminating if possible the cases referred to a medical team known as being particularly competent in the study of FMF-associated diseases. (vi) The use of literature studies or meta-analysis may detect cases that have been published several times. Bias is inevitable, for instance, co-morbidity clearly increases the chance of a disease being detected.

For each associated or candidate associated disease, it is important to research (i) clinical or genetic FMF characteristics, (ii) differences in the presentation of the associated disease from its usual presentation, (iii) the prevalence of *MEFV* gene mutations in an homogeneous population of patients with the associated disease alone, and without any symptoms of FMF.

1.1. Vasculitis

International definitions of the forms of vasculitis considered to be associated with FMF [22] are as follows:

- PAN is a necrotizing inflammation of the medium and small arteries, without associated glomerulonephritis, pulmonary capillaritis, or disease of other arterioles, capillaries or venules.
- Microscopic polyangiitis (microscopic polyarteritis) is a necrotizing vasculitis with few or no immune deposits, affecting small vessels (capillaries, venules, arterioles). Necrotizing arteritis of the small or medium-sized arteries may be present, pulmonary capillaritis often occurs, and necrotizing glomerulonephritis is very common. This disease is often associated with anti-neutrophil cytoplasm antibodies (ANCA).
- Henoch-Schonlein purpura is a vasculitis characterized by predominantly IgA immune deposits affecting the small vessels (capillaries, venules or arterioles). This form of vasculitis affects the skin, the gut, and the glomerules, and is associated with arthralgia or arthritis.

Other forms of vasculitis are not observed in patients with FMF. Since their aetiology and pathogenesis are rarely known, and their clinical and

histological features overlap, a definitive classification of the forms of systemic vasculitis cannot be reached.

I.1.1. Polyarteritis Nodosa and Microscopic Polyangiitis in Patients with FMF

Polyarteritis nodosa (PAN) is definitely associated with FMF. The two first cases were described in 1954 by Benhamou *et al.* in Algiers [23]. PAN fulfils all criteria for inclusion in the category of an FMF-associated disease [24-30]. Thirty-one cases have been published in 1999 [28]. The prevalence of PAN in FMF patients is about 1% [24, 28], while its prevalence in the general population is 6 per 100,000 [31], and 4 per 100,000 in Turkey [28]. FMF symptoms precede the appearance of those of PAN by many years [28, 29]: in a large study series, the mean age at onset of FMF was 6.8 yrs [15-17] and the mean age at the onset of PAN-FMF was 24.3 yrs (9-44) [29]. However, PAN vasculitis may provide the occasion for the diagnosis of FMF. These patients have regular FMF symptoms and are generally well treated with colchicine. This treatment has a low resistance frequency. Genotypes have been obtained in about twenty cases of FMF-PAN, and the mutations discovered were common within the study populations.

The characteristic features of PAN are numerous. PAN is often associated with microscopic polyangiitis, and therefore this vasculitis is often an overlap syndrome. Renal involvement with typical aspects of microscopic vasculitis, either with necrotizing glomerulonephritis or focal and ischemic glomerulonephritis [28] and a severe arterial hypertension have been frequently observed [28, 29]. Several studies have shown that between 38 to 50% [28, 29] of patients had perirenal hematomas, which were occasionally bilateral [26], and were sometimes revealed by rupture and dramatic internal hemorrhage. Hepatic [30] and splenic [26] hematomas, associated with perirenal hematoma, have been also described. Arterial aneurysms are responsible for these hematomas and are easily seen on angiography, CT scan, MRI and echography. They are usually numerous and are present even in patients with microscopic polyangiitis. Their spectacular disappearance upon treatment has been reported.

The age at the time of the PAN diagnosis is particularly young in patients with FMF. In classical PAN, the mean age at diagnosis is between the fourth and fifth decades. In FMF-associated PAN cases the mean age at the time of the PAN diagnosis is in the third decade, for instance 24.3 yrs (9-44) versus 37.5 (10-63) years in classical PAN cases [29]. As in classical PAN, the PAN-FMF association is more frequent in males (M:F 6:2 vs 15:5 for classic PAN [29]). The Hbs antigen is present in only 16% to 18% of cases [28, 29] instead of 20-30% of patients as seen in classical PAN.

Recovery is observed in the majority of FMF-PAN patients after a year of treatment with a combination of corticosteroids and cyclophosphamide. The use of flash treatment with corticosteroids and/or cyclophosphamide is rarely necessary and long term immunosuppressive treatment is rarely used. Arterial embolization has been used with success for the treatment of hematomas due to aneurysms [30]. Death from FMF-PAN is rare and may in fact be due to other causes than PAN [28].

In summary, FMF-PAN occurs in young patients, involves more visceral injuries, and results in more articular pain and cutaneous vasculitis than classical PAN, which presents with more peripheral neuropathy and myalgia, as well as higher frequencies of positive ANCA and Hbs Ag scores. Data from evolutionary studies also gives the impression that PAN is less severe in FMF patients [29].

In PAN patients belonging to the ethnic groups where there is a high frequency of FMF, the FMF prevalence varies from 7.6 to 22% [32]. The prevalence of PAN has been studied in individuals heterozygotic for *MEFV* mutations, considered as FMF carriers [21]. Amongst 70 *MEFV* heterozygotes, two cases of PAN were found; however there were two possibilities for bias in this study; firstly, amongst the 70 heterozygotes only 15 were normal, while 24 had clinical FMF (and thus perhaps a rare mutation on the other allele) and 28 others had rheumatic diseases. Secondly, patients with complaints similar to those relevant to FMF and with "unusual features" were included in this study.

An interesting feature in patients with FMF-PAN is the high prevalence of antistreptolysin O antibody elevation [21, 33]. This prevalence excludes coincidence. *Streptococcus* infections are indeed frequent in Turkey. However, *Streptococcus* has already been implicated in the genesis of PAN in young subjects [34].

Finally, some authors are in favour of the integration of PAN with or without microscopic polyangiitis amongst the symptoms of FMF as "protracted febrile myalgia" [35, 36], a syndrome seen in 10% of FMF

cases. The severe pain, sensitivity to corticosteroids and, occasionally the histological features, are common to this syndrome and vasculitis.

I.1.2. Henoch-Schonlein Purpura (HSP)

The association of HSP with FMF is well known [24, 33, 37, 38, 39], and fulfils the above-mentioned criteria for diseases associated with FMF. Twenty years ago, 20 cases had already been published [37]. The prevalence of HSP in FMF patients varies from 2.6% to 3.6% [36, 40] and 7% in Turkey [24].

The HSP prevalence in the general population varies from 0.05% to 0.8% [24, 36]. There are no differences in the clinical features of FMF-HSP and classical FMF. The age at diagnosis is perhaps younger, but the age of onset is the same [24]. HSP is often the first disease revealing FMF; so the diagnosis of HSP in a patient belonging to an ethnic group where FMF is frequent should be followed by a search for FMF symptoms. There are no specific *MEFV* mutations associated with HSP-FMF [33, 39], and no clinical difference between HSP associated with FMF and typical HSP [24]. Spontaneous resolution is observed in two out of three cases. In one in three cases resolution is obtained with corticosteroid treatment. Chronic renal involvement is rare [38]. Interestingly, Schlesinger *et al.* observed that five cases out of ten manifested after penicillin administration [38].

There are no studies of the prevalence of HSP amongst heterozygotic carriers of *MEFV* mutations. Guershoni *et al.* recently observed nine cases with simple *MEFV* heterozygosis (17.3%) and five cases who were either homozygotic or composite heterozygotes (9.6%) in a series of 52 HSP cases [39]. As 25% of the general population in Israel carry simple heterozygote *MEFV* mutations one can conclude that the HSP population in Israel is not outside the norm in this respect, but that the prevalence of a positive genotype (homozygosity or compound heterozygosity) is certainly more frequent than usual. As in patients with PAN-FMF, the prevalence of antistreptolysin O antibody elevation is high [33, 37].

II. Diseases Possibly Associated with FMF

II.1. Behcet Disease and FMF

Behcet disease (BD) is an inflammatory disorder with a genetic background, characterized by oral and genital ulcers, uveitis, cutaneous pustular erythematous lesions, arthritis, central nervous system involvement, and/or vascular manifestations, such as venous thrombosis, arteritis and aneurysms [41]. Like FMF, Behcet disease consists of recurrent attacks of acute inflammation. Its common manifestations are self-limited except for the ocular attacks; repeated attacks of uveitis can cause blindness. Susceptibility to Behcet disease is strongly associated with the presence of the HLA-B51 allele. Behcet disease and FMF share some common symptom such as fever, arthritis, abdominal pain, and acute scrotum pain due to orchitis or epididymitis. Colchicine has a positive effect on some of the articular manifestation of Behcet disease.

Both FMF and Behcet disease are observed all around the Mediterranean basin, but Behcet disease clusters along the ancient Silk Road which extends from Asia to the Mediterranean. The prevalence of Behcet disease is variable, from 0.12 to 0.33 per 100,000 in the United States to 0.64 per 100,000 in the United Kingdom, with 13.5 to 20 cases per 100,000 in Asia and Saudi Arabia, and 80 to 370 per 100,000 in Turkey [41]. Males are those more frequently affected in Middle Eastern Countries. The onset is typically in the third or fourth decade of life [41].

Behcet disease can not be considered with certainty as a disease associated with FMF. This is due to the contrary results and/or to methodological issues in the literature to date. Schwartz *et al.* [42] conducted a retrospective study in which FMF patients also suffering from Behcet disease (FMF-BD) were recruited mainly from approximately 4,000 registered FMF patients. The prevalence of BD was 16 per 4,000; that is 400 per 100,000. This result was considered as significant compared to the prevalence of BD in Japan (10 per 100,000). This work has been the subject of discussions concerning the criteria of inclusion and the control populations [43, 44]. In this study, nevertheless, as in most studies to date, in most cases the FMF was of intermediate severity and the patient genotypes were not given. More FMF cases than controls were of Iraqi or Turkish origin, and they responded less favourably to colchicine. The extension of their BD was limited. A higher proportion of FMF-BD cases than cases with BD alone had skin, central nervous system, and gastrointestinal manifestations. These patients originated from North Africa, and had a family history of BD. HLA-B5 antigen was present in 53% of the FMF-BD cases and 40% of the BD controls.

Ben-Chetrit *et al.* [45] identified two BD patients among a group of 355 FMF patients (563 per 100,000) and two (the same patients) FMF

patients among 53 BD patients, a relatively high frequency, approximately 10 times higher than the frequency of FMF among Non-Ashkenazi Jews. Statistical analysis supported their findings that the association between FMF and BD was higher than expected in both directions (FMF in BD patients and *vice versa*). Nevertheless, the small number of patients with both diseases was of concern in this study [45].

Bakkaloglu *et al.* [40] analysed the presence of associated diseases in 2,838 Turkish FMF patients: the frequency for BD was 0.5% i.e. 500 per 100,000. They claimed that this frequency was significantly higher than in a control population. However the comparative group was probably represented by 46,813 children in Turkey among whom not a single case of BD was found [44]. On the other hand, the mean age for the population studied was 23 (ranging from 2-87 years), making the quoted statistic rather meaningless [44].

In a well-conducted study, Fresco *et al.* [46] did not find a higher than expected number of FMF patients among 344 BD patients. The prevalence of FMF was similar among a RA patient cohort as well as among the group of healthy controls. More recently, the same group found that none out of 108 patients with FMF fulfilled the diagnostic criteria for BD [47]. Thus the issue of the prevalence of concomitant FMF and BD deserves further studies. Whether there is an association between these two diseases or whether the occurrence is due to the high prevalence of these two diseases in the same area is not clear.

Toutou *et al.* [18] studied a cohort of 114 chromosomes from diagnosed BD patients from an ethnically mixed population for common FMF mutations, and screened an ethnically-matched cohort of FMF and control chromosomes in parallel. The M694V, V726A, and E148Q mutations tended to be more frequent in BD (2.6%, 2.6% and 5.2% respectively) than in controls (0%, 0% and 2.2% respectively). Because *MEFV* mutations were more frequent in BD than in controls the authors suggest that they may act as additional susceptibility factors for BD.

Atagunduz *et al.* [48] further investigated the presence of *MEFV* mutations in a homogeneous population of BD patients from Turkey, a country (see above) with a high prevalence of both BD and FMF. The frequencies of three *MEFV* mutations (M694V, V726A and M680I) were investigated in 57 BD patients. Fifteen BD patients were found to carry one single *MEFV* mutation (26%) compared with 9.1% in the control group ($p=0.0003$). Moreover in the same study, amongst 20 patients with vascular involvement, 11 (55%) had *MEFV* mutations compared to four patients (11%) in the group not showing vascular involvement ($p=0.001$). Severe vascular complications such as Budd-Chiari syndrome, superior vena cava thrombosis and vascular neuro-BD were found to be present only in the group positive for *MEFV* mutations. M694V was the dominant mutation (11 out of 15 patients with mutated alleles) Finally, six out of seven female patients with vascular involvement carried *MEFV* mutations in contrast to five out of 13 male patients ($p=0.07$). Since male BD patients are described as having a more severe clinical course of disease and a greater degree of vascular involvement, the authors conclude that *MEFV* mutations might be a more crucial genetic factor in female patients with vascular involvement. No association with other clinical manifestations was observed.

In another study, a similar frequency of *MEFV* mutations among BD patients (30.2%) had been previously reported [45]. However, this study originates from Israel, a country where *MEFV* mutations are more common than in Turkey. When the authors compared the carrier rate of each ethnic group in the BD cohort with the expected carrier rate in the same ethnic groups in the general population the only group found to have a significant value were the Arabs. None of the BD patients with a single *MEFV* mutation had any clinical manifestation of FMF. BD patients with and without *MEFV* mutations had similar ages at onset of BD, a similar frequency of HLAB51, and similar clinical manifestations such as the numbers of sites or organs involved [45].

From these studies one can conclude that BD patients have a higher frequency of *MEFV* mutations than controls, and that this high prevalence provides a further argument to support the proposal that *MEFV* mutations may participate in the manifestation of inflammatory disorders other than FMF. One can also conclude from these studies that those BD patients with a mutation of one *MEFV* allele do not have any symptoms of FMF. This last conclusion is in discord with the results and interpretation of the study by Livneh *et al.* [49]. These authors reported a thorough study of eight FMF-BD patients who were heterozygous for the M694V mutation and in whom no additional mutation was found on the non-carrier chromosome. Further widening their conclusion, they claimed that these findings "may mirror a more generalized rule that FMF may be precipitated in carriers of

a single mutated gene by environmental factors or genetic factors not directly associated with *MEFV*". This hypothesis requires further studies.

II.2. Inflammatory Bowel Diseases (IBD)

In 1962 one case of Crohn's disease was observed in a cohort of 50 FMF patients [50]. Two years later this case was completely described [51]. The patient, a 56 old Jewish woman from Morocco suffered from severe Crohn's disease and died from an intestinal perforation. Thirty-five years later, one FMF case with sacroileitis (SA), and three familial cases showing association between FMF and Crohn's disease (one with FMF in the two grandparents, one with FMF in the father and one with FMF in a sister) were identified in a French cohort of 832 Crohn's disease patients (whose ethnic origins were not mentioned) [52].

More recently 173 French non-Ashkenazi Jewish families with FMF (300 patients) were assessed for inflammatory bowel diseases [17]. Within nine families 14 patients had FMF, three patients had both diseases (one with Crohn's disease, the other two with ulcerative colitis) and eight FMF patients had IBD cases in their families (six with Crohn's disease, one with ulcerative colitis and one with unclassified colitis). In five of these families FMF and IBD existed in first-degree relatives. Familial IBD was seen in two families. Severe IBD was detected in seven patients. In the three patients with both diseases, their ages at diagnosis of IBD were 18, 16, and 9 years, and their ages at radical surgery (ileo-colectomy, colectomy, and total colectomy) were 21, 18, and 14 years, respectively. In the IBD patient group (11 cases) the mean age at diagnosis of IBD was 18 (9-25) years and the average age at surgery (seven patients) was 22 (14-31) years. These seven patients underwent 12 major procedures, and five of them were in clinical relapse 2, 7, 14, 11 and 6 years after the last surgical procedure. SA was present in two of the Crohn's disease patients. Amyloidosis was present in two FMF patients, one of whom had also IBD. Patients were genotyped in the two mutation hotspots present in the *MEFV* gene (exon 2 and 10), and all but one patient had at least one M694V mutation. Homozygosity for M694V was detected in 12 of 15 FMF patients, including the three patients with IBD. This study did not investigate a comparative control group, or a group of normal subjects. The NOD/CARD15 gene, a susceptibility gene for Crohn's disease [53] is being studied in these and other FMF-IBD patients.

Recently Fidler *et al.* [19] discovered seven patients with Crohn's disease (CD) in a registry of 4,978 FMF patients. Two control groups, one with 20 age-matched FMF patients without CD and the other with CD alone were analysed. FMF in FMF-CD patients was characterized by a higher frequency of attacks and increased prevalence of amyloidosis, but the overall severity score was said to be similar in both groups. Nevertheless, ankylosing spondylitis was observed in two patients, polyarthritis in one, muscular dystrophy in one, mixed connective tissue disease with primary sclerosing cholangitis and nephritis in a fifth patient. Finally, four patients were diagnosed with a third inflammatory condition. Three of these seven FMF-CD patients had amyloidosis. Genotyping for FMF showed homozygosity for M694V in three of the seven patients, compound heterozygosity in one (M694V/V726A), and only one mutated allele (M694V) in another. One patient refused genetic analysis and in another patient no mutations were found in any of the hotspots analysed. All but one patient had a family history of FMF. Crohn's disease presented at a significant later age in the FMF-CD group (40.6 years versus 26.2 years in the Crohn's disease-only group). In this patient group, the severity and other characteristics of their Crohn's disease were comparable to those seen in the Crohn's disease alone control group.

Are these data sufficient to admit IBD or CD as FMF-associated diseases? These two studies are retrospective; they do not involve control groups (in the first study), or a control group with an unrelated digestive disorder (the second study). The prevalence of IBD in Israel is well known [54-57]. The last published prevalence of Crohn's disease in this country (25.5 per 100,000 in 1987 and 65.1 per 100,000 in 1997) [54] is certainly lower than the 7 per 4,000 (175 per 100,000) FMF patients seen by Fidler *et al.* [19]. However the low number of patients in their series, the heterogeneity of the origins of their patients (two Iraqis, two Libyans, two North Africans and one Kurd) must be underlined. The prevalence of IBD in the French Jewish population is not known, and is perhaps not the same as in Israel, the environmental factors in France being different. The severity of IBD or CD in patients carrying one or two *MEFV* mutations, almost all in the first study, deserves comment. *MEFV* gene mutations may act as modifiers, affecting the expression of IBD. Other inflammation-associated genes may trigger FMF and IBD. For instance, MICA (major histocompatibility complex class I chain related gene A) has been associated with IBD [58] and with FMF [59].

III. Miscellaneous

III.1. Streptococcus Associated Diseases

The presence of high levels of antistreptolysin O (ASO) antibodies and streptococcus-associated diseases, such as acute post-streptococcal glomerulonephritis and acute rheumatoid fever [60, 61], have been reported in patients with FMF. In Turkey, the prevalence of rheumatic heart disease is higher in FMF patients than in the normal population [61]. The ASO levels are higher in FMF patients with arthritis attacks than in FMF patients with other kinds of attacks, and higher in these patients than in patients in a FMF attack-free interval [61]. ASO and antideoxyribonuclease B levels in patients with FMF and without history or clinical evidence of upper respiratory tract infection are higher than those in healthy controls [60]. ASO and antideoxyribonuclease B titres were higher in FMF patients than in controls four weeks after a documented group A beta- haemolytic streptococcal pharyngitis [60]. Patients with FMF certainly have an exaggerated response to streptococcal antigens and may be more prone to the late complications of streptococcal infection.

III.2. Juvenile Inflammatory Arthritis

Juvenile inflammatory arthritis is not statistically associated with FMF. However particular severity has been observed in two patients (one homozygous for the M694V mutation and the other a compound heterozygote for M694V-E148Q/ E148Q mutations). These two patients had a polyarticular course with destructive arthritis and a rapid evolution necessitating orthopedic surgery [21]. Other similar cases have been observed but not published.

III.3. Systematic Lupus Erythematosus

Langevitz *et al.* [62] found a low prevalence of systemic lupus erythematosus (SLE) in a series of FMF patients (3 per 6,000). These patients presented a low-grade SLE associated with low/normal levels of anti-SAP, in contrast to the elevated anti-SAP levels in most active SLE patients. This finding possibly sheds light on the low incidence and mild course of SLE in FMF patients.

III.4. Multiple Sclerosis

Mutations in *MEFV* seem to increase the risk of rapid deterioration in non-Askenazi multiple sclerosis patients [63].

PUTATIVE SELECTIVE ADVANTAGES IN CARRIERS OF *MEFV* MUTATIONS

The fact that around 70 *MEFV* gene mutations have appeared since pre-Biblical times, and have expanded in the countries of the Mediterranean basin suggests that the large number of carriers of *MEFV* mutations observed (1/3-1/5 of the population) in the ethnic groups involved is not only the result of founder effects and/or the effect of a well

known endogamy [64]. A very important advantage has perhaps helped these carriers to survive better than the non-carriers, similar to the heterozygote advantage against malaria seen for the sickle cell trait. It has been shown that a small increase in heterozygote fitness, less than 5%, is sufficient to sustain a high frequency of the deleterious allele. This advantage for the carriers of *MEFV* mutations is not obvious. It has been suggested that heterozygotes were possibly better protected against asthma [65, 66] and allergic rhinitis [67]. The decreased allergic response in FMF patients and carriers, if proven, may be a result of, rather than the cause of, underlying patho-physiology. Moreover, allergy is not a fatal disease, and one wonders whether this was just a bystander effect rather than a causative factor in the selection [66]. It may be that this putative advantage was operative against diseases that are now well-treated or have disappeared from the areas of interest. Devastating tuberculosis, terrible epidemics of smallpox, cholera, plague, exanthematic typhus and even malaria are well known in the history of the Mediterranean basin.

The analysis of the pyrin/marenostrin protein raises arguments in favour of a role in the defense against one or several bacterial species [20]. Most patients with FMF carry missense mutations in the C-terminal half of the pyrin protein. To study the physiologic role of pyrin, Chae *et al.* generated mice expressing a truncated pyrin molecule that, similar to FMF patients, retains the full PYRIN domain. When stimulated, macrophages from these mice produce increased amounts of activated caspase-1 and consequently, elevated levels of mature IL-1 β . These data support a critical role for pyrin in the innate immune response, possibly by acting on ASC, and suggest a biologic basis for the selection of hypomorphic pyrin variants in man.

On the other hand, hyperexpression of TH1 has been observed in patients with FMF and in heterozygotes for a *MEFV* mutation [6]. Th1 clones produce the pro-inflammatory cytokines IL2, INF γ , and TNF β , favour cell-mediated cellular immunity and activate monocytes. Centola *et al.* [9] have shown that the *MEFV* gene plays a role in INF- γ -mediated inflammation, being a downstream element in this cascade, and is effective in the phagocyte response in general. They concluded that *MEFV* functions in a negative feedback loop for TH1 [9]. A TH1 profile has been found to be predominant in infections due to intracellular bacteria such as tuberculosis [68], typhus [69], and the malaria parasite [70].

Finally, the role of natural selection by infectious diseases in shaping human evolution is a subject of considerable importance and growing interest. Major causes of constant mortality such as tuberculosis, or episodes of great mortality during epidemics of other infectious diseases, have certainly had considerable potential to exert selective pressure in favour of human gene mutations that confer protection against them. For instance, population variations in susceptibility to tuberculosis have been associated with polymorphisms in a number of genes [71].

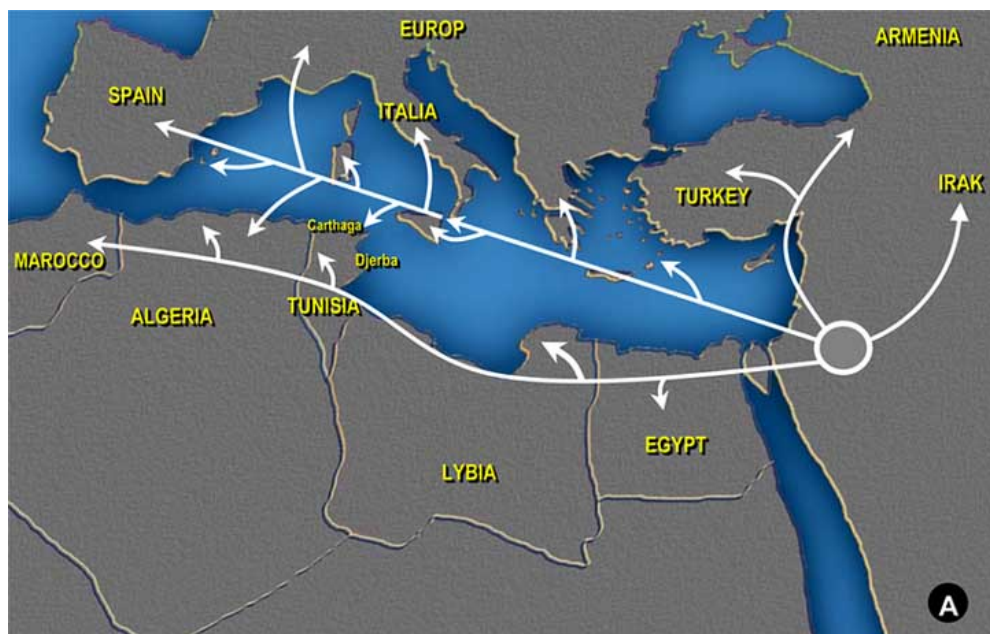


Fig. (1A). The Diaspora began in the VIth century B.C., and accelerated after the second destruction of the Temple in the year 70 A.D.

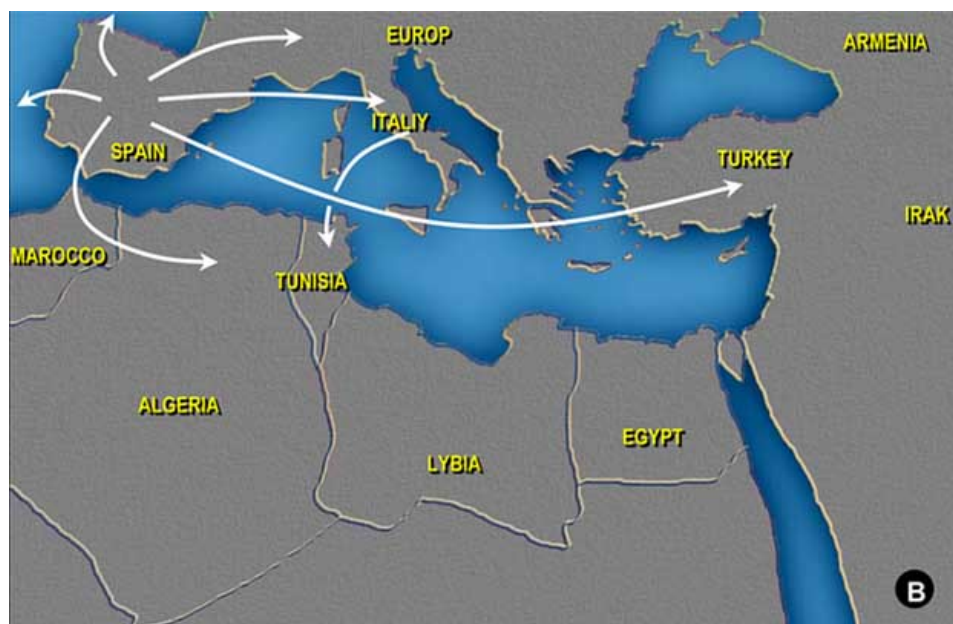


Fig. (1B). Destinations of the Iberian peninsula Jews during the Inquisition.

The study of mortality from infectious diseases in FMF patients is difficult, because antibiotic treatments appeared when FMF was first described [1]. Nevertheless an indirect approach, but an approach not to be neglected, could be the comparative and historical study of causes of mortality from the beginning of the XXth century in Mediterranean cities, where the causes of mortality can be retrospectively studied in ethnic groups, and where one of these groups is known to have had a high rate of MEFV mutations for centuries. Such a study requires several methodological criteria including firstly, a serious and regular census of the population as a whole, and then by ethnic groups. Secondly, death certificates with obligatory inscriptions of the main cause of death before authorisation for burial should be available, and thirdly, analyses and statistical descriptions of these data should be made by epidemiologists.

This type of study is possible in the city of Tunis (Tunisia) during the French Protectorate. The administrative records from this period allow the study of the different ethnic populations (French, Arab, Jewish, Italian, Maltese) owing to a census taken approximately every five years (1911, 1921, 1926, 1931, 1936, 1946) ; furthermore, since 1885 a death certificate stating the main cause of death and the ethnic group of the deceased was obligatory before burial. The annual "Statistiques démographiques et médicales; Régence de Tunis, Protectorat français" and the "Annuaire de la Tunisie" published by the Institut Pasteur of Tunis with Charles Nicolle (Nobel Prize laureate for the discovery of the typhus vector), Ernest Conseil and their students contain these data for each year from 1909 to 1955. Socio-economic data for the same period (analysis of the conditions of life: housing types, population density, drips, access to water, incomes and so on) are given by historians [72]. Statistics for the infant mortality rate (IMR), that is the number of infants dead before the age of one year per 1000 infants born alive, a good socio-economic index, are also available [73]. In addition to the mortality data, the morbidity rate is available for certain diseases except, curiously, for tuberculosis. Partial statistics on tuberculosis morbidity are available in the form of data from civil and military physicians, universities, and some administration medical services, for the period following the Second World War.

Jews had been living in Tunis for at least five centuries before the second destruction of the Temple. The majority of this Jewish population originates from Palestine, rather than Spain, and is improperly called the Sefarad. Spanish Jews, choosing the Mahgreb as a land of hope during the Inquisition, migrated to Morocco and Algeria, and more rarely to Tunisia, from 1492 on. However, in the XVIth and XVIIth centuries some Spanish and Portuguese Jews came to Tunis from the cities of Tuscany, in Italy, where their ancestors had been welcomed by the Medicis during the Inquisition. This small part of the Jewish population of Tunis (about 1/10) came to Tunis from Italy firstly for Christian slave ransoming, then for trade, and stayed there in a somewhat difficult cohabitation with the ancient Jews (Fig. 1; A,B). This ancient Jewish population shared the same traditions with other Oriental Jews, including the custom of endogamy, as

for all the Jews of the Mediterranean basin, and probably shared the same genetic peculiarities. It is highly probable that in the first half of the XXth century the prevalence of MEFV mutations in this ancient Jewish population was the same as that we observe now in French or Israeli Jews born in North Africa (or whose parents were born in North Africa); that is 1/3-1/5 of this group [74, 75].

In Tunis, the general mortality per 1,000 from 1909 to 1956 was 33.02 (19.1-47.7) for Muslims, 15.4 (8.6-18.8) for Europeans and 20.1 (9.6-29.8) for Jews [76]. The IMR was 204.3 (155-241) for Muslims, 89.9(37-175) for Europeans and 118.5(54-189) for Jews. These differences between the three groups are statistically significant. Thus, the general mortality and the IMR were very high in Muslims (the IMR in Muslims was higher than the IMR in a list of 15 European countries in the years 1937-1938). The general mortality was lower in Jews than in Muslims (nevertheless, the IMR in the Jewish population heads the same list in the period). The lowest general mortality and IMR were observed in Europeans.

The most interesting results concern mortality from tuberculosis [76]. Tuberculosis was the only disease for which mortality was constantly lower in Jews than in the French living in Tunis; exactly half that of the rate for French living in France, (Fig. 2) and significantly lower than the mortality rate in other Europeans (Italians and Maltese) The mortality from tuberculosis per 100,000 was 506 (160-785) in Muslims, 133.4 (13-184) in Europeans and 76.4 (25-109) in Jews. The number of deaths from tuberculosis per 100 deaths in the same groups was 13.6 (7.3-18.2), 7.21 (1.3-11) and 4.3 (2.2-8.3) respectively. The percentage of pulmonary tuberculosis in the total mortality from tuberculosis (pulmonary and extra-pulmonary) was 83.8 (75.6-88.2) in Muslims, 78.3 (71.4-81.2) in Europeans and 67.3 (57-79.3) in Jews. All of these differences were statistically significant. Data are available for the period 1919-1938 (except for 1933 and 1934) for the French, Muslim, Jewish, Italian, and Maltese populations. Interestingly the mortality from tuberculosis in Italians (immigrants from the south of Italy; Puglia, Calabria and Sicily) and in Maltese was significantly lower than that seen in Muslims and French, and statistically higher than the mortality rate in Jews [76]. The reasons for this low mortality from tuberculosis in the Jewish (and to a lesser degree in the Italian and Maltese) patients of Tunis are not known. Such a low mortality rate was not observed for the other infectious diseases. From numerous data, we know that the prevalence of tuberculosis was the same, and according to some reports higher, in Jews than in Muslims, and certainly higher than in the French before the appearance of antibiotic treatments [77]. The low mortality from tuberculosis in Jews cannot be explained by differences in the socio-economic status among the study groups: indeed the standard of living of the Jews and the Muslims did not differ, and was much lower than that of the Europeans. Jews were living as an isolated group in a ghetto, La Hara, in the Muslims' Medina, whereas Europeans were living inside the modern city. Thus, in Tunis in the first half of the XXth century the IMR was very

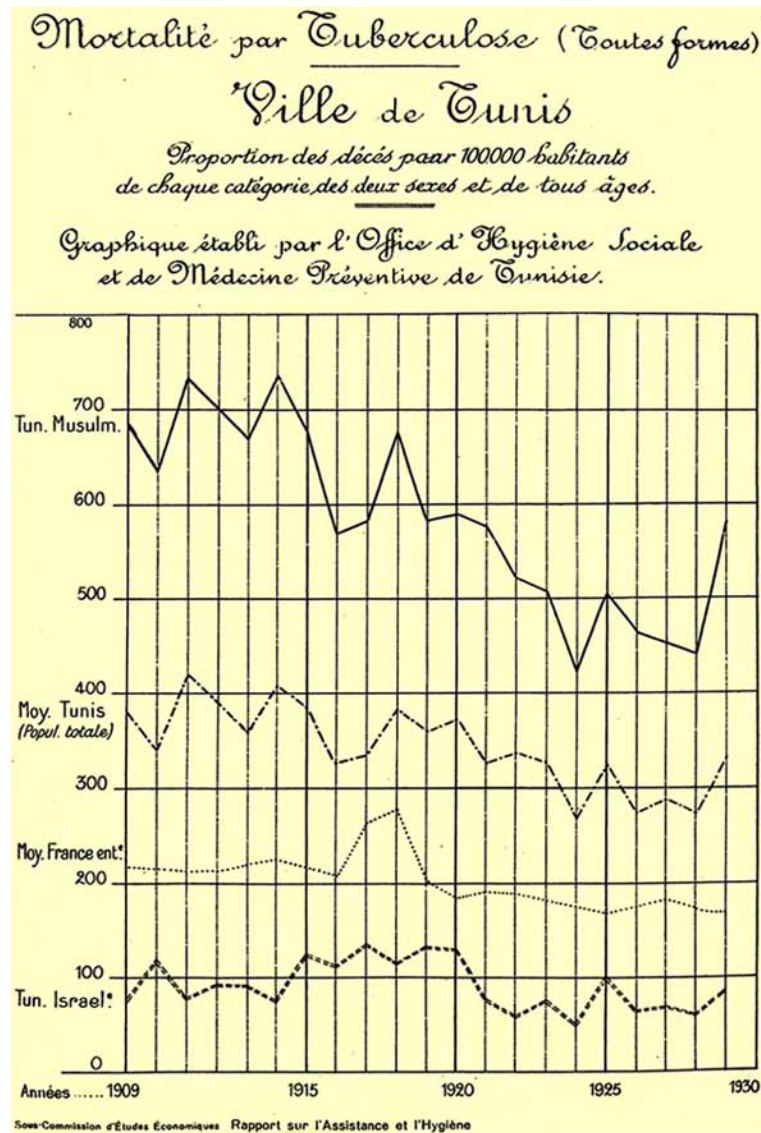


Fig. (2). Mortality from tuberculosis in Tunis (Tunisia) from 1909 to 1930. The official document from the Tunisian Office of Social Hygiene and Preventative Medicine is shown. Tun. Musulm., Tunisian Muslims; Moy. Tunis, Mean of the total population of Tunis; Moy. France ent., Mean of the French living in France; Tun. Israel., Tunisian Jews.

much higher in Jews than in Europeans (in particular in the French), but mortality from tuberculosis was very much lower. For instance, in Jews from Tunis in the 1930s the mortality from tuberculosis was half of that in France (Fig. 2), but the IMR was three times higher.

The explanations advanced for this well-known low mortality from tuberculosis in Jews include: wet rather than dry sweeping, strict control of meat, and low milk consumption [72, 78-80]. However, these explanations are not satisfying since they do not explain the discrepancy between morbidity and mortality and the low mortality from tuberculosis in the Italian and Maltese patients. As was raised in Gibraltar [81, 82], misreporting of tuberculosis due to the stigma attached to the disease is a known source of error but this hypothesis supposes a medical complicity favoring one ethnic group over another and is denied by the surviving doctor interviewed by the author. Finally, it is possible that tuberculosis was taken in charge later in Arab population than in the Jewish population, but this hypothesis does not hold for the French.

However, according to 24 studies collected by Arnould [83] and compiled 25 years later by Rakower [84], the mortality, but not the morbidity, of tuberculosis in Jews living in various cities of Eastern and Western Europe, North America and the Maghreb, appeared to be lower than in other groups from the same cities regardless of their economic status. Specific genetic advantages were invoked, for instance, heterozygosity for Tay-Sachs and Gaucher diseases in Ashkenazi Jews. In

Gibraltar the low tuberculosis mortality in the Jewish population has been well studied, and was linked to the low IMR in the very rich Gibraltar Jewish families [81, 82]. However, this is an exception, and what is important is that the low tuberculosis mortality in Jews did not depend on the socio-economic status.

S. Özen *et al.* [66] analyzed a Turkish population for whether the presence of *MEFV* gene mutations increased resistance to tuberculosis in the mutation carriers. The carrier frequency among tuberculosis patients was almost 1/6, and the difference with healthy controls was not significant. However *resistance* to tuberculosis, lower *severity* of tubercular disease and lower *mortality* from tuberculosis remain a good hypothesis for *MEFV* gene mutation carriers. To the indirect arguments given by Özen *et al.* we can add that Ethiopian and Yemeni Jews do not carry FMF or *MEFV* mutations [85, 86] and had, in the fifties, high rates of severe and lethal pulmonary and extra-pulmonary tuberculosis [84, 87].

Has the Koch bacillus, in the past, been one of the possible triggers of FMF characterized by polymorphonuclear serositis attacks? Have the M694V and E148Q *MEFV* gene mutations in Sephardic Jews, the other mutations in Ashkenazi Jews, and their chronic increased acute phase inflammatory response provided selective advantages against tuberculosis in homozygotes and heterozygotes? It will be interesting to assess the prevalence of *MEFV* mutations in Muslims living in Tunisia (a mixed population with Berbers, Andalous, Mamlûks, Moors, blacks from Africa)

and compare this with the low prevalence of *MEFV* mutations observed in North African Muslims living in France (1 North African Muslim with a *MEFV* mutation for 6 North African Jews, the North African population in France being 5 million and the Jewish population 500,000), and also to assess the prevalence of *MEFV* mutations in the south of Italy, Sicily [88] and Malta. Finally, the prevalences of *MEFV* mutations in Sicily, Calabria and in Malta are perhaps as high as in Cyprus [89]. Indeed, in 1492, at the beginning of the Spanish Inquisition, 150,000 Jews lived in South Italy, Sicily and Malta. Half of them accepted to convert to Catholicism to stay at home.

In tuberculosis the immune response to the intracellular pathogen *Mycobacterium tuberculosis* is based on TH1. Macrophage activation and granuloma formation are TH1-dependent. Markedly enhanced susceptibility to tuberculosis in mice deficient for interferon [90, 91] or IL12 [92] has been observed. Hence FMF patients could be more resistant to mycobacterium due to their increased TH1 response.

IL-1 signaling is essential for host defense during murine pulmonary tuberculosis [93]. The IL-1 type 1 receptor is essential for IL-1 mediated signaling events in mycobacterial infection [94] and IL-1 has a protective role in mycobacterial infection in IL-1 α/β double-knockout mice [95]. IL-1 is produced at the site of infection during tuberculosis in man [93]. Interleukin IL-1 β and TNF α are produced by apoptotic cells during the course of *Mycobacterium tuberculosis* induced apoptosis [96]. Lipoarabinomannan, a cell wall component of *Mycobacterium tuberculosis* may be an important stimulator of innate immunity in infection with *Mycobacterium tuberculosis* via mechanisms that involve endogenous IL-1 activity [97]. A 19-kDa cell wall *Mycobacterium tuberculosis* lipoprotein is the main signal required to trigger both apoptosis and the release of IL-1 β during the early stages of mycobacterial infection [98]. It is now generally admitted that FMF is due to mutation in pyrin/marenostrin which normally inhibits prointerleukin-1 β cytokine processing to the active form [99].

Actually nothing can prove the hypothesis that the low mortality from tuberculosis observed in Jews living in Tunisia in the first half of the XXth century is explained by the high prevalence of *MEFV* mutations. The facts reported here are indirect and not demonstrative arguments. They do, however, give a strong indication of the direction for further research.

ACKNOWLEDGEMENTS

Professor Paul Sebag, historian, *in memoriam*. Professor Mohamed El Aziz Ben Achour, Mayor of the Medina of Tunis; Professor André Abitbol, University of Tunis.

REFERENCES

- Mamou, H.; Cattani, R. *Sem. Ho. Paris*, **1952**, *28*, 1061-1070.
- Sohar, E.; Gafni, J.; Pras, M.; Heller, H. *Am. J. Med.*, **1967**, *43*, 227-253.
- Tunca, M.; Kirkali, G.; Soyuturk, M.; Akar, S.; Pepsy, M.B.; Hawkins, P.M. *Lancet*, **1999**, *353*, 1415.
- Gang, N.; Drenth, J.P.; Langevitz, P.; Zemer, D.; Brezniak, N.; Pras, M.; van Meer J.W.; Livneh, A. *J. Rheumatol.*, **1999**, *26*, 890-897.
- Oktem, S.; Yavuzsen, B.; Sengui, B.; Akhunar, H.; Resmi, H.; Kirkali, G.; Akar, S.; Tunca, M. *Clin. Exp. Rheumatol.*, **2000**, *18*, 73. (Abstract)
- Aypar, E.; Ozen, S.; Okur, S.; Kutluk, T.; Besbas, N.; Bakkaloglu, A. *J. Rheumatol.*, **2003**, *30*, 3011-2013.
- The French FMF consortium. *Nat. Genet.*, **1997**, *17*, 25-31.
- The International FMF Consortium. *Cell*, **1997**, *90*, 797-807.
- Centola, M.; Wood, G.; Frucht, D.M.; Galon, J.; Aringer, M.; Farrell, C.; Kingma, D.W.; Horwitz, M.F.; Mansfield, E.; Holland, S.M.; O'Shea, J.J.; Rosenberg, H.; Malech, H.I.; Kastner, D. *Blood*, **2000**, *95*, 3223-3231.
- Drenth, J.P.H.; van der Meer, J.W.M. *N. Engl. Med.*, **2001**, *345*, 1748-1757.
- Hazenbergh, B.P.C.; Limbyrg, P.C.; Bijzet, J.; Rijswijk, M.H. *Ann. Rheum. Dis.*, **1999**, *58*, 96-102.
- Lachmann, H.J.; Booth, D.R.; Booth, S.E.; Bybee, A.; Gallimore, R.; Soyuturk, M.; Akar, S.; Sengul, B.; Yavuzsen, T.U.; Tunca, M.; Hawkins, P.N. *Clin. Exp. Rheumatol.*, **2000**, *18*, 72. (Abstract)
- Poland, D.C.W.; Drenth, J.P.H.; Rabinovitz, E.; Livneh, A.; Bijzet, J.; van het Hof, B.; Van Dijk, W. *Ann. Rheum. Dis.*, **2001**, *60*, 777-780.
- Notarnicola, C.; Didelot, M.N.; Seguret, F.; Demaille, J.; Touitou, I. *Genes Immun.*, **2002**, *3*, 43-45.
- Booth, D.R.; Lachmann, B.L.; Gillmore, J.D.; Booth, S.E.; Hawkins, P.N. *QJM*, **2001**, *94*, 527-531.
- Ozdogan, H.; Sayman, M.; Melikoglu, M.; Altiparmak, M.R.; Kasapcapur, O.; Arisoy, N.; Tuzuner, N.; Ereke, E.; Yazici, H. *Clin. Exp. Rheumatol.*, **2000**, *18*, 96. (Abstract)
- Cattan, D.; Notarnicola, C.; Molinari, N.; Touitou, I. *Lancet*, **2000**, *355*, 378-379.
- Touitou, I.; Magne, X.; Molinari, N.; Navarro, A.; Quellec, A.L.; Picco, P.; Seri, M.; Ozen, S.; Bakkaloglu, A.; Karaduman, A.; Garnier, J.M.; Demaille, J.; Kone-Paut, I. *Hum. Mutat.*, **2001**, *16*, 271-272.
- Fidder, H.H.; Chowers, Y.; Lidar, M.; Sternberg, M.; Langevitz, P.; Livney, A. *Medicine*, **2002**, *81*, 411-416.
- Chae, J.J.; Komarow, H.D.; Cheng, J.; Wood, G.; Raben, N.; Liu, P.; Kastner, D. *Mol. Cell*, **2003**, *11*, 591-604.
- Ozen, S.; Bakkaloglu, A.; Yilmaz, E.; Duzova, A.; Balci, B.; Topaloglu, R.; Besbas, N. *J. Rheumatol.*, **2003**, *30*, 2014-2018.
- Savage, C.O.S.; Harper, L.; Cockwell, P.; Adu, D.; Howie, A.J. *BMJ*, **2000**, *320*, 1325-1328.
- Benhamou, E.; Albou, A.; Destaing, F.; Ferrand, B. *Bull. Mem. Soc. Med. Hop. Paris*, **1954**, *70*, 47-254.
- Ozdogan, H.; Arisoy, N.; Kasapcapur, O.; Sever, L.; Caliskan, S.; Tuzuner, N.; Mat, C.; Yazici, H. *J. Rheumatol.*, **1997**, *24*, 323-327.
- Tinaztepe, K.; Gucer, S.; Bakkaloglu, A.; Tinaztepe, B. *Eur. J. Pediatr.*, **1997**, *156*, 505-506.
- Basaranoglu, M.; Mert, A.; Tabak, F.; Apaydin, S.; Aktuglu, Y.; Ozdogan, H. *Rheumatology*, **1999**, *38*, 794-796.
- Ozen, S. *Curr. Opin. Rheumatol.*, **1999**, *11*, 393-398.
- Ozen, S.; Ben-Chetrit, E.; Bakkaloglu, A.; Gur, H.; Tinaztepe, K.; Calguneri, M.; Turgan, C.; Turkmeh, A.; Akpolat, I.; Danaci, M.; Besbas, N.; Akpolat, T. *Semin. Arthritis Rheum.*, **2001**, *30*, 281-287.
- Hatemi, G.; Masatlioglu, S.; Gogus, F.; Seyahi, E.; Ozdogan, H. *Clin. Exp. Rheumatol.*, **2002**, *20*, S103(Abtract)
- Akar, S.; Goktay, Y.; Akinci, B.; Tekis, D.; Biberoglu, K.; Birlik, M.; Onen, F.O.; Tunca, M.; Akkoc, N. *Rheumat. Int.*, **2004** (on line)
- Michet, C.J. *Rheum. Dis. Clin. North Am.*, **1990**, *16*, 261-268.
- Gur, H.; Tchakmakjian, L.; Eherentfeld, M.; Sidi, Y. *Am. J. Med. Sci.*, **1999**, *11*, 393-398.
- Tekin, M.; Yalcinkaya, F.; Tumer, N.; Akar, N.; Misirlioglu, M.; Cakar, N. *Acta Paediatr.*, **2000**, *89*, 177-182.
- David, J.; Ansell, B.M.; Woo, P. *Arch. Dis. Child*, **1993**, *69*, 685-688.
- Langevitz, P.; Zemze, D.; Livneh, A.; Shemer, J.; Pras, M. *J. Rheumatol.*, **1994**, *21*, 1708-1709.
- Tekin, M.; Yalcinkaya, F.; Tumer, N.; Cakar, N.; Kocak, H.; Ozkaya, N.; Gencgonul, H. *Nephro. Dial. Transplant*, **1999**, *14*, 475-479.
- Flatau, E.; Kohn, D.; Schiller, D.; Lurie, M.; Levy, E. *Arthritis Rheum.*, **1982**, *25*, 42-47.
- Schlesinger, M.; Rubinow, A.; Vardy, P.A. *Isr. J. Med. Sci.*, **1985**, *21*, 83-85.
- Gershoni-Baruch, R.; Broza, Y.; Brik, R. *J. Pediatrics*, **2003**, *143*, 658-661.
- Bakkaloglu, A.; Ozen, S.; Topaloglu, R.; Dusunsel, R.; Simsek, H. *Clin. Exp. Rheumatol.*, **2002**, *20*, S90 (Abstract)
- Sakane, T.; Takeno, M.; Suzuki, N.; Inaba, G. *NEJM*, **1999**, *341*, 1284-1291.
- Schwartz, T.; Langevitz, P.; Zemer, D.; Gazit, E.; Pras, M. Livneh, A. *Semin. Arthritis Rheum.*, **2000**, *29*, 286-295.
- Livneh, A. *Clin. Exp. Rheumatol.*, **2003**, *21*, 266.
- Ben-Chetrit, E.; Yazici, H. *Clin. Exp. Rheumatol.*, **2003**, *20*, S1-2.
- Ben-Chetrit, E.; Cohen, R.; Chajek-Shaul, T. *J. Rheumatol.*, **2002**, *29*, 530-534.
- Fresko, I.; Masatoglu, S.; Melitlioglu, M.; Tunc, R.; Biyikli, H.; Ozdogan, H.; Yazici, H. *Clin. Exp. Rheumatol.*, **2000**, *18*, 301.
- Tunc, R.; Hulan, A.; Malikoglu, M.; Ozyazgan, Y.; Ozdogan, H.; Yazici, H. *Clin. Exp. Rheumatol.*, **2001**, *19*, S54-S47.
- Atagunduz, P.; Ergun, T.; Direskendi, H. *Clin. Exp. Rheumatol.*, **2003**, *21*, S35-S37.
- Livneh, A.; Aksentijevich, I.; Langevitz, P.; Torosyan, Y.; G-Shoham, N.; Shinar, Y.; Pras, E.; Zaks, N.; Padeh, S.; Kastner, D.L.; Pras, M. *Eur. J. Hum. Genet.*, **2001**, *9*, 191-196.
- Cattan, R.; Khayat, G.; Hirsh-Marie, H. *Bull. Mem. Soc. Med. Hop. Paris*, **1962**, *113*, 1137-1155
- Cattan, D. *Thèse de Médecine AGEMP, Paris*, **1964**.
- Beaugerie, L.; Lamy, P.; Ganne, N.; Carbonnel, F.; Le Quintrec, Y.; Cosnes, J.; Gendre, J.P. *Presse Med.*, **1997**, *26*, 892-89.
- Hugot, J.P.; Chamaillard, M.; Zouali, H.; Lesage, S.; Cezard, J.P.; Belaiche, J.; Almer, S.; Tysk, C.; O'Morain, C.A.; Gassul, M.; Binder, V.; Finkel, Y.; Cortot Modigliani, R.; Laurent-Puig, P.; Gower-Rousseau, C.; Macry, J.; Colombel, J.F.; Sahbatou, M.; Thomas, G. *Nature*, **2001**, *411*, 599-603.
- Niv, Y.; Abuksis, G.; Fraser, G.M. *Am. J. Gastroenterol.*, **1999**, *94*, 2961-2965.
- Niv, Y.; Abuksis, G.; Fraser, G.M. *Am. J. Gastroenterol.*, **2000**, *95*, 693-698.
- Odes, H.S.; Fraser, J.; Krawiec, J. *Scand. J. Gastroenterol.*, **1989**, *170*, 36-38.
- Odes, H.S.; Locker, C.; Neumann, L.; Zirkin, H.J.; Weizman, Z.; Sperber, A.D.; Fraser, G.M.; Krugliak, P.; Gaspar, N.; Edelman, L. *Am. J. Gastroenterol.*, **1994**, *89*, 1859-1862.
- Orchard, T.R.; Dhar, A.; Simmons, J.D.; Vaughan, R.; Welsh, K.I.; Jewel, D.P. *Clin. Exp. Immunol.*, **2001**, *126*, 437-440.
- Touitou, I.; Picot, M.C.; Domingo, C.; Notarnicola, C.; Cattani, D.; Demaille, J.; Kone-Paut, I. *Arthritis Rheum.*, **2002**, *44*, 163-169.

- [60] Yalcinkaya, F.; Ucar, U.T.; Ozkaya, N.; Tekin, M.; Elhan, A.H.; Tutar, E.; Guriz, DH; Aysev, D.; Gokdemir, R.; Dogtu, U.; Tumer, N. *Clin. Rheumatol.*, **2002**, *21*, 378-381.
- [61] 61. Tekin, M.; Yalçinkaya, F.; Tümer, N.; Cakar, N.; Koçak, H. *Clin. Rheumatol.*, **1999**, *18*, 446-449.
- [62] Langevitz, P.; Zandman-Goddard, G.; Blank, M.; Pras, M.; Livneh, A.; Sjhonfeld, Y. *Clin. Exp. Rheumatol.*, **2002**, *20*, S82. (Abstract)
- [63] Shinar, Y.; Livneh, A.; Villa, Y.; Pinhasov, A.; Zeitoun, I.; Achiron, A. *Clin. Exp. Rheumatol.*, **2002**, *20*, S75. (Abstract)
- [64] Pras, M. *Clin. Exp. Rheumatol.*, **2002**, *20*(Suppl. 26), S66.
- [65] Brenner-Ullman, A.; Melzer-Ofir, H.; Daniles, M.; Shohat, M. *Am. J. Med. Genet.*, **1994**, *53*, 172-175.
- [66] Ozen, S; Balli, B; Ozkara, S. *Clin. Exp. Rheumatol.*, **2002**, *20*, S57-S58.
- [67] Sackesen, C.; Bakaloglu, A.; Sekerel, B.E.; Ozaltin, F.; Besbas, N.; Yilmaz, E.; Adalioglu, G.; Ozen, S. *Ann. Rheum. Dis.*, **2004**, *63*, 187-190
- [68] Wang, J.; Wakeham, J.; Harknass, R.; Xing, Z. *J. Clin. Invest.*, **1999**, *103*, 1023-1029.
- [69] Walker, D.H.; Popov, V.L.; Feng, H.M. *Lab Invest.*, **2000**, *80*, 1361-1372.
- [70] Torre, D.; Speranza, F.; Giola, M.; Matteelli, A.; Tambini, R.; Biondy, G. *Clin. Diagn. Lab Immunol.*, **2002**, *9*, 348-351.
- [71] Lipsitch, M.; Sousa, A.O. *Genetics*, **2002**, *161*, 1599-1607.
- [72] Sebag, P. (1959) in *La Hara de Tunis*. Presses Universitaires de France, pp. 58-65. (1998) in *Tunis, histoire d'une ville. L'Harmattan*. p 443.
- [73] Reidpath, D.D.; Allotey, P. *J. Epidemiol. Community Health*, **2003**, *57*, 344-346.
- [74] Kogan, A.; Shinar, Y.; Lidar, M.; Revivo, A.; Langevitz, P.; Padeh, S.; Pras, M.; Livneh, A. *Am. J. Med. Genet.*, **2001**, *102*, 272-276.
- [75] Stoffman, N.; Magal, N.; Shohat, T.; Lotan, R.; Koman, S.; Oron, A.; Danon, Y.; Halpern, G.J.; Lifshitz, Y.; Shohat, M. *Eur. J. Hum. Genet.*, **2000**, *8*, 307-310.
- [76] Cattán, D. *Clin. Exp. Rheumatol.*, **2003**, *21*, S53-S54.
- [77] Bloch, E. Le dispensaire antituberculeux; Incidences Tunisiennes *La Tunisie Medico-sociale*, **1951**, *18*, 3-11.
- [78] Cattán, A. Bulletin de la Revue Tunisienne des Sciences Medicales de Tunis. **1914**, pp. 3-20.
- [79] Toistivint, A.; Remlinger, J. *Rev. Hyg.*, **1900**, *4*, 128-129.
- [80] Valensi, J.; Conseil, E. *Rev. Polit. Parlementaire*, **1913**, *77*, 102-117.
- [81] Sawchuk, L.A.; Herring, D.A. *Hum. Bio.*, **1984**, *56*, 291-306.
- [82] Sawchuk, L.A.; Herring, D.A.; Walks, L.R. *Am. Anthropol.*, **1985**, *87*, 616-625.
- [83] Arnould, E. *Rev. Phtisiol.*, **1934**, *15*, 466-495.
- [84] Rakover, J. *Am. Re. Tuberc.*, **1953**, *67*, 85-93.
- [85] Rozenbaum, M.; Portnoy, E.; Rosner, I. *Clin. Exp. Rheumatol.*, **2002**, *20*, S89. (Abstract)
- [86] Sohar, E.; Pras, M.; Heller, J.; Heller, H. *Arch. Int. Med.*, **1961**, *107*, 529-538.
- [87] Dolberg, O.T; Alkan, M.; Schaleffer, F. *Isr. J. Med. Sci.*, **1991**, *27*, 386-389.
- [88] La Regina, M.; Nucera, G.; Diaco, M.; Procopio, A.; Gasbarini G.; Notarnicola, C.; Kone-Paut; Touitou, I.; Mana, R. *Europ. J. Hum. Genet.*, **2003**, *11*, 50-56.
- [89] Booth, D.R.; Booth, S.E.; Rowczmeio, R.; Lachman, H.J.; Hajrioussos, V.J.; Hawkins, P.N. *Clin. Exp. Rheumatol.*, **2002**, *20*, S97. (Abstract)
- [90] Cooper, A.M.; Dalton, D.K.; Stewart, T.A.; Grffin, J.P.; Russel, D.G.; Orme, I.M. *J. Exp. Med.*, **1993**, *178*, 2243-2247.
- [91] Flynn, J.L.; Chan, J.; Triebold, K.J.; Dalton, D.K.; Stewart, T.A.; Bloom, B.R. *J. Exp. Med.*, **1993**, *178*, 2249-2254.
- [92] Cooper, A.M.; Magram, J.; Ferrante, J.; Orme, I.M. *J. Exp. Med.*, **1997**, *186*, 39-45.
- [93] Juffermans, N.P.; Florquin, S.; Camoglio, L.; Verbon, A.; Kolk, A.H.; Speelman, P.; van Derenter, S.J.H.; van der Poll, T. *J. Infect. Dis.*, **2000**, *182*, 902-908.
- [94] Sugawara, I.; Yamada, H.; Hua, S.; Mizuno, S. *Microbiol. Immunol.*, **2001**, *45*, 743-750.
- [95] Yamada, H.; Mizuno, S.; Horai, R.; Iwakura, Y.; Sugawara, I. *Lab Invest.*, **2000**, *80*, 759-767.
- [96] Ciaramella, A.; Cavone, A.; Sanyucci, MB. *J. Infect. Dis.*, **2002**, *186*, 1277-1282.
- [97] Juffermans, NP; Verbon, A; Belisle, JT; Hill, PJ; Speelman, P; van Deve, SJ; van der Poll, T. *Am. J. Respir. Crit. Care Med.*, **2000**, *162*, 486-489.
- [98] Ciaramella, A.; Cavone, A.; Santucci, MB; Garg, SK; Sanarico, N; Bocchino, G.; Galati, G.; Martino, A.; Aurichio, G.; D'Orazio, M.; Stewart, GR.; Neyrolles, o.; Young DB.; Colizi, V; Fraziano, M. *J. Infect. Dis.*, **2004**, *190*, 1167-1176.
- [99] McDermott, M. *Trends in Immunol.*, **2004**, *25*, 457-460.