

# The Suspicious Relationship Between Familial Mediterranean Fever (FMF) and Tuberculosis

## TÜBERKÜLOZ VE AİLEVİ AKDENİZ ATEŞİ ARASINDA ŞÜPHELİ İLİŞKİ

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### Summary

*Familial Mediterranean Fever (FMF) is a familial disease which affects people of Mediterranean or Middle Eastern origin. It is characterized by paroxysmal attacks of fever, peritonitis and/or pleuritis. The diagnosis of FMF is put usually clinically. J'though the exact basis of the disease is unknown, some parameters have been subject to intense investigation like tumor necrosis factor, enhanced dopamine beta hydroxylase activity and elevated serum free fatty acids. We recognized there had been an interesting co-existence of FMF and tuberculosis bacilli in three patients. This observation was made during another study examining the diagnosis of disseminated tuberculosis by PCR. In that study the PCR positivity was present in only one of 17 ppd positive healthy controls, while in all of these three cases. We present here the detail of these cases.*

**Key Words:** Tuberculosis, FMF, PCR

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Familial Mediterranean Fever (FMF) is a familial disease which almost exclusively affects people of Mediterranean or Middle Eastern origin. It is characterized by paroxysmal attacks of fever, peritonitis and/or pleuritis often occurring in association with migratory nondeforming arthritis. The diagnosis of FMF is a clinical one (1). Although the genetic/biochemical basis of the disease is unknown, several biochemical derangements known to occur in individuals with the disease process are under investigation as possible causes or genetic

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### Özet

*Ailevi Akdeniz Ateşi (FMF), Akdeniz ve Ortadoğu kökenli insanları daha çok etkileyen, tekrarlayan karın ağrısı, ateş, peritonit ve/veya plevrit ile karakterize olabilen bir hastalıktır. Tanı genellikle klinik olarak konur. Genetik ve biyokimyasal temel sebepler henüz tam aydınlatılmamıştır. Bununla birlikte tümör nekrozun faktör, artmış beta hidroksilaz aktivitesi, artmış serbest plazma yağ asitleri gibi bazı parametreler araştırılan konulardır. Dissémine tüberküloz tanısının polimeraz zincir reaksiyonu (PCR) ile tanısının incelenmesi konulu bir araştırma sırasında tesadüfen 3 kontrol vakasında FMF kliniğinin ve tüberküloz basilinin birlikteliği dikkatimizi çekti. Ppd pozitif sağlıklı 17 kontrolün birinde PCR pozitifken bu üç vakanın üçünde de pozitif olması ilginç bulundu. Bu yazıda vakalar ayrıntılı olarak sunuldu.*

**Anahtar Kelimeler:** Tüberküloz, FMF, PCR

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markers of the disease. Among these are inadequate tumor necrosis factors production (2), enhanced dopamine beta hydroxylase activity (3) and elevated serum free fatty acids (4) which are injurious to cell membranes and may contribute to the polyserositis which occurs during an attack. It is quite unclear, however, whether any of these biochemical derangements seen in cases of FMF are either the results or the cause of the disease process. Recently we have identified positive PCR signals for Mycobacterium tuberculosis DNA in bone marrow biopsy specimens obtained from three patients with FMF. In contrast in 17 healthy control cases, all with a positive tuberculin skin test, as a result of BCG vaccination only 1 PCR positive case was identified. The detection limit for the PCR technique utilized is 50 M. tuberculosis genomes/ml of sample. The detection of M. tuberculosis DNA in

all 3 of the FMF patients studied and only 1 of the 17 ppd positive controls suggests that *M. tuberculosis* may be important in the pathogenesis of FMF possibly serving as a triggering factor for the disease or that FMF may be a clinical variant tuberculosis infection in people of Mediterranean or Middle Eastern descent.

### The Presentation of Cases

**Controls and cases:** As a control group, 17 paraffin embedded BM biopsy specimens from patients with a positive mantoux skin test (Age; 35.3

13.5 yrs, range 19-56 yrs) but no active tuberculosis clinically and normal BM histology were included. Each of 3 cases are presented in detail below.

**Primers:** Each sample was amplified with two different sets of primers. The first primer amplified a 123-base pair (bp) fragment of the insertion element IS6110 of the genome of *M. tuberculosis* (5). The second primer amplifies a 240-bp region in the gene encoding the MBP 64 protein (6).

**Sample preparation:** Sections of formalin-fixed, paraffin embedded tissues were scraped with a sterile surgical blade (a new one for each sample) and the scrapings were collected in a 1.5-ml eppendorf tube. After deparaffinization with xylene and ethanol, the samples were incubated at 55°C in a buffer containing 10mM Tris pH 8.0, 0.5% SDS, 100mM NaCl, 25 mM EDTA and 0.5 mg/ml proteinase-K for 24 hours followed by Phenol-chloroform extraction and ethanol precipitation. The resultant dried pellets were suspended in 30 ml distilled water. 5 ml from the aqueous suspension was used for PCR amplification. A crude lysate of 1000 *M. tuberculosis* bacteria obtained from a clinical isolate was prepared by boiling 10 min in 1 ml TE solution (10 mM Tris-HCl pH 8.0, 1mM EDTA). This material was used as the positive control for each PCR run.

**Amplification:** Identical amplification procedures were used with both primer sets. The sample amplified consisted of a 50 µl volume containing 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1% Triton X-100, 2.0 mM MgCl<sub>2</sub>, 50 mM of each dNTP, 20 pmol of each primer, 1 unit of taq polymerase (Promega) and 5 µl of study sample. Forty cycles of 94°C-30 sec, 55°C-1 min and 72°C-1 min were

performed in an automated thermalcycler. Reaction mixtures without DNA were used as negative controls. After adjusting the turbidity of the clinical isolate of *M. tuberculosis* to a 0.5 McFarland standard, 10-fold dilutions of the resultant solution were cultured on Lowenstein-Jensen medium. The *M. tuberculosis* detection limit for the PCR procedure was determined by PCR amplification of crude cell lysates prepared from these samples.

**Detection of amplification product:** The presence of the 123 and 240 bp amplification products was determined by electrophoresis of a 10 µl aliquot of the amplified mixture on a 2% agarose gel. The gels were stained by ethidium bromide and photographed using a UV-transilluminator (Figure 1). Lane 2 and 3 displayed positive results from case 1 and case 2 as 240 bp amplification products. Lane 8, 9 and 10 displayed positive results of case 1, 2 and 3 as 123 bp amplification products. Detection of an amplified band with either one of the primer sets was considered as a positive result. As a result all three cases with clinical FMF were positive by PCR for *M. tuberculosis*. The PCR results for each sample were repeated after changing the sample code numbers and only consistently positive samples were reported as positive.

**Clinical description of 3 cases:** The first patient was a 21 year old male who was followed with a 5 year old diagnosis of FMF. His family history was positive for FMF. Specifically, two of his cousins had FMF. He had developed amyloidosis secondary to FMF which was readily detectable in his bone marrow biopsy. *M. tuberculosis* DNA was also detected by PCR in his marrow biopsy specimen. His intermediate ppd reaction was 15 mm of induration.

The second patient was a 52 year old female. She had been hospitalized for abdominal and joint pain with recurring febrile episodes. After an intensive search for a cause for her fever, no specific etiology was identified. She was started empirically on antituberculosis treatment and initially improved. Two months later, she developed recurrent fever. Since then her febrile attacks have been episodic. Each subsides spontaneously after 24-48 hours. Importantly, her attacks are accompanied by

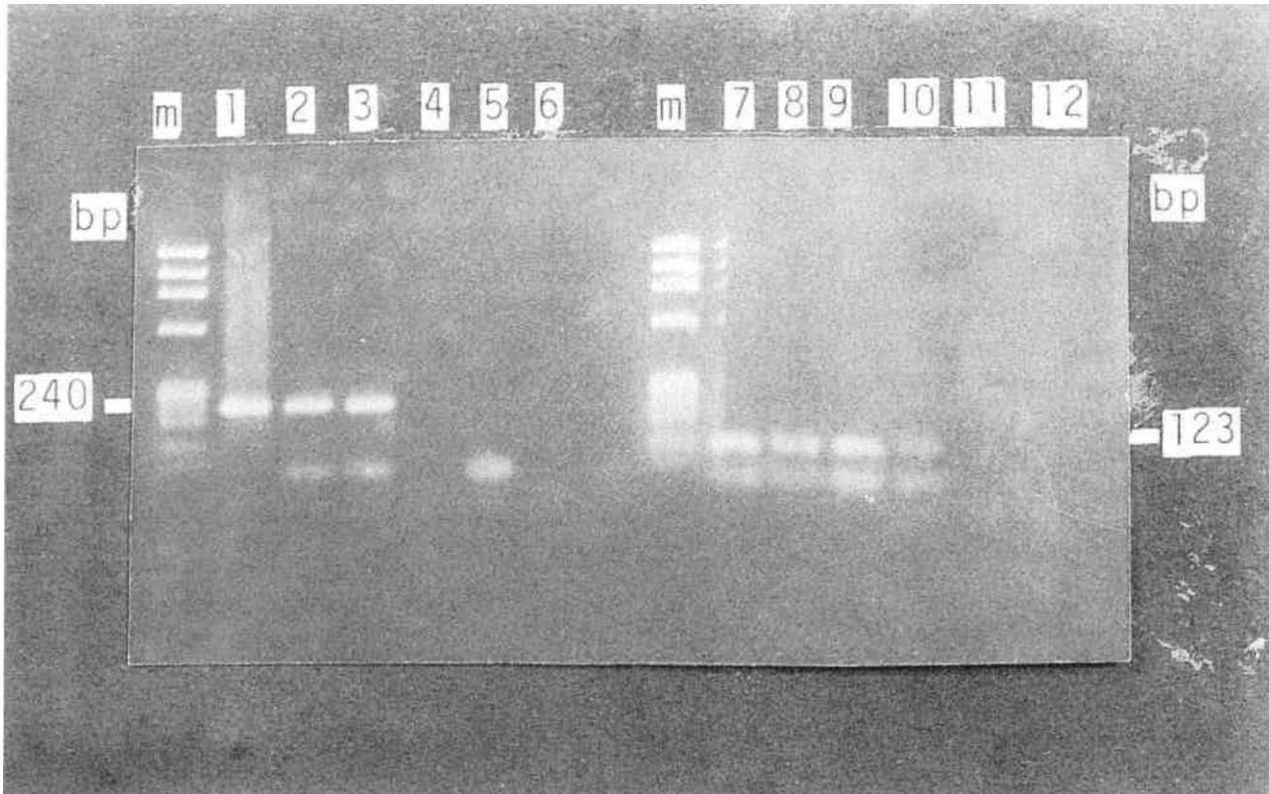


Figure 1. Amplification products (in agarose gel by.

A: Amplification with MPB 64 protein coding gene specific primers (240 bp).

Lane 1 : Positive control (1000 M. tuberculosis crude lysate)

Lane 2,3 : Positive samples (Case 1 and 2)

Lane 4 : Negative sample (Case 3)

Lane 5,6 : Negative controls (reaction mixture w/o DNA)

B: Amplification with ISO 110 specific primers (123 bp).

Lane 7 : Positive control (1000 M. tuberculosis crude lysate)

Lane 8,9,10 : Positive samples (Case 12 and 3)

Lane 11,12 : Negative controls (reaction mixture w/o DNA)

Lane m: Molecular weight marker (Hae III digested FX 174 phage DNA).

abdominal pain. A clinical diagnosis of FMF was made. She improved with the colchicine treatment consisting of 0.6 mg/lid. Prior pathologic specimens of bone her marrow and lymph nodes were studied and were found to be negative. Specifically no granulomas or malignant cells were detected. Subsequent more recent bone marrow biopsy samples obtained from this woman have been found to be positive for M. tuberculosis DNA. Subsequently, her tuberculin skin test was found to be positive with a 25 mm diameter area of enduration.

The third patient is a 19 year old male who was admitted with night sweats and fever. After a thor-

ough diagnostic evaluation was found to be negative except for a 20 mm in diameter ppd reaction and an erythrocyte sedimentation rate of 105 mm/hour, he was started on a trial of antituberculous treatment. He initially improved. Three months into his antituberculous treatment, he began to experience episodic attacks of abdominal pain with fever lasting 24-48 hours. His fibrinogen level during these attacks was found to be increased at 620 mg/dl. A clinical diagnosis of FMF was made. He was started on colchicine 0.6 mg/tid along with antituberculous treatment. At the end of six months of treatment, he was symptom free. A bone marrow biopsy obtained during his initial diagnostic

evaluation was assessed for *M. tuberculosis* by PCR. Positive results were obtained.

### Discussion

Inborn errors of catecholamine metabolism (7), circulating immune complexes (8), leukocyte chemotaxis (9) and complement system abnormalities (10) have all been investigated as potential etiologies for FMF. There are reports of an elevated T4/T8 ratio in FMF which is corrected by colchicine therapy (12). This might represent a hypernormal T helper cell response to an unknown antigen. It is generally accepted that the gene responsible for FMF is linked to the alpha-globin complex present on chromosome 16p (13). A triggering factor, perhaps an infectious agent such as *M. tuberculosis*, may initiate the clinical sequence of events terminating in a syndrome consistent with a diagnosis of FMF. Based upon the finding of *M. tuberculosis* DNA in the bone marrow of 3 cases of FMF, it is possible to speculate that a relationship between *M. tuberculosis* infection and FMF may exist. *M. tuberculosis* may be but one of several different triggering factors responsible for a clinical syndrome consistent with a diagnosis of FMF. Alternatively, FMF may simply be a clinical variant of tuberculosis infection in uniquely susceptible individuals.

Based upon these observations we speculate that a gene on chromosome 16p, alone or in combination with some other unidentified hereditary factors, may regulate the immune system in such a way that individuals with this gene(s) respond to *M. tuberculosis* bacilli in a manner consistent with the clinical picture of FMF.

Both FMF and tuberculosis are endemic in Turkey (14,15). Thus, the association observed may merely represent a consequence of dual endemicity. On the other hand, the fact that only one of 17 healthy ppd positive control cases had a positive PCR response to *M. tuberculosis* suggests that a true relationship exists between *M. tuberculosis* and FMF.

There are several other reasons directs our attention to this relationship. There are some points common in both tuberculosis and FMF in our ob-

servations. First of all both are epidemic in our country (14-16). Both could result in AA type amyloidosis. Hypersensitivity type autoimmune reaction takes place somewhere in their course, on which, for example, tuberculin skin test was based (17,18). For these reasons and our observation in which we had demonstrated *M. tuberculosis* bacilli present in the bone marrow biopsies of patients with FMF in a quantity more than healthy controls, we feel that it is necessary to elucidate whether this finding is just a coincidence or *Mycobacterium tuberculosis* might be a cause for the development of FMF. To achieve this aim, prospectively designed studies are essential.

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