

*Full Length Research Paper*

## Early age onset familial Mediterranean fever associated with compound heterozygote M680I /M694V mutation

Safar Farajnia<sup>1,2\*</sup>, Ailar Nakhband<sup>1</sup>, Mandana Rafeey<sup>3</sup> and Kazem Sakha<sup>3</sup>

<sup>1</sup>Drug Applied Research Center, Tabriz University of Medical Science

<sup>2</sup>Biotechnology Research center, Tabriz University of Medical Science

<sup>3</sup>Department of Medicine, Tabriz University of Medical Science, Daneshgah Ave., Tabriz. Iran.

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**Familial Mediterranean fever (FMF) is an autosomal recessive genetic disorder characterized by acute episodes of fever accompanied by severe abdominal pain, pleurisy, arthritis, and skin rash. The clinical variability of the disease has been mainly attributed to MEFV gene allelic heterogeneity and partly to the influence of additional genetic and/or environmental factors. We present a 6-month-old boy who suffered from recurrent fever accompanied by abdominal pain and skin rashes. Molecular screening by polymerase chain reaction (PCR) and sequencing for common mutations causing FMF revealed presence of a 694V/680I compound heterozygote mutation in exon 10 of the related gene. This is the first report of early onset and severe phenotype FMF case associated with a 694V/680I compound heterozygote mutation.**

**Key words:** FMF, mutations; compound heterozygote, early onset.

### INTRODUCTION

Familial Mediterranean fever (FMF; MIM249100) is an autosomal recessive disease characterized by recurrent attacks of fever and peritonitis, pleuritis or erysipelas-like erythema. The most serious complication of FMF is the development of systemic amyloidosis, ultimately leading to renal failure (Samuels et al., 1998). Symptoms can appear during the first decade of life and more than 80% of patients experience the disease during childhood and adolescence. A typical attack of disease consists of fever and serositis lasting for 1- 3 days, with a spontaneous recovery. Between attacks FMF patients are usually free of symptoms. The severity and frequency of the attacks varies between patients and the male to female ratio is considered to be 1.2:1 (Tunca et al., 2005).

FMF predominantly affects the population surrounding the Mediterranean basin (Ben-Chetrit and Levy, 1998). The disease occurs most commonly in Turks, Arabs,

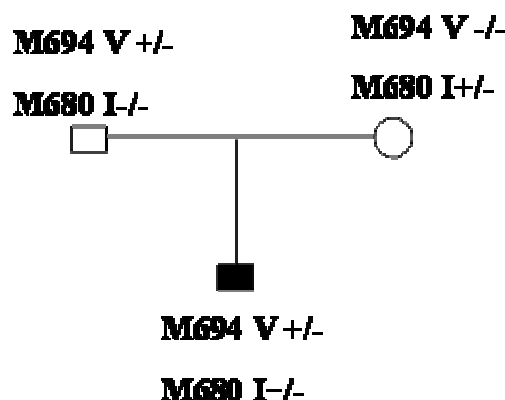
Armenians, and Sephardic Jews (Bakkaloglu, 2003). However, cases from other ethnic groups in other parts of the world are increasingly being reported. The prevalence of FMF in Iranian population has not well defined but in Iranian Jews it has been reported to be about 6% (Stoffman et al., 2000).

Until recently, the diagnosis of FMF was based on clinical manifestations, ethnicity, family history and response to colchicines. In 1992, the gene responsible for FMF was localized on the short arm of chromosome 16 (Pras et al., 1992) and in 1997, two independent groups cloned the specific gene for FMF (MEFV) (The French FMF Consortium and The International FMF Consortium 1997). The gene encodes pyrin/marenostrin protein that is a member of the RoRet gene family and is expressed predominantly in granulocytes. On the basis of the FMF phenotype, pyrin is likely to be a negative regulator of granulocyte-mediated inflammation (Pars 1998). To date, more than 50 FMF associated pyrin gene mutations have been identified; most of them are mapped on exon 10 and four of these missense mutations (M680I, M694V, M694I, and V726A), are responsible for about 70-86% of mutations depending on ethnic group. It has

\*Corresponding authors E-mail: farajnias@tbzmed.ac.ir. Tel: 0098411-3363234.

**Table 1.** Results of genetic analysis for common MEFV gene mutations.

Subject	694V	694I	680I	726A
Child	M/N	N/N	M/N	N/N
Father	M/N	N/N	N/N	N/N
Mother	N/N	N/N	M/N	N/N

**Figure 1.** Pedigree illustrating mode of mutation inheritance. Open boxes are related to parents that is asymptomatic FMF gene carrier, black box is related to child with FMF due to M694V/M680I compound heterozygote mutation.

been shown that some of the MEFV gene mutations have low penetrance (E148Q, P396S) (Cazeneuve et al., 1999), whereas some (V726A) lead to a milder disease (Aksen-tijevich et al., 1999). The M694V mutation is the most prevalent mutation, known to be associated with a severe FMF and frequently seen among people suffering from amyloidosis in homozygote state (Livneh et al., 1999; Shohat et al., 1999). In this report we present mutation analysis of an Iranian patient with early onset and severe FMF, which contain a 694V/680I compound heterozygote genotype.

## MATERIAL AND METHODS

### Patient

A six-month-old male baby presented with periodic attacks of fever, abdominal pain, and erysipelas-like erythema. His periodic attacks was noted at 4<sup>th</sup> month for the first time, which was appeared as fever of up to 40°C about once a week lasting 1-2 day and accompanied with abdominal pain in most cases. The patient's parents never had periodic febrile attacks and there was no history of consanguineous marriage. Chest and abdominal imaging failed to detect any abnormalities. Laboratory findings at the time of febrile attacks were as follows: Hemoglobin 12.8 g/dl, white blood cell count 15300/L (68% granulocytes, 26% lymphocytes, 4% band and 2% monocytes), platelet count 320000/L, erythrocyte sedimentation rate 54 mm/h, fibrinogen level 255 mg/dl (normal: 200-400 mg/dl) and C-reactive 3+. Blood chemistry and urinalysis were normal. Autoantibodies, including ANA and RF were negative. Nonsteroidal antiinflammatory drugs had been administered without

remarked clinical efficacy. Treatment with 0.5 mg/day of colchicines showed no significant improvement but 1 mg/day of colchicines was associated with good therapeutic response.

### Genetic analysis

Molecular testing was carried out on DNA extracted from peripheral blood leukocytes of the patient and his parents by standard methods (Sambrook et al., 1989). The four common mutations (Met694Val, Met680Ile, Val726Ala, and Met694Ile) were investigated with the aid of amplification refractory mutation system (ARMS) using primers designed to amplify the normal or altered alleles. Each set of primers consisted of three oligonucleotides, their sequences were as follows: 694V common: 5'-TATCATTGTTCTGGGCTC-3', mutant: 5'-TGGTACTCATTTCCTTCAC-3', normal: 5'-TGGTACTCATTTCCTTCAT-3', 726A common: 5'-TGGAGGTTGGAGACAAGACAGCATGGATCC-3', mutant 5'-TGGGATCTGGCTGTCACATTGTAAAAGGAGATGCTTCCTG-3', normal: 5'-TGGGATCTGGCTGTCACATTGTAAAAGGAGATGCTTCCTA-3', 694I common: 5'-TATCATTGTTCTGGGCTC-3', normal: 5'-CTGGTACTCATTTCCTTC-3', mutant: 5'-CTGGTACTCATTTCCTTT-3', and 680I: common: 5'-GGAAACAAGTGGGAGAGGCTGC-3', mutant 5'-GTAGCCATTCTCTAGCGACAGTGCC -3', normal: 5'-GTAGCCATTCTCTAGCGACAGTGCG -3'. The primers for amplification of complete exon 10 were as follow: E10 forward; 5'-CCCATGGACCCCTACCTAGG-3'; E10-Reverse 5'-AAGAGAGATGCAGTGTGGGC-3'. Polymerase chain reaction (PCR) were carried out in 25 microliter reaction volumes containing 100 ng genomic DNA, 25 pmols primers, 0.2 mM dNTPs, 2.5 µL reaction buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM MgCl<sub>2</sub>) and 1 U Taq DNA polymerase (Fermentas). Cycling condition were 94°C 4 min, followed by 30 cycles of 94°C 1 min, 58°C 30 s, 72°C 30 s and a final extension of 72°C 5 min. PCR products were separated by electrophoresis on a 1.5% agarose gel. Ethidium bromide staining of the agarose gel was used to detect the amplified fragments. Entire amplified exon 10 was subjected for sequencing by dideoxy method.

## RESULTS AND DISCUSSION

The results of genetic analysis have shown in Table 1 and Figure 1. Genetic analysis using ARMS-PCR revealed that the patient was compound heterozygote for 694V/680I mutation and his father and mother were heterozygote for 694V and 680I mutation respectively. Sequencing of the complete exon 10 coding region confirmed the results of ARMS-PCR and did not show any other mutation.

FMF is a genetic condition inherited in an autosomal recessive fashion. Mutations in the MEFV gene (short for Mediterranean fever) on chromosome number 16 are the underlying cause of FMF (The French FMF Consortium, 1997). The clinical characteristics of FMF including fever, peritonitis, pleurisy, rashes, arthritis, and occurrence of amyloidosis are variable in their pattern, frequency, intensity and age of onset. Extensive attempts have been spent to understand the base of such variability and, types of mutations, ethnic background, additional genetic modifier and environmental factors possibly play a role in

this phenotype difference. Initial phenotype-genotype studies suggest the presence of a correlation between some mutations and severity of the disease (Pras et al., 1997; Dewalle et al., 1998). Patients who are homozygous for M694V mutation are reported to have particularly severe FMF disease and possibly a greater propensity to develop AA amyloidosis (Cazeneuve et al., 1999; Shohat et al., 1999; Dewalle et al., 1998). Recent studies reported that M694V are the most common mutation in those developing secondary amyloidosis and that the M694V mutation is an important factor in predicting the development of amyloidosis (Balci et al., 2002). Appearance of FMF in which *MEFV* mutation affect only a single allele remains a subject of controversy. While there are reports indicating FMF disease in individuals are heterozygote for known *MEFV* mutations (suggest an autosomal dominant inheritance (Yuval et al., 2001), the presence of mutations without phenotypic association are relatively common observation and referred as phenotype III FMF (Kogan et al., 2001). The M680I mutation, one of the other common mutations prevalently seen in Armenian patients, was suggested to be associated with a milder phenotype (Pars, 1998).

The patient described herein both has inappropriate ethnic background and their clinical presentations specially the age of onset were unusual. The tests for detecting common mutations responsible for FMF showed that he was compound heterozygote for the M694V/M680I mutation. The father and mother of the patient that were heterozygote for 694V and 680I mutations respectively have never experienced clinical signs of FMF, whereas in child the association of these two mutations has lead to a severe FMF. It has been shown that many heterozygote individuals including the father of our patient with 694V mutation in one allele did not show clinical expression of FMF. In the other hand clinical manifestations associated with mutation M680I are considered less severe. Our data indicate that the association of these two mutations even in compound heterozygote form might lead to a severe FMF characterized by the early age onset and high frequency of painful episodes.

Although established clinical criteria for diagnosis of FMF exist (Livneh et al., 1997), many patients remain undiagnosed because of rather nonspecific symptoms. Therefore, molecular genetic analysis could substantially improve early and correct diagnosis of FMF and helps for making decision for initiation of lifelong prophylactic treatment with colchicine which is known to prevent the insidious onset of end-stage renal disease.

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