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A novel *MEFV* gene mutation (A511V) in a Chilean FMF patient

Nejat Akar [a,*], **Ayşenur Öztürk** [a], **Çiğdem Arslan** [a], **Ece Akar** [a],
Silvia Castillo Taucher [b,c], **Cristobal Passalacqua** [b]

[a] *Faculty of Medicine, Pediatric Genetics Department, Ankara University, Ankara, Turkey*
[b] *Genetic Section, Medicine Department, Hospital Clinico Universidad de Chile, Chile*
[c] *Cytogetetics Section, Laboratorio Clinica Alemana, Chile*

Abstract Familial Mediterranean fever (FMF) is an autosomal recessive disease which is characterized by recurrent fever and inflammation of serous membranes. A Chilean FMF patient was investigated for *MEFV* mutations. After DNA extraction, exons 3, 5, 10 and 3′UTR region of *MEFV* gene were analyzed by DNA sequencing while E148Q and R202Q mutations of exon 2 were detected by RFLP. A novel missense mutation, A511V (c.1532C > T, p.Ala511Val), was found in a heterozygous state in exon 5 of *MEFV* gene. Also, R202Q (c.605G > A, p.Arg202Gln) was detected in heterozygous state. R202Q was of clinical value in the diagnosis of FMF when combined with a disease causing mutation. In this patient, A511V was detected in compound heterozygous state with R202Q and this association may play an important role in FMF.

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1. Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease which is characterized by recurrent fever and inflammation of serous membranes (peritoneum, synovium and pleura [1]). FMF, having the most frequent occurrence between hereditary periodic diseases, was first identified as a clinic entity by Siegal in 1945 [2]. The most important complication of FMF is amyloidosis. Colchicine has been used in treatment since 1972 [3]. It reduced the frequency and severity of FMF attacks and prevented the development of amyloidosis [4–6].

FMF most commonly occurs in Mediterranean populations especially in Jews, Turks, Arabs and Armenians [1,7]. Although the disease is seen in other populations rarely, it was defined

in Greeks, Italians, Germans, Polishes, Australians, Cubans, Belgians, Brazilians [8], and also Egyptians [9]. The prevalence was reported as 1/256–1/500 in non-Ashkenazi Jews [10] and as 1/1073 in Turks [11]. On the other hand carrier frequency was estimated in North African Jews, Ashkenazi Jews, Armenians and Turks as 1/5, 1/11, 1/7 and 1/5, respectively [10,12,13]. No data are available regarding the proportion of *MEFV* mutation in Chilean FMF patients.

MEFV gene, responsible for FMF, in 16p13.3 [14], was cloned by International FMF Consortium and French FMF Consortium independently and indicated that it comprises 10 exons, including 3505 nucleotides and 15 kb length. The protein encoded by *MEFV* was called as pyrin/marenostrin which contains 781 amino acids [15,16]. Its expression in neutrophils and functions in inflammation were shown in studies [17].

After cloning of the gene, M694V, M680I and V726A mutations were the first to be determined in exon 10 [15,16]. To date, more than 180 mutations and/or polymorphisms related to FMF were identified in the *MEFV* gene [18].

A patient from Chilie, diagnosed as FMF case according to the Tel-Hashomer criteria was investigated for *MEFV* mutations. The patient had periodic fever, abdominal pain lasting for several years.

2. Subjects and methods

Blood sample of the patient was transferred from Chile to Ankara/Turkey. DNA was extracted from peripheral blood lymphocytes according to standard procedures. Exons 3, 5, 10 and 3′UTR were analyzed by DNA sequencing whereas E148Q and R202Q mutations in exon 2 were detected by Restriction Fragment Length Polymorphism (RFLP) method. For polymerase chain reactions (PCR) of exon 2, 3, 5 and 10-3′UTR, F:5′-ATCTTGGGCCCTAAACGTGG-3′ and R:5′-TCC-TTCAGGTCCGCAGATGC-3′, F:5′-TCTGTGTAAGCAA-CTTGGGTTTG-3′ and R:5′-TTGGGAAAATGAAGT-AAGGCCC-3′, F:5′-TATCGCCTCCTGCTCTGGAATC-3′ and R:5′-CACTGTGGG TCACCAAGACCAAG-3′, F:5′-GAGGTGGACGTTGGAGACAA-3′ and R:5′-TCCT CCT-CTGAAATCCATGG-3′ primers were used and 658, 480, 526, and 614 bp fragments were amplified, respectively. The amplicons of exon 2 were analyzed using Eco88I and PvuII

restriction endonucleases (Fermentas, Lithuania) for mutations E148Q and R202Q. For sequencing, PCR products were purified using a PCR purification kit (Metis, Turkey) and then analyzed by automated fluorescence DNA sequencing method (CEQ8000XL, Beckman Coulter, USA) [19,20].

3. Results

A new variant, the A511V (Alanine–Valine) missense mutation, caused by a C > T transition at nucleotide 1532 in exon 5, which results in a alanine–valine exchange at codon 511, was found in the heterozygous state by sequencing (Fig. 1), and also this mutation was confirmed by RFLP. Amplified 526 bp product digested with HhaI (Fermentas, Lithuania) at 37 °C and subjected to 3% agarose gel electrophoresis. After digestion, in sample of wild type there were 3 fragments (199, 165 and 162 bp) while 4 fragments in heterozygote sample (361, 199, 165 and 162 bp) (Fig. 2). On the other hand, R202Q mutation in exon 2, R314R in exon 3, E474E, Q476Q and D510D polymorphisms in exon 5 were detected in heterozygous state. But no mutation was found after sequencing of other exons and 3′UTR of the *MEFV* gene.
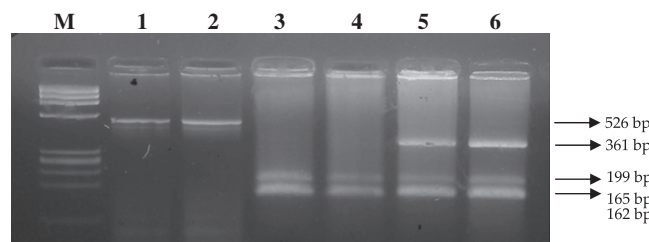
Figure 2 PCR–RFLP of novel MEFV mutation A511V analyzed by agarose gel electrophoresis. PCR products 526 bp of exon 5 were digested by HhaI restriction endonuclease and band profiles were examined in 3% agarose gel. After digestion, 361, 199, 165 and 162 bp fragments were observed in heterozygosity whereas 199, 165 and 162 bp fragments in wild-type. M: X174 DNA-HaeIII Marker, 1–2: Uncut, 3–4: wild-type, 5–6: Heterozygous (these are the same sample).
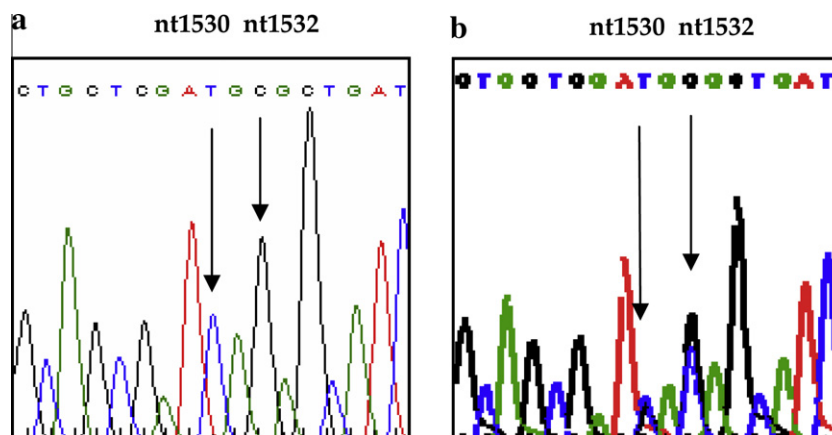
Figure 1 Electrophoregram of the new A511V MEFV variant. (a) Wild-type (b) The C > T transition at nucleotide 1532 in exon 5 converts alanine to valine exchange in codon 511. Also 1530 C > T polymorphism is seen in heterozygous state (nt: nucleotide).

4. Discussion

Molecular analysis of the *MEFV* gene revealed that a novel missense mutation A511V, located in exon 5 of the gene, was present in the heterozygous state. It causes amino acid replacement of alanine to valine. Polymorphisms and FMF causing mutations have been described in exon 5 suggesting the functional importance of this domain [21].

E474E (1422GA), Q476Q (1428AG), and D510D (1530TC) polymorphisms in exon 5 and R314R polymorphism in exon 3 were first defined by Bernot and colleagues (1998) and has been used as markers in haplotype analysis [22,23]. Cazeneuve et al. (1999) showed that these three polymorphisms were associated in cis position, in addition to R501R (1503CT) polymorphism [24]. Also, Aldea and his colleagues (2004) constituted haplotypes including 1422GA, 1428AG and 1530TC polymorphisms [21].

R202Q in exon 2 of *MEFV* gene was first identified by Bernot et al. (1998) and it has been proposed to be a common polymorphism [22]. Additionally, in FMF database, R202Q was indicated as a polymorphism in linkage disequilibrium with M694V [18]. In a study of 26 Greek FMF patients, R202Q homozygosity has been shown in 4 patients but was not detected in 60 healthy controls and statistically significant difference was observed between the two groups. R202Q gene alteration may be a mutation more than a polymorphism and because of the heterozygote controls, dose-dependent effect may occur in homozygous FMF patients [25]. In the following years, homozygosity of R202Q was reported to be associated with the disease and could be a mutation but has not been mentioned the relation with the M694V mutation [26]. Miyoshi et al. (2008) reported E148Q/R202Q compound heterozygosity in a Japanese patient which may be responsible for the disease [27]. Although, R202Q heterozygosity was detected in 163 healty Japanese controls of 170, homozygosity could not be detected [28]. In our previous study, we found that there is one more haplotype, which was not in linkage disequilibrium with M694V. This result showed that R202Q might be associated with the disease at least in some FMF patients. R202 homozygosity could not be detected in our control group but, high frequency of heterozygosity showed that it has no effect when it is in heterozygous state. However, when carrying R202Q in compound heterozygous with another disease causing mutation, the clinical spectrum appears [29]. So, this emphasizes importance of R202Q for diagnosis. Also our Chilean patient carries R202Q and A511V in compound heterozygote state. This association may create a defect on protein function and could play an important role in FMF progression.

We were not able to screen A511V mutation in Chilean population. Five hundred sixteen Turkish & Egyptian FMF patients were analyzed for exon 5 of *MEFV* gene by sequencing and A511V was not present. In conclusion, our results show that compound heterozygosity of R202Q and A511V may have a role in expression of the clinical findings. The relevance of the novel mutation with the disease should be confirmed by studying with FMF in large patient groups and by functional studies.

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