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LETTER TO THE EDITOR

Common Mediterranean Fever Gene Mutations in the Azeri Turkish Population of Iran

Mortaza Bonyadi,[1,2] Mohsen Esmaeili,[1,2] Abbas Karimi,[1,2] and Saeed Dastgiri[3]

Familial Mediterranean fever (FMF) is an autosomal recessive disorder primarily affecting the Mediterranean populations. It is characterized by recurrent attacks of fever and inflammation of serosal membranes and gradual development of nephropathic amyloidosis. More than 70 disease-associated mutations have been identified in the Mediterranean fever gene (*MEFV*) responsible for FMF. The aim of this study was to determine the mutation carrier rate in the Iranian Azeri Turkish population. A cohort of 200 unrelated healthy individuals was screened for the five most common *MEFV* mutations (M694V, V726A, M680I, M694I, and E148Q) using the amplification refractory mutation system for the first four and by polymerase chain reaction–restriction-digestion testing for E148Q. Genotyping revealed that the carrier rate in the Azeri Turkish population was 25.5%, with E148Q being the most common mutation (11.5%) followed by V726A (1.75%). The remaining common mutations were not found in this cohort. Our data indicate that the FMF carrier rate and E148Q mutation frequency are high in the Iranian Azeri Turkish population.

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder mainly affecting the Mediterranean populations such as Arabs, Armenians, Jews, and Turks, although recently it has been described in many other populations (Touitou, 2001; Onen, 2005). It is characterized by recurrent self-limited attacks of fever with serositis, synovitis, or erysipelas-like skin lesions. A less frequent but very severe complication of FMF is the development of renal amyloidosis, ultimately leading to the end-stage kidney failure (Lidar and Livneh, 2007). The responsible gene, Mediterranean fever gene (*MEFV*), located on chromosome 16p13.3 encodes for pyrin (marenostrin) (The French FMF Consortium, 1997; The International FMF Consortium, 1997). Pyrin belongs to a class of proteins involved in the regulation of apoptosis and inflammation (Lidar and Livneh, 2007). More than 70 FMF causing mutations have been reported in *MEFV* (Infevers, 2009). However, five common mutations (namely E148Q, M680I(G/C), M694V, M694I, and V726A) account for more than 70% of the deleterious alleles in the Mediterranean ethnic groups (Papadopoulos *et al.*, 2008). Recently, molecular genetic studies showed that FMF is no longer a rare disease in the northwestern Iran (Esmaeili *et al.*, 2008). There is no available data on the *MEFV* mutation carrier rate in the Iranian Azeri Turkish population. This study aims to determine the carrier frequency of the five common *MEFV* mutations in this ethnic group.

Subjects and Methods

A total of 200 unrelated apparently healthy Azeri Turkish DNA samples were obtained from couples undergoing carrier screening for spinal muscular atrophy, Duchenne muscular dystrophy, cystic fibrosis, deafness, and beta-thalassemia. Informed consent was obtained from all the participants. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols (Miller *et al.*, 1988).

The presence of the five most common *MEFV* mutations was determined using amplification refractory mutation system–polymerase chain reaction (PCR) and PCR–restriction fragment length polymorphism methods as previously described (The French FMF Consortium, 1997; Eisenberg *et al.*, 1998; Livneh *et al.*, 1999; Medlej-Hashim *et al.*, 2002). The appropriate positive and negative controls were employed for each test. The positive results were repeated to ensure reproducibility. PCR products and restriction enzyme–digested fragments were electrophoresed in a 2% agarose gel and observed by ethidium bromide staining. Chi-squared test was used for comparison between discrete parameters.

[1]Faculty of Natural Sciences, Center of Excellence for Biodiversity, University of Tabriz, Tabriz, Iran.
[2]Liver & Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Department of Community and Family Medicine, School of Medicine, National Public Health Management Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Results

Genotyping of 200 Iranian Azeri Turkish control individuals for the five common *MEFV* mutations showed no carriers for M694V, M694I, and M680I, whereas 11.5% and 1.75% of the tested chromosomes carried the E148Q and V726A mutations, respectively. One 12-year-old female was diagnosed to be homozygous for E148Q mutation. She had no symptoms of FMF attacks or amyloidosis. Carrier frequency has been estimated to reach 1:4 (25.5%) in this ethnic group.

Discussion

This is the first report about the carrier frequency of FMF mutations in Azeri Turks living in northwestern Iran. This ethnic group, constituting 25% of the Iranian population, is ethnically identical to Azeris and closely related to Turks. Our results revealed that carrier rate in this cohort is 25.5%, higher than that previously reported in Jews (22%) (Stoffman *et al.*, 2000), Arabs (18.5%) (El-Shanti *et al.*, 2006), Turks (20%) (Yilmaz *et al.*, 2001), and Armenians (21%) (Sarkisian *et al.*, 2008). It is noteworthy that 86% of the carriers from this ethnic group had E148Q/wt genotype.

Even though M694V was the most common mutation in the Azeri Turkish FMF patients (28%) (Esmaeili *et al.*, 2008), it was not detected in the general population sample ($p < 0.001$). In contrast, E148Q was observed frequently (11.5%) among the healthy population, but was found in 7% of the FMF patients ($p < 0.05$) (Esmaeili *et al.*, 2008). This may suggest that the E148Q mutation has reduced penetrance, and a considerable proportion of the genetically affected individuals who were homozygous for E148Q remain asymptomatic (Aksentijevich *et al.*, 1999; Stoffman *et al.*, 2000). In addition, the contribution of the E148Q mutation to FMF morbidity is currently under debate (Ben-Chetrit *et al.*, 2000; Gershoni-Baruch *et al.*, 2002; Tchernitchko *et al.*, 2003; Topaloglu *et al.*, 2005).

The V726A was found to be the second most frequent mutation among the FMF patients (9%) (Esmaeili *et al.*, 2008) and the healthy population (1.75%) in this ethnic group ($p < 0.001$).

The null frequency of M694V, M694I, and M680I mutations was also previously reported in other population studies (Bernot *et al.*, 1998; Aksentijevich *et al.*, 1999; Medlej-Hashim *et al.*, 2005; Mattit *et al.*, 2006; Fragouli *et al.*, 2008), which probably points to their higher penetrance (Medlej-Hashim *et al.*, 2005; El-Shanti *et al.*, 2006).

Molecular genetic studies on the Turkish population showed that the *MEFV* mutation spectrum and frequency is somewhat different from that of our cohort (Yilmaz *et al.*, 2001). The frequency of E148Q in Azeri Turks is twice more than that of the Turkish population, whereas the frequency of V726A in these two populations is almost similar. In healthy individuals of Azeri Turks, the frequencies of M694V and M680I were null; however, these mutations in Turks were reported to be with the frequencies of 1.5% and 2.5%, respectively.

Because of high carrier rate of E148Q and V726A mutations in our population, the detection of these mutations in heterozygous state is not necessarily enough to diagnose FMF. Therefore, diagnosis should be made on clinical grounds when encountering these genotypes.

It is noteworthy that the five common mutations studied in our general population are only partially accountable for the prevalence of FMF in this ethnic group because approximately half of the alleles investigated in the Azeri Turkish FMF patients have unidentified *MEFV* mutations (Esmaeili *et al.*, 2008).

The high frequency of carriers of *MEFV* in our population may also imply a selective advantage for heterozygotes of this gene. Positive selection pressure favoring heterozygosity of *MEFV* mutations may reflect the need for better response to intracellular pathogens (Schaner and Gumucio, 2005) or protection against development of atopic sensitization and allergic rhinitis (Sackesen *et al.*, 2004).

This study clearly indicates a high incidence of *MEFV* gene mutations in the Iranian Azeri Turkish population (gene frequency of 0.1325 and carrier rate of 0.255). Further, previous study showed that FMF is no longer a rare disease in this ethnic group (Esmaeili *et al.*, 2008). These data could offer additional incentive to the Iranian health system policy makers to plan for the management of the disease in future.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Mortaza Bonyadi, Ph.D.
Center of Excellence for Biodiversity
Faculty of Natural Sciences
University of Tabriz
Tabriz 51638-68639
Iran

E-mail: jabbarpour@tabrizu.ac.ir

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