Association of Missense Mutations of Mediterranean Fever (*MEFV*) Gene with Multiple Sclerosis in Turkish Population

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Abstract Genetic risk factors are known to contribute to the etiology of multiple sclerosis (MS). Patients with familial Mediterranean fever (FMF) have susceptibility to develop MS. Mediterranean fever (*MEFV*) gene has already been identified as being responsible for FMF. The aim of this study was to explore the frequency of missense mutations of *MEFV* gene in a cohort of Turkish patients with MS. The study included 100 patients with MS and 160 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction and restriction fragment length polymorphism analyses for the five *MEFV* gene mutations (M694V, M680I, V726A, E148Q, and P369S). There were statistically significant differences of the *MEFV* gene mutation carrier rates and allele frequencies between MS patients and healthy controls ($p=0.0008$, odds ratio (OR) 2.6, 95 % confidence interval (CI) 1.47–4.77 and $p=0.0002$, OR 2.6, 95 % CI 1.55–4.48, respectively). The results of this study suggest that *MEFV* gene mutations are positively associated with predisposition to develop MS.

Keywords Multiple sclerosis · *MEFV* gene mutation · FMF

Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, and axonal degeneration and is believed to arise from complex interactions of both environmental and genetic risk factors (Hoffjan and Akkad 2010). Patients with familial Mediterranean fever (FMF) have susceptibility to the develop MS. Inflammation, disruption of blood–brain barrier, mitochondrial energy deficit, demyelination, and axonal damage, which play an important role in the pathogenesis of MS, may occur during the course of FMF.

FMF is the most common autoinflammatory disorder having an autosomal recessive inheritance pattern characterized by lifelong recurrent, self-limiting attacks of fever and inflammation in the peritoneum, synovium, or pleura, accompanied by pain mostly seen in people of the Middle Eastern and Mediterranean regions, including non-Ashkenazi Jews, Middle Eastern Arabs, Turks, and Armenians (Ben-Chetrit and Levy 1998; Samuels and Ozen 2006; Onen 2006). During the FMF attack, body temperature is about 39–40 °C (Onen 2006). The attacks typically occur every few weeks to months and last for 1–3 days (Meinzer et al. 2011). Mediterranean fever (*MEFV*) gene has already been identified as being responsible for FMF. In persons with *MEFV* gene mutations, it was suggested that upregulation of the inflammatory response most likely favors inflammation in general and as such predisposes *MEFV* mutant carriers to inflammatory diseases (Booth et al. 2000). The upregulation of the inflammatory response in carriers of *MEFV* mutations can also affect the severity of the accompanying chronic diseases such as rheumatoid arthritis (RA), Behcet's disease (BD), palindromic rheumatism (PR), and ulcerative colitis (UC) (Rabinovich et al. 2005, 2007; Giaglis et al. 2006; Canete et. al. 2007). Patients with the inflammatory burden of these chronic diseases appear to be highly susceptible to develop a more severe disease if they also have a mutated *MEFV*. A possible association of MEFV gene mutations and MS has also been suggested in cohorts from Israel, Turkey, and Germany

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(Shinar et al. 2003; Unal et al. 2010; Yahalom et al. 2011; Kümpfel et al. 2012).

The *MEFV* gene is located on the short arm of chromosome 16p13.3, comprises 10 exons, and encodes a 781-amino acid protein called marenostrin or pyrin (Pras et al. 1992). Pyrin is only expressed in neutrophils and monocytes; the cell types involved in innate immune responses. Pyrin has a key role in the regulation of inflammasome activity and pro-interleukin-1β processing and this cytokine play a part in MS pathogenesis (Ting et al. 2006; Papin et al. 2007; Carpintero and Burger 2011). Due to high body temperature that arises during the FMF attacks, damage can occur in myelin and mitochondria proteins that also cause MS development. Thus, MS findings may occur in the FMF patients especially with irregular use of colchicine (Alpayci et al. 2012). To date, more than 221 mutations have been identified in MEFV gene and 100 of these mutations are known to be associated with FMF phenotype (http://fmf.cnrs.fr/infevers/). Of these mutations, five account for more than 70 % of FMF cases (i.e., V726A, M694V, M694I, M680I, and E148Q) and have different frequencies in classically affected populations (Yepiskoposyan and Harutyunyan 2007).

Considering that FMF is very common in Turkey and MS findings may occur in the FMF patients, it is important to investigate the frequency of *MEFV* mutations in patients with MS. Therefore, we aimed to study and compare the frequencies of missense mutations (M694V, M680I, V726A, E148Q, and P369S) of *MEFV* gene in patients with MS and healthy controls in a cohort of Turkish population.

Material and Methods

Study Population

The study population comprised 100 unrelated MS patients (mean age 36.56±8.84 years; 26 males, 74 females) who had taken part in our previous study (Ates et al. 2010). All MS patients were registered at the outpatient clinic of the Neurology Department at Gaziosmanpasa Medical Faculty and they all fulfilled the 2005 Revised McDonald Multiple Sclerosis criteria for classification (Polman et al. 2005). A total of 160 unrelated healthy subjects (mean age 37.68± 11.39 years; 62 males, 98 females) were recruited consecutively. All participants, patients and healthy controls, were of Turkish origin, from the inner Central Black Sea region of Turkey. The protocol of this study was approved by the Institutional Ethics Committee, and all participants gave written informed consent before entering the study. All the participants were evaluated for the clinical findings of FMF, and none of them had symptoms or family history of FMF. The MS patients were assigned to two groups as *MEFV* mutation carriers and non-carriers.

Analysis of *MEFV* Gene Mutations

Genomic DNA was isolated from peripheral blood lymphocytes using a commercial kit (Sigma-Aldrich, Taufkirchen, Germany), according to the manufacturer's instructions. The most frequently observed four mutations (E148Q [rs3743930; c.442G>C], M694V [rs61752717; c.2080A>G], M680I [rs28940580; c.2040G>C or c.2040G>A], and V726A [rs28940579; c.2177T>C]) and a rare mutation (P369S [rs11466023; c.1105C>T]) in the *MEFV* gene were screened in this study. These five mutations were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. PCRs of M694V, M680I, and V726A mutations were performed by using previously described protocols (Gersoni-Baruch et al. 1998). HinfI, HphI, and AluI restriction enzymes were used for RFLP of M694V, M680I, and V726A mutations, respectively. PCRs of E148Q and P369S mutations were performed by using previously described protocols (Aksentijevich et al. 1999). E148Q and P369S mutations were analyzed using primers F: 5′-CCT GAA GAC TCC AGA CCA CCC CG-3′, R: 5′-GGC CCT CCG AGG CCT TCT CTC TG-3′ and F: 5′-TCC CCG AGG CAG TTT CTG GGC ACC-3′, R: 5′-TGG ACC TGC TTC AGG TGG CGC TTA C-3′, respectively. BstNI and AluI restriction enzymes were used for RFLP of E148Q and P369S mutations, respectively. The amplified and restricted products were separated by electrophoresis on a 2 % agarose gel. Ethidium bromide staining was used to detect the amplified and restricted fragments.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 13.0) and the OpenEpi Info software package version 2.3.1 (www. openepi.com). The χ^2 test was used to evaluate the Hardy–Weinberg equilibrium for the distribution of the genotypes of the patients and the controls. The relationships between MS patients and healthy controls were analyzed by using chi-square test. Chi-square test and Fisher's exact test were used to compare categorical variables appropriately, and odds ratio (OR) and 95 % confidence interval (CI) were used for the assessment of risk factors. All p values were two-tailed, and CIs were set at 95 %. p values less than 0.05 were considered as significant.

Results

Table 1 shows the demographic and clinical characteristics of MS patients and healthy controls. There was no significant difference in the mean ages and gender between

Table 1 The demographic characteristics of patients with multiple sclerosis and healthy controls

Demographic features	MS patients ($n=100$)	Healthy controls ($n=160$)
Age (years), mean (SD)	36.56 (8.84)	37.68 (11.39) ($p=0.40$)
Sex (female/male)	74/26	98/62 ($p=0.06$)
MS types		
Relapsing–remitting	79	N/A
Primary progressive	3	
Secondary progressive	18	
Disease duration (years), mean(SD)	7.91 (6.59)	N/A
EDSS score (SD)	3.34 (2.04)	N/A

MS multiple sclerosis, *EDSS* expanded disability status scale, *SD* standard deviation, *N/A* not assessed

groups. The distribution of *MEFV* gene mutations in MS patients and healthy controls were given in Table 2. In healthy control group, mutation analysis showed that 27 (16.9 %) of the subjects were carrying one mutated *MEFV* allele. The frequencies of M694V, M680I, V726A, E148Q, and P369S mutation carriage were 6.3 % (with 10/320 allele frequency), 3.1 % (5/320), 1.9 % (3/320), 5 % (8/320), and 0.6 % (1/320), respectively (Table 2). No compound heterozygous or homozygous mutation was detected in healthy controls. In the patient group, mutation analysis showed that 35 (35 %) of the patients were carrying at least one mutated *MEFV* allele (Table 2). One homozygous and two compound heterozygous mutations were observed in the patient group. The frequencies of M694V, M680I, V726A, E148Q, and P369S mutation carriage in patients with MS were 14 % (with 14/200 allele frequency), 6 % (6/200), 4 % (4/200), 11 % (12/200), and 3 % (3/200), respectively. There were statistically significant differences of the *MEFV* gene

mutation carrier rates and allele frequencies between MS patients and healthy controls ($p=0.0008$, OR 2.6, 95 % CI 1.47–4.77 and $p=0.0002$, OR 2.6, 95 % CI 1.55–4.48, respectively) (Table 2). The observed and expected frequencies of the mutations in both patient and control group were in Hardy–Weinberg equilibrium. When the *MEFV* gene mutations were separately compared between patients and controls, no association was observed.

Discussion

MEFV gene mutations were suggested to upregulate inflammatory response and predispose *MEFV* mutation carriers to inflammatory diseases (Booth et al. 2000). *MEFV* gene has already been identified as being responsible for FMF. The upregulation of inflammatory response in *MEFV* gene mutation carriers can also affect the severity of the accompanying

Table 2 Distribution of *MEFV* mutations in patients with MS compared with healthy controls

	MS patients ($n=100$) (%)	Healthy subjects ($n=160$) (%)	*p* value	OR (95 % CI)
No. heterozygous				
M694V/WT	12 (12)	10 (6.3)	0.105	2.0 (0.88–5.06)
M680I/WT	5 (5)	5 (3.1)	0.444	1.6 (0.43–6.20)
V726A/WT	4 (4)	3 (1.9)	0.517	2.2 (0.36–15.16)
E148Q/WT	7 (7)	8 (5.0)	0.501	1.4 (0.48–4.18)
P369S/WT	3 (3)	1 (0.6)	0.320	4.9 (0.39–259.7)
No. homozygous/compound heterozygous				
M680I/E148Q	1 (1)	–		
M694V/E148Q	2 (2)	–		
E148Q/E148Q	1 (1)	–		
Total mutations (*n*)	35 (35)	27 (16.9)	*0.0008*	*2.6 (1.47–4.77)*
Total allele frequency (*n*)	39/200	27/320	*0.0002*	*2.6 (1.55–4.48)*
M694V allele	13/200	10/320	0.068	2.2 (0.92–5.17)
M680I allele	7/200	5/320	0.152	2.3 (0.69–7.96)
E148Q allele	11/200	8/320	0.076	2.2 (0.88–6.00)

The results that are statistically significant are shown in italics

MEFV Mediterranean fever, *MS* multiple sclerosis, *WT* wild type

chronic diseases such as RA, BD, PR, and UC (Rabinovich et al. 2005, 2007; Giaglis et al. 2006; Canete et. al. 2007). Patients with these chronic diseases appear to be highly susceptible to develop a more severe disease if they also have a *MEFV* mutation. A possible association of *MEFV* gene mutations and MS has also been suggested in cohorts from Israel, Turkey, and Germany (Shinar et al. 2003; Unal et al. 2010; Yahalom et al. 2011; Kümpfel et al. 2012). In concordance with the results of these studies, in our study, we also found a high association between *MEFV* gene mutations and MS. In a previous study from Turkey with a small sample size (53 MS patients and 66 healthy controls), *MEFV* mutations (E148Q, M680I, M694V, M694I, and V726A) was shown to be significantly higher in patients (38 %) than controls (11 %) (Unal et al. 2010). In our study, the frequency of *MEFV* gene mutations was 16.9 % in healthy controls and 35 % in MS patients. While the total frequency of the *MEFV* mutations was found about two times higher in the MS patients than the healthy control group in our study, it was estimated 3.5 times higher in previous Turkish study (Unal et al. 2010). The frequency of MS was also higher in patients with *MEFV* mutations compared to the normal population in two studies from Israel population (Shinar et al. 2003; Yahalom et al. 2011). In the study of Shinar et al., it was demonstrated that non-Ashkenazi MS patients carrying one mutated *MEFV* gene, particularly M694V, showed rapid progression to disability (Shinar et al. 2003). In the other study from Israeli, in concordance with the first study, MS was shown to be more common in FMF than in the general Israeli population and homozygosity for the M694V *MEFV* mutation may aggravate the phenotype of MS and predispose FMF patients to develop MS (Yahalom et al. 2011). In a study from Germany, Kümpfel et al. (2012) investigated for mutations in exons 2, 3, and 10 of the *MEFV* gene in 157 MS patients and screened selectively for the low-penetrance *MEFV* mutations E148Q and K695R in 260 independent MS patients and 400 unrelated Caucasian controls. Kümpfel et al. (2012) suggested that E148Q mutation is a promising candidate risk factor for MS. According to these four different studies and our study with similar results, we suggest that *MEFV* mutations can increase the risk of MS development. Although a prospective study will increase the validity of our results, the similar findings obtained in several studies from two different ethnic populations strongly support our outcome. In a retrospective screening study, Akman-Demir et al. (2006) reported 12 patients with FMF among total 2,800 patients with inflammatory and demyelinating CNS disease and suggested that FMF increased four times among the MS patients. Akman-Demir et al. (2006) found that the rate of FMF in their MS patients was almost four times the expected prevalence in Turkey, while Kalyoncu et al. (2010) found MS to be twice higher in FMF compared to the general population, supporting the association between the two diseases. There were many patients reported to have both FMF

and MS symptoms mostly from Turkey (Topçuoglu and Karabudak 1997; Yücesan et al. 2004; Guinet et al. 2008; Unal et al. 2009; Ugurlu et al. 2009; Kalyoncu et al. 2010; Sayin et al. 2011).

In conclusion, our study and several others suggest that *MEFV* gene mutations are positively associated with predisposition to develop MS. *MEFV* gene mutations should be investigated in other ethnic populations especially where FMF and MS is common.

Conflict of Interest   None

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