TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 967-971

doi:10.3906/sag-1505-131

MEFV mutations in Iranian Azari Turkish patients with Henoch–Schönlein purpura

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Received: 30.05.2015 • **Accepted/Published Online:** 01.09.2015 • **Final Version:** 23.06.2016

Background/aim: The aim of the current study was to screen the rate of *MEFV* mutations in Henoch–Schönlein purpura (HSP) and to investigate the association of these mutations plus clinical symptoms with HSP disease in the Iranian Azari Turkish ethnic group.

Materials and methods: The study groups included 40 unrelated HSP patients and 200 apparently healthy people without any kind of inflammatory diseases as a control group. Molecular screening was performed for eight main mutations, namely M694V, M694I, M680I, V726A, E148Q, R761H, P396S, and R408Q, using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and sequencing.

Results: Out of the 40 studied patients, 27 subjects (67.5%) did not show any mutation, whereas 10 patients (25%) were heterozygotes for one of the following mutations: M694V, M680I, V726A, E148Q. Moreover, three patients (7.5%) were compound heterozygotes for P369S and R408Q. The significant differences between the patient and control groups for M680I, V726A, E148Q, P396S, and R408Q were P = 0.0043, P = 0.0324, P = 0.0145, P = 0.0043, and P = 0.0043, respectively. Furthermore, no significant difference in clinical manifestations was observed between the two groups of patients with and without mutations.

Conclusion: Based on the results, *MEFV* mutations could be considered effective genetic factors for development of HSP in the Iranian Azari Turkish ethnic group.

Key words: *MEFV* mutations, Henoch–Schönlein purpura, vasculitis, Azari Turkish, Iran

1. Introduction

Henoch–Schönlein purpura (HSP) is one of the most common types of systematic small vessel vasculitis during childhood (1), with an incidence of 10–20 subjects per 100,000 people (2). HSP is a multifactorial inflammatory disease that occurs from the age of 3 to 15, and it is more prevalent among boys (2). HSP is normally described by deposition of immunoglobulin A within the small blood vessel wall (3) and, according to the diagnostic criteria of HSP, this disease would include nonthrombocytopenic palpable purpura and at least one of the other manifestations such as colicky abdominal pain, arthritis, and renal involvement with hematuria and proteinuria, which can progress towards renal failure (1). Although HSP is generally self-limiting and the major pathogenic cause of HSP is still unclear, it seems that inflectional, environmental, and hereditary factors could play important roles in the development and progress of the disease (4).

Given the genetic variations in genes related to cytokines and cell adhesion molecules, which are involved in modulating inflammatory responses and endothelial cell activation, several researchers have focused on polymorphisms in related genes to study the probable associations with HSP (2). In addition, recently the connection between vasculitis such as HSP, Behçet disease (BD), polyarteritis nodosa (PAN), and *MEFV* mutations has been reported, i.e. the gene mutations in *MEFV* could be regarded as an aspect that could have a considerable effect on susceptibility to HSP and its progress (1). The *MEFV* gene is located in 16p13.3 and has 10 exons encoding a protein (pyrin/marenostrin) with multiple

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domains. To date, more than 80 different mutations in the *MEFV* gene have been recognized and several mutations in it are associated with the severity of diseases (5). Pyrin could only be found in granulocytes and is involved in inflammation and cell death regulation (5,6). This protein is considered a crucial part of inflammatory response that could have impact on gene expression, especially the genes involved in immune and inflammatory responses (2).

The present study was planned to investigate the prevalence of common and rare mutations of the *MEFV* gene in Iranian Azari Turkish patients with HSP who had not shown any sign of familial Mediterranean fever (FMF) or other inflammatory diseases.

2. Materials and methods

2.1. Patients

Forty sequential unrelated patients with HSP who had received a clinical diagnosis of HSP and 200 subjects who were apparently healthy without any kind of inflammatory diseases from the Iranian Azari Turkish ethnic group were genetically screened in this study. All the patients were informed about the study purposes and written consent was obtained from the subjects or their parents. In addition, the medical report and family history were collected for each subject. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols (7). This study was also approved by the Ethical Committee of Tabriz University of Medical Sciences.

2.2. PCR amplification and digestion

In this research, eight main mutations in the *MEFV* gene were analyzed using polymerase chain reaction (PCR) amplification as previously described (6). In brief, the E148Q, P369S, and R408Q mutations were detected by the PCR and restriction fragment length polymorphism (PCR-RFLP) technique, while M680I(G/C), M694I, M694V, V726A, and R761H mutations were detected by the amplification refractory mutation system (ARMS-PCR) (6). In this process, positive and negative controls were used for each test and the positive results were repeated to ensure reproducibility (8). Finally, the accuracy of results was verified by direct sequencing.

2.3. Statistical analysis

To compare the frequency of gene carriers, chi-square test and Fisher's exact test were applied (9). The odds ratios (OR) and confidence intervals (CI) at the significance level of 95% were calculated and reported for all the data (10). Significant difference has been assigned to less than 0.05 (P-values < 0.05). Furthermore, the descriptive statistics including percentage and median were used to analyze the obtained data (1,11). Lastly, the chi-square test of goodness-of-fit was utilized to test the Hardy–Weinberg equilibrium.

3. Results

In this study, ARMS-PCR, PCR-RFLP, and sequencing procedures were exploited for molecular diagnosis of eight mutations in HSP patients. The experimental group consisted of 40 HSP patients (24 males and 16 females). The ratio of males to females was 1.5 and the average of age was 7.3 years (ranging from 3 to 13) at the time of HSP diagnosis.

In the first step, eight *MEFV* mutations were screened (12). Out of the 40 patients, 27 (67.5%) did not have any mutations, while 13 (32.5%) had at least an *MEFV* mutation. Molecular screening of the eight *MEFV* mutations, namely M694V, M694I, M680I, V726A, E148Q, R761H, P396S, and R408Q, revealed 10 people (25%) were heterozygotes for one of the mutations (M694V in 1 patient, M680I in 3 patients, V726A in 4 patients, and E148Q in 2 patients), while three people (7.5%) were compound heterozygotes (P369S/R408Q in 2 patients, V726A/P369S/R408Q in 1 patient) and none of the patients were homozygous. In this studied ethnic group, the mutations of M694V, M680I, V726A, E148Q, P396S, and R408Q were observed in 2.5%, 7.5%, 12.5%, 5%, 7.5%, and 7.5% of the patients, respectively. Our results showed a significant difference in frequencies of *MEFV* mutations between the HSP patient and control groups (P-value = 0.0353). Moreover, it was revealed that there was also a significant difference between the mutation distributions in the two groups: M680I (P = 0.0043), V726A (P = 0.0324), E148Q (P = 0.0145), P396S (P = 0.0043), and R408Q (P = 0.0043). In addition, the frequency of M694V, M694I, and R761H mutations in the patient group was not statistically different from that of the control group (P = 0.1666, P = 1, and P = 1, respectively). Totally, 17 mutated alleles were observed in 13 patients, revealing that the mutated allele frequency was 0.21 in this cohort. The distribution of *MEFV* mutations in the HSP patient and control groups are shown in Table 1.

In the second step, the clinical parameters of the patients with/without mutations were analyzed, which showed that there was no significant difference between the considered groups (Table 2). Moreover, in comparison with the patients without *MEFV* mutations, arthritis and arthralgia were more frequent, but insignificant, in the patients with mutated alleles (10/13:15/27, P-value = 0.298 and 5/13:5/27, P-value = 0.246, respectively) (Table 2). Although the patients with *MEFV* mutations were averagely younger than the patients without any mutation, it did not show a significant difference (6.6 years and 7.9 years, respectively). This result is compatible with previous reports (12,13) (not shown in the tables). The main clinical characteristics of the patients were as follows: palpable purpura in 40 patients (100%), joint involvement in 29 patients (72.5%), abdominal pain in 24 patients (60%), renal involvement in 4 patients (10%), orchitis in 2 patients

Table 1. Distribution and P-values of *MEFV* mutations in HSP patients and the control group.

Mutation	Patient group (n = 40)	Control group (n =2 00)	OR (95% CI)	P-value
M694V	1	0		0.1666
M694I	0	0		1
M680I	3	0		0.0043*
V726A	5	7	3.9388	0.0324*
E148Q	2	45	0.1813	0.0145*
R761H	0	0		1
P396S	3	0		0.0043*
R408Q	3	0		0.0043*
Total	17	52	2.1037	0.0353*

*P-values < 0.05

Table 2. Comparison of clinical features between patients with and without mutations.

Clinical feature	HSP with mutation (n = 13)	HSP without mutation (n = 27)	OR (95% CI)	P-value
Palpable purpura	13(100.0)	27(100.0)		1.0000
Arthritis	10(76.9)	15(55.5)	2.666	0.298
Arthralgia	5(38.4)	5(18.5)	2.750	0.246
Joint involvement	12(92.3)	17(62.9)	7.0588	0.0678
Abdominal pain	8(61.5)	16(59.2)	1.1000	0.8414
Renal involvement	1(7.6)	3(11.1)	0.6667	1.0000
Orchitis	0(0.0)	2(7.4)	0.0000	0.5496
Fever	1(7.6)	2(7.4)	1.0417	1.0000
Nausea	0(0.0)	1(3.7)	0.0000	1.0000
Vomiting	1(7.6)	4(14.8)	0.4792	0.6532

*P-values < 0.05

(5%), fever in 3 patients (7.5%), nausea in 1 patient (2.5%), and vomiting in 5 patients (12.5%).

4. Discussion

HSP is an inflammatory multisystemic disorder with unknown etiology. It seems that genetic and environmental factors such as infections trigger the disease (9). Based on some studies, genetic variants in human leukocyte antigen (HLA), angiotensin converting enzyme (ACE), and the *MEFV* gene may confer susceptibility on HSP (4). The *MEFV* gene encodes the pyrine protein, which plays an important role in inflammatory pathways by decreasing inflammation, particularly in neutrophils; therefore, the mutated protein might then cause uncontrolled inflammation and predisposing development of HSP and other types of vasculitis (13). It is proposed that mutation carriers may show higher inflammatory responses with severe clinical symptoms; moreover, research has indicated that the abnormal clearance of immune complexes and dysregulation of inflammatory response were due to defective genetic loci (13). Hence, investigation of *MEFV* mutations in HSP patients would be a useful approach to follow up susceptible patients that could lead to early diagnosis and treatment of HSP sufferers (13).

Additionally, it has been reported that *MEFV* mutations are more common in children with HSP symptoms, which could have influential effects on our understanding about the clinical manifestations and molecular mechanisms of HSP (5). Previously, a comprehensive study described a significant association between HSP and FMF (13). In this regard, the high frequency of vasculitis in FMF patients persuaded the researchers to study the *MEFV* mutations in vasculitis, including HSP, PAN, Behçet disease, inflammatory bowel disease, and rheumatic disease (14). It is suggested that carrying out more investigations, especially in populations with high prevalence of FMF mutations, would lead to better understanding (4). However, more efforts are necessary to identify the risky patients to implement preventive treatment (4). With regard to these observations, the present study investigated the frequency and clinical significance of *MEFV* mutations in HSP patients (14).

In the present paper, the spectrum and distribution of eight *MEFV* mutations were screened in Iranian Azari Turkish HSP patients. Observation showed some *MEFV* mutations (M680I, V726A, E148Q, P396S, and R408Q) could act as an additional genetic susceptibility factor in HSP development. To confirm the suggested association, it was proposed that further investigation should be carried out to determine the ethnic differences in allelic frequency of *MEFV* gene among HSP patients (8).

Molecular screening of eight *MEFV* mutations revealed that ten people (25%) were heterozygous, whereas three people (7.5%) were compound heterozygous for them. In another study, Ozcakar et al. reported that 34% of 80 HSP patients had heterozygous *MEFV* mutations whereas none of the patients showed homozygous or compound heterozygous mutations (1). Other studies reported that 8% homozygous and 43% heterozygous subjects were found in a Turkish population and the rates of homozygous and heterozygous subjects in a Chinese population were 13% and 45%, respectively (2,5). Furthermore, Gershoni-Baruch stated that 27% of Israeli patients had at least a single mutation in the *MEFV* gene (11).

In our cohort, 32.5% of HSP patients were carriers of *MEFV* mutations compared with 26% in the control group with the same ethnic background. The current study is in agreement of Turkish and Chinese populations; the prevalence of *MEFV* mutations in HSP patients was significantly higher than in the normal population (Table 3) (5). Current findings were different from the result obtained by Nikibakhsh et al., in which a high rate of M694V mutations in HSP subjects was reported (15). In contrast to the current findings, two other studies conducted by Altug et al. and Bayram et al. revealed the lower mutation rate for M694V in the HSP patient group (12,13). However, none of the patients with HSP showed the M694V mutation in a Chinese population (12). In this study, the carrier rate of *MEFV* mutations in the control group was 26% and more than half of these individuals had the E148Q mutation. E148Q, located in exon 2, is necessary to determine the cytoplasmic and nuclear localization of pyrin protein within cells (12). With respect to the insignificant differences of the E148Q mutation between the experimental and control groups, it should be noted that it may not be fully penetrant (9). It has been suggested that the E148Q mutation is a mild mutation with reduced penetrance, and a considerable number of patients who were homozygous for the E148Q mutation remain asymptomatic (9). In a study conducted in China, a 30% mutation rate has been reported for E148Q in HSP patients (12). They concluded that E148Q can be a contributory genetic factor for HSP and its related syndrome and it plays an important role in clinical phenotypes associated with HSP (2,12). Given these findings, both M694V and E148Q mutations seem to act very differently in HSP

Table 3. The distribution of mutations in previous studies.

Authors	Country	HSP (n)	Mutation (+) n(%)
Gershoni et al.	Israel	52	14(26.9)
He et al.	China	78	27(34.6)
Özçakar et al.	Turkey	80	27(33.7)
Bayram et al.	Turkey	107	47(43.9)
Dogan et al.	Turkey	76	18(23.6)
Altug et al.	Turkey	68	18(26.4)
Nikibakhsh et al.	Iran	50	12(24.0)
Present study	Iran	40	13(32.5)

patients from different ethnicities with different genetic backgrounds; therefore, it is suggested they may not be specifically related to HSP (12).

Although R761H was the most frequent mutation among the rare mutations, no R761H mutation was found in the patients with HSP (8). This means that it is possible our patients carry new mutations (15). This study did not detect any of the eight mutations in 63 of the 80 alleles studied (78.75%). This could be due to multiple factors, including the presence of unknown mutations in the promoter or within intron regions, genetic heterogeneity, presence of modifier genes, and unknown environmental factors (16). The obtained results and a study performed in Israel indicate that there is no statistically significant difference in clinical manifestations between the patient groups with and without mutations (5) but the patients with *MEFV* mutations were on average younger than the patients without any mutation. This result is compatible with previous reports that mutation carrier patients were younger and more frequent, but not statistically significant as $P < 0.05$ (12,14). Another study revealed the *MEFV* mutations do not influence the clinical symptoms of HSP (5). On the other hand, three other studies (1,12,14) conducted in Turkey indicated that *MEFV* mutations are important predisposing factors for HSP and these mutations also could affect the clinical symptoms (2). In conclusion, our study supports the notion that Iranian Azari Turks are supposed to have a high risk of HSP development and *MEFV* mutations can be a predisposing factor (8).

Acknowledgments
We thank all the colleagues in the project.

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