



Original Article

## Prevalence of the MEFV gene mutations and their clinical correlations in Azeri Turkish patients with childhood Henoch-Schonlein purpura: The role of M680I and E148Q mutations

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### Abstract

**Introduction:** Patients with Henoch-Shonlein purpura (HSP) have higher rates of Mediterranean fever (MEFV) mutations comparing general population. To our knowledge, there is no report in this regard among Azeri Turkish children. In this study, we evaluated the prevalence of MEFV mutations and their clinical and laboratory correlations in Azeri Turkish children with HSP.

**Methods:** In this case-control study, we included 40 unrelated patients from Azeri Turk origin diagnosed with HSP between January 2010 and March 2011. The control group consisted of 100 healthy unrelated subjects. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using standard protocols. Each sample was initially analyzed for the five common mutations (M694V, M694I, M680I, V726A and E148Q).

**Results:** From 40 patients with HSP, 10 patients (25.0%) had one MEFV mutation. Both patient groups (with and without mutation) were similar regarding clinical manifestations and age at the onset of disease. Frequency of female gender was higher in patients with the mutation. MEFV mutations were found in 26.0% of control group among them 19.2% had V726A and 80.8% had E148Q mutation. There was no significant difference in total mutations between patients and controls. Frequency of M680I mutation was significantly higher in HSP patients than controls ( $P = 0.020$ ). E148Q mutation was much higher in the control group than HSP patients, but the difference was not statistically significant ( $P = 0.053$ ).

**Conclusion:** There was no difference in the clinical spectrum of patients with and without MEFV mutation. M680I mutation may have a probable predisposing role for HSP.

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### Introduction

Henoch-Shonlein purpura (HSP) is the most common systemic vasculitis of small vessels in childhood leading to involvement of the skin, gastrointestinal (GI) tract, joints, and kidneys.<sup>1</sup> The incidence of this disease is 12.9-15.0 per 100000 children.<sup>2</sup> Its peak incidence is between 2-8 years of age and is more common in males

than females.<sup>3</sup> Clinical signs include nonthrombocytopenic purpura with mass distribution in lower extremities and buttocks, arthritis, especially in large joints, GI symptoms, and renal involvement.<sup>3,4</sup> The cause is unknown, but infectious agents, medications, food allergies and insect bites have been implicated in its occurrence.<sup>5</sup>

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This disease was first described about 200 years ago by Heberden. Rare manifestations include involvement of the nervous system, heart, eyes, pseudo rheumatoid nodules, pancreatitis, and pulmonary hemorrhage.<sup>4</sup> Although HSP is a self-limited vasculitis with a good prognosis, but sometimes it may lead to long-term complications and even death.<sup>6</sup>

Familial Mediterranean fever (FMF) which is characterized by fever and polyserositis is caused by mutations in the MEFV gene.<sup>7,8</sup> Five changes in the sequence of the Mediterranean fever (MEFV) gene have been known in most patients. Mutations in V726A, M694I, M694V and M680I in exon 10, and E148Q in exon 2 are seen in most patients with FMF.<sup>9-11</sup>

In some recent studies, a relationship between presence of MEFV mutations and vasculitis diseases such as, HSP, Polyarteritis nodosa and Behcet's disease has been proposed widely.<sup>12-14</sup> HSP has been seen in 7.0% of patients suffering from FMF.<sup>15</sup> Review of literature shows that patients with HSP in Turkey,<sup>16-18</sup> Iran<sup>19</sup> and China<sup>20</sup> carry higher mutations of MEFV. A recent study in Iran indicates that FMF gene (MEFV) mutations are frequent in Azeri Turkish population but with some differences in the frequency of individual mutations. Genotyping revealed that the carrier rate in the Azeri Turkish population was 25.5%.<sup>21</sup> However, there is few information about MEFV mutations in children in our area. This study was carried out to work on varieties of MEFV gene mutations in HSP children and evaluate the association between these mutations with clinical characteristics.

## Methods

In this case-control study, 40 unrelated patients from Azeri Turk origin diagnosed to have HSP were studied. These patients were referred by pediatricians, gastroenterologists, and rheumatologists for counseling and genetic testing to the Molecular-Medical Genetic Center of Tabriz, Iran, between January 2010 and March 2011. All patients fulfilled the criteria for the diagnosis of HSP established by American College of Rheumatology published in 1990.<sup>1</sup>

An accurate and detailed family history

was obtained for each patient. No patient had a family history or suspected diagnosis of FMF. Data were collected from medical charts and records including: sex, age, cutaneous manifestations, arthritis, GI tract or renal involvement and laboratory findings at the time of diagnosis [cell blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), urine analysis (UA) and occult blood on stool]. We also recorded their therapeutic course including the use of oral or intravenous corticosteroids. The control group consisted of 100 apparently unrelated subjects who referred for genetic evaluation of deafness without any inflammatory disease.

All patients and control group and their parents were informed about the study. A written consent was obtained from the parents of all participating subjects for blood sampling and deoxyribonucleic acid (DNA) extraction. The study protocol was approved by the Ethic Committee of Tabriz University of Medical Sciences. Peripheral blood leukocytes were used for genomic DNA extraction. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP) methods were used for the initial analysis of the five common mutations (M694V, M694I, M680I, V726A and E148Q),<sup>9-11</sup> using proper positive and negative controls.

To warrant reproducibility, positive results were repeated. PCR products and restriction enzyme-digested fragments were electrophoresed in a 2.0% agarose gel (Sigma Aldrich, Germany) and observed by ethidium bromide staining. To detect the amplified fragments we used ethidium bromide staining of the agarose gel. Sequencing of randomly selected samples was used for checking the results of PCR-RFLP and ARMS-PCR. HSP patients were primarily divided into, two groups of MEFV gene mutations carriers and non-carriers and various data were compared between them. Furthermore, each mutation was compared separately between two groups of patients and controls.

Statistical analyses were performed using the SPSS software (version 16, SPSS Inc., Chicago, IL, USA). Continuous values were expressed as mean  $\pm$  standard deviation (SD) and categorical variables were expressed as percentages. The categorical parameters were compared by  $\chi^2$  tests or Fisher's exact test. The continuous variables were compared by paired samples t-tests.  $P < 0.050$  was considered as significant.

## Results

The patient population consisted of 40 Azeri Turkish children diagnosed with HSP (26 boys and 14 girls). The main demographic and clinical data have been shown in table 1. The male: female ratio was 1.85. The mean age at the disease onset was  $6.73 \pm 2.86$  years (range, 2-13 years). Other than purpura, manifested by all patients, joint and GI manifestations were more frequent. No neurological or cardiac involvement was seen in our patients. Most patients (45.0%) had involvement in 2 sites and only in 2 patients (5.0%) four sites were involved.

Leukocytosis [white blood cell (WBC)  $> 10000$ ] was seen in 26 (65.0%) patients. Elevated values of ESR ( $> 10$  mm/h) were found in 75.0% of patients and CRP was positive in 30.0% of patients. 30 patients (75.0%) had a negative stool exam for occult blood, 4 patients (10.0%) had 1+, 4 patients (10.0%) had 2+ and 2 patients (5.0%) had 3+ occult blood.

Genetic analysis showed that 30 out of 40 patients with HSP (75.0%) had not MEFV mutation, and 10 patients (25.0%) had one MEFV mutation (Table 2). All these 10 mutations were heterozygous. Altogether 10 mutant alleles were seen in 10 patients. None of the patients or controls had M694I

mutation. MEFV mutations were found in 26.0% of control group among them 5 cases (19.2%) had V726A mutation, and 21 cases (80.8%) had E148Q mutation. M694V and M680I mutations were not observed in the control group (Table 2). Although there was no significant difference in total mutations between patients and controls, but the frequency of M680I mutation was significantly higher in HSP patients than controls ( $P = 0.020$ ). Also, E148Q mutation was much higher in the control group than HSP patients but the difference was not statistically significant ( $P = 0.053$ ) (Table 2).

**Table 1. Demographic and clinical data in 40 children with HSP**

Variable	n (%)
Gender	
Male	26 (65.0)
Female	14 (35.0)
Mean age at onset of disease (years)	$6.73 \pm 2.86$
Palpable purpura (%)	40 (100)
Arthritis/arthralgia	31 (77.5)
GI involvement	21 (52.5)
Renal involvement	8 (20.0)
Mean leukocytes ( $\times 10^9/l$ )	$12.73 \pm 4.4$
Mean thrombocytes ( $\times 10^3/ml$ )	$382.3 \pm 1.52$
Mean ESR (mm/h)	$23.07 \pm 14.45$
Positive CRP	
1+ (%)	6 (15.0)
2+ (%)	5 (12.5)
3+ (%)	1 (2.5)
Treatment with steroids	16 (40.0)

HSP: Henoch-Shonlein purpura; GI: Gastrointestinal; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein

The comparison between two groups of HSP patients, with and without MEFV mutation, has been demonstrated in table 3. There was only a statistical significant difference in their gender and frequency of female gender was higher in patients with mutation ( $P = 0.007$ ).

**Table 2. MEFV mutations in two groups of patients and controls**

Mutation	Patients [n (%)]	Controls [n (%)]	P
M694V	1 (2.5)	0 (0)	0.280
M680I	3 (7.5)	0 (0)	0.020*
E148Q	3 (7.5)	21 (21)	0.053
V726A	3 (7.5)	5 (5)	0.560
Total	10 (25)	26 (26)	0.900

\*P is two-sided significant. MEFV: Mediterranean fever

**Table 3. Comparison of clinical of 40 patients with HPS and laboratory features of 40 HSP patients with and without MEFV mutations**

Patients with HSP	With mutation	Without mutation	P
Gender (male) [n (%)]	23 (76.7)	3 (30.0)	0.007*
Mean age (year) (mean $\pm$ SD)	6.98 $\pm$ 3.11	6.00 $\pm$ 1.85	0.350
Arthritis [n (%)]	22 (73.3)	9 (90.0)	0.270
Palpable purpura [n (%)]	30 (100)	10 (100)	-
GI involvement [n (%)]	16 (53.3)	5 (50.0)	0.850
Renal involvement [n (%)]	2 (6.7)	0 (0.0)	> 0.999
Leukocytosis [n (%)]	17 (56.7)	9 (90.0)	0.056
Thrombocytes (mean $\pm$ SD)	369.3 $\pm$ 1.20	421.2 $\pm$ 93.88	0.220
Elevated ESR [n (%)]	22 (73.3)	8 (80.0)	0.670
Positive CRP [n (%)]	7 (23.3)	5 (50.0)	0.110

\*P is two-sided significant. MEFV: Mediterranean fever; ESR: Erythrocyte sedimentation rate; GI: Gastrointestinal; CRP: C-reactive protein; HSP: Henoch-Shonlein purpura; SD: Standard deviation

Both groups were similar in age at onset of disease and clinical manifestations. Frequency of leukocytosis was considerably higher in patients with the mutation, but this difference was not statistically significant ( $P = 0.056$ ).

### Discussion

In the present study, the frequency of MEFV gene mutations in children with HSP and its association with clinical and demographic characteristics were evaluated. Mean age of patients was  $6.73 \pm 2.86$  years and 65.0% of them were male. Similar to our results, age of patients in other studies<sup>2-4</sup> is between 5-7 years and 55.0-65% of patients are males.<sup>2-4</sup> In this study, skin involvement was seen in 100%, joint involvement in 77.5%, GI involvement in 52.5%, and renal involvement in 10.0% of patients. Frequency of different organs involvement in previous studies<sup>2-5</sup> is more or less similar to our study. However in the study of Spasojevic-Dimitrijeva et al.<sup>6</sup> renal involvement was observed in 51.0% of patients that is higher than other studies and 2.0% of their patients had orchitis that was not found in our patients.

In the laboratory findings, leukocytosis was seen in 65.0%, high ESR ( $> 10$ ) in 75.0%, and positive CRP in 30.0% of our HSP patients. In accordance to our findings, in the study of Kolnik et al. high ESR was reported in 66.0% and a high CRP ( $> 8$ ) in 28.0% of patients.<sup>22</sup> In our study, although leukocytosis, elevated ESR, positive CRP and high platelet count were more frequent in patients carrying MEFV mutations, but this difference was not statistically significant. In

the Kolnik et al.<sup>22</sup> and Dogan et al. studies<sup>18</sup> similar results were reported. However in the study of Ozcakar et al.<sup>23</sup> ESR and CRP levels were significantly higher in the group with mutations.

In a genetic study of patients, only 25.0% showed the mutations in MEFV gene, among them E148Q, M680I, V726A mutations had the equal frequency and M694V mutation had the least frequency. Similar to the present study, Gershoni-Baruch et al.<sup>16</sup> observed that 26.9% of HSP patients had MEFV mutations with a higher frequency in E148Q mutation (11.5%). Bayram et al. study<sup>17</sup> determined that 43.9% of HSP patients had one of MEFV mutations, and the most frequent mutation in their patients was M694V. In Ozcakar et al. study MEFV gene mutation was found in 34.0% of patients, and most common mutation was M694V (20.0%) and the least mutation was E148Q (75.3%).<sup>23</sup> In the Nikibakhsh et al. study<sup>19</sup> M680I mutation was the most frequent mutation.

In our study, there was no significant correlation between age, arthritis and GI involvement among patients with or without mutations. Also, Dogan et al.<sup>18</sup> did not observe a significant relationship between HSP patients with and without MEFV mutations in terms of age, sex, joint, GI and renal involvement. Another study performed by He et al. showed a statistically significant association between E148Q mutation and joint involvement.<sup>20</sup>

In the present study, no significant difference was found between patient and control groups regarding the total frequency of MEFV gene mutations. However in

examining each mutation separately the frequency of M680I mutation was significantly higher in HSP patients in comparison with the control group in whom no M680I mutation was found. This finding probably indicates that M680I mutation may be a predisposing factor to HSP. Similar to our results, Kolnik et al.<sup>22</sup> showed that the prevalence of MEFV mutations in patients with HSP was not more than general population, but study of Dogan et al.<sup>18</sup> showed that the prevalence of MEFV mutations was 9 fold more in HSP patients than general population. These different results may be attributed to genetic variations in different geographical areas.

### Conclusion

There was no difference in clinical features of HSP patients with and without MEFV

mutation except female gender, which was higher in patients with the mutation. There was not any significant difference between patient and control group regarding the total frequency of MEFV gene mutations. However, the frequency of M680I mutation was significantly higher in HSP patients. This finding may suggest that M680I mutation is a predisposing factor to HSP. More investigations with larger samples are needed in future in Azeri Turk population.

### Conflict of Interests

Authors have no conflict of interest.

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