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Prevalence of *Mefv* Gene Mutations and their Association with Clinical Phenotypes in 102 Caucasian Children with Henoch-Schonlein Purpura

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Abstract

Aim: To assess the prevalence of *MEFV* mutations in Caucasian children with Henoch-Schönlein purpura (HSP) and to investigate a possible association between the two diseases in a population with presumably low incidence of familial Mediterranean fever (FMF).

Methods: One hundred and two children diagnosed with HSP between January 2002 and February 2009 were included in the study. Clinical data were obtained from medical charts. Children were tested for 6 common *MEFV* mutations. To find out the carrier rate of mutations in *MEFV* gene in Slovenian population a control group of 105 apparently healthy adults was screened.

Results: Heterozygous *MEFV* gene mutations were found in 6% of children with HSP and in 7% of apparently healthy adults. The most common allelic variants found in both groups were as follows: V726A in 5 participants, K695R in 4 participants, E148Q in 3 participants and M694V in 1 participant. No significant differences in HSP clinical picture between the group of children with and without mutations in *MEFV* were found. HSP patients with *MEFV* mutations were younger than patients without *MEFV* mutations.

Conclusion: In contrast to previously published researches, *MEFV* mutations are not more frequent in children with HSP comparing to apparently healthy population and have no influence on the clinical presentation of HSP.

Keywords: Familial Mediterranean fever; Henoch-Schonlein purpura; *MEFV* mutations

Abbreviations: CRP: C-reactive protein; DNA: Deoxyribonucleic Acid; ECE Countries: Eastern and Central European Countries; ESR: Erythrocyte Sedimentation Rate; EULAR: European League Against Rheumatism; FMF: Familial Mediterranean Fever; GIT: Gastrointestinal; HSP: Henoch- Schönlein Purpura; *MEFV: F*amilial Mediterranean Fever Gene; PRES: Paediatric Rheumatology European Society

Introduction

Henoch-Schönlein purpura (HSP) is the most common systemic vasculitis in childhood with estimated incidence of 12.9-20.1/100.000 children [1,2]. It is characterized by palpable purpura, arthritis and arthralgias, abdominal pain, occult or manifest gastrointestinal bleeding and nephritis ranging from microscopic hematuria and proteinuria to nephrotic or nephritic syndrome or even acute renal failure [3,4]. HSP is also the most common vasculitis in Slovenian children. On the other hand Familial Mediterranean Fever (FMF), the most common periodic fever syndrome, is a very rare disease in Slovenia with only few diagnosed cases in adult population.

FMF is an autosomal recessive disease caused by mutations in *MEFV* gene localized on chromosome 16. *MEFV* gene is composed of 10 exons encoding a 781 amino acid protein known as pyrin, which is expressed mainly in innate immune cells, but not lymphocytes. Pyrin has several domains that have a distinct role in interactions with different proteins related to inflammation [5]. Mutation in *MEFV* gene causes the clinical picture of FMF, which is characterized by recurrent fever accompanied by arthritis, serositis, myalgias and rash [6,7]. So far, 100 disease associated mutations have been described [8].

Studies investigating the *MEFV* mutations in children with HSP performed in populations, where FMF prevalence is high, reported *MEFV* gene mutations to be more common in patients with HSP than in general population, thus making it an important predisposing factor for HSP [9-11]. It was also shown that FMF patients develop vasculitides, most commonly HSP, more often than general population [6]. Aims of the present study were to determine the prevalence of *MEFV* mutations in children with HSP in a population with presumably low incidence of FMF and to investigate the possible associations between the two diseases.

Methods

Study design was a retrospective data collection of children diagnosed with HSP between January 2002 and February 2009 at the University Children's Hospital, Ljubljana, Slovenia. Study population consisted of 102 children who were available for genetic testing and were willing to participate in the study. To be diagnosed as having HSP, children had to fulfil the EULAR/PReS endorsed consensus criteria for classification of childhood vasculitides that were valid at the time of diagnosis and had to be younger than 18 years at the time of diagnosis [12,13]. The exclusion criteria were leukocytoclastic vasculitis of other aetiology or suspected periodic fever syndrome. Data were collected from medical charts, including sex, age at diagnosis, cutaneous manifestations, joint, GIT and/or renal involvement, and laboratory parameters at the time of diagnosis- ESR, CRP and the number of leukocytes. Control group consisted of 105 apparently healthy adults, who donated their DNA for research purposes.

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Genotype	Patients with HSP	Controls	Total (% of all persons with mutations*)
null/null	96	98	194
M694V/null	1	0	1 (8)
M694I/null	0	0	0
M680I/null	0	0	0
V726A/null	1	4	5 (38)
K695R/null	2	2	4 (31)
E148Q/null	2	1	3 (23)
Total	102	105	207

* the number of all persons with mutations is 13

Table 1: *MEFV* genotypes.

A written informed consent for participation in the study, drawing of blood for DNA isolation and genetic testing of *MEFV* mutations was obtained from the parents of the children or from the patients themselves if older than 18 years at the time of drawing blood. Patients were invited for a control visit. Blood sample was obtained at the time of a clinical examination. The study was approved by the Ethics' Committee of the Slovenian Ministry of Health and was conducted according to the principles of the Helsinki Declaration.

Clinical and laboratory data were collected using Microsoft Excel 2003 and statistical tests were performed using SPSS 13.0. A p value <0.05 was considered statistically significant.

Molecular analysis

DNA isolation was performed from peripheral blood using FlexiGene isolation kit (Qiagen, Germany), following the manufacturer's instructions. Polymerase chain reaction of part of exons 2 and 10 was performed using the AmpliTaq polymerase (Applied Biosystems, USA) and corresponding reagents. Six most common mutations in MEFV gene were tested including V726A, K695R, M694V, M694I, M680I in exon 10 and E148Q in exon 2 [15]. Primers used for the amplification were FMFe2f 5' AAAACGGCACAGATGATTCC 3' / FMFe2r 5'

CCTTCTCTCTGCGTTTGCTC3' and FMFe10f5'

TTGGAGACAAGACAGCATGG 3'/FMFe10r5'

AGCAGGAAGAGAGATGCAGTG 3' (Invitrogen, USA).

Our protocol mixture consisted of 4 ng/μL double stranded DNA, 0.2 μM of each deoxynucleoside triphosphate (dNTP), 0.4 μM primers, 1 mM $MgCl_2$, 1/10 of corresponding reaction buffer and 0.08 U/ μL Ampli*Taq* DNA polymerase (Applied Biosystems, USA).

After the initial preincubation of DNA for 5 minutes at 95°C, 35 cycles of amplification were performed. Three steps PCR protocol was chosen, with DNA denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and primer extension at 72°C for 30 seconds. Seven minutes primer extension at 72°C was allowed at the end of 35 cycles.

PCR products (201bp for exon2 and 400bp for exon10) were purified and concentrated using the QIAquick PCR purification kit (Qiagen, Germany), following the manufacturer's instructions and subjected to direct nucleotide sequencing using the Big Dye Terminator cycle sequencing kit and ABI PRISM 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). We compared the obtained sequences with the gene sequence published in GenBank (NG_007871.1) [14].

Results

MEFV mutations in the group of children with HSP and in the control group

Heterozygous mutations were found in 6/102 (6%) of children with HSP and in 7/105 (7%) subjects in a control group. The distribution of *MEFV* genotypes in both groups is shown in Table 1. There were no homozygous or compound heterozygous mutations found in either group. The difference in the frequency of mutations between the groups was not statistically significant (χ^2=0.048, p= 0.8271).

Clinical features of HSP and association with MEFV gene mutations

Among 102 children with medical history of HSP 48 patients were male and 54 female. The mean age at diagnosis was 7.1 ± 3.9 years; range 0.5–17.3 years. Palapable purpura was present in all patients (100%), arthritis or periarticular edema in 58 patients (57%), mild gastrointestinal involvement in 22 patients (22%), severe gastrointestinal involvement necessitating methylprednisolone therapy in 11 patients (11%) and kidney involvement in 11 patients (11%). The mean value of leukocytes at the time of hospitalization was 9.7 ± 3.8 × 10^9/L. Elevated values of ESR (>15 mm/h) were found in 66% (38/58) of patients, mean value was 32.8 ± 19.6 mm/h and CRP values (>8 mg/L) were elevated in 28% (28/100) of patients, mean value was 31.8 ± 21.7 mg/L.

Patients with *MEFV* mutations were in average younger than patients without *MEFV* mutations, but the difference was not statistically significant. No other statistically significant difference was found between patients with and without mutations. The clinical manifestations in the group of children with MEFV mutations and in the group of children without mutations are shown in Table 2.

Clinical presentation of HSP in children, carriers of MEFV mutations, was very diverse and their clinical data is shown in Table 3.

Discussion

Present study is the first to evaluate the prevalence of *MEFV* mutations in children with HSP in a population where FMF is very rare. There was no difference in the *MEFV* carrier rate between the group

	Patients without MEFV mutations n=96 n (%)	*Patients with MEFV mutations n=6 n (%)*
Mean age at diagnosis (years):	7,1±3,9	5,8 ±2,6
Gender, n (%)		
Female	51 (53)	3 (50)
Male	45 (47)	3 (50)
Clinical features, n (%)		
Palpable purpura	96 (100)	6 (100)
Arthritis or periatricular edema	55 (57)	3 (50)
Mild GIT involvement	21 (22)	1 (17)
Severe GIT involvement	11 (12)	0
Renal involvement	10 (10)	1 (17)
Laboratory findings:		
Leukocytes, n1=96, n2=6	9,7±3,9	9,1±2,9
Elevated ESR, n1= 51, n2=4	26,0±19,3	26,8±21,1
Elevated CRP, n1=27 n2=1	32,3±21,9	18*

* CRP value was higher than 5mg/L in only one patient with MEFV mutation
n1- number of data available for patients without MEFV mutations
n2- number of data available for patients with MEFV mutations

Table 2: Clinical manifestations in group of children with and without MEFV mutations.

Patient - gender	genotype	age at diagnosis (y)	Clinical manifestations
M	M694V/null	7,6	purpura
F	V726A/null	5,0	purpura, articular involvement, mild gastrointestinal involvement
M	K695R/null	8,9	purpura, articular involvement, kidney involvement
F	K695R/null	3,1	purpura
F	E148Q/null	2,7	purpura
M	E148Q/null	7,6	purpura, articular involvement

M- male, F- female, y- years

Table 3: Clinical manifestations in individual children with MEFV mutations

of children with HSP and apparently healthy controls. Six out of 102 (6%) patients and 7 out of 105 (7%) apparently healthy controls were found to be heterozygous for one of the screened MEFV mutations. No homozygous or compound heterozygous mutations were found in either group.

Studies evaluating *MEFV* mutations in children with HSP have, so far, only been published in Israeli and Turkish populations where the carrier rate of *MEFV* mutation is high [9-11]. In an Israeli population, Gershoni-Baruch et al. found a single mutation in 17.3% and two mutations in 9.6% of patients with HSP and concluded that the prevalence of mutated alleles significantly exceeds the prevalence in general Israeli population [9]. *MEFV* mutations as an important predisposing factor for HSP were reported also in a study published by Özçakar et al. who found that the proportion of *MEFV* mutation carriers among Turkish patients exceeds that in general population [10]. Recently, another study conducted in Turkish population confirmed that *MEFV* mutations are more frequent in HSP patients than in the general population [11]. Our results do not support findings of previously published studies.

The present study is also the first study in the region of eastern and central European (ECE) countries evaluating carrier rate of *MEFV* mutations in a population where FMF is a rare disease. FMF mainly affects ethnic groups living around the Mediterranean basin, especially Jews, Arabs, Turks and Armenians. In a meta-analysis study published in 2008, based on 16.756 chromosomes from FMF patients and normal individuals from 14 affected populations, where carrier rate is estimated to 1:3 to 1:5, the mean overall carrier rate was found to be 18.6% with peak values in Arabs, Armenians, Jews and Turks. It is rare in other populations. Greeks were found to have a low *MEFV* carrier rate of 1, 4% [15]. FMF was thought to be rare also in Italy but it was found differently when the search of *MEFV* mutations was conducted among patients of Italian origin referred to Italian-French medical centers for fever of unknown origin or surgical emergencies [16]. However, it appears that in western European caucasian patients *MEFV* analysis is of particularly weak diagnostic value for recurrent fevers [17]. First data about the number of FMF patients in ECE countries was published recently and the number of genetically confirmed FMF patients was extremely low. Only 11 genetically confirmed patients were found in a population of 121.5 million from 14 ECE countries [18]. The prevalence of mutations in our study population- 7% is unusually high considering Slovenia's geographic position and extremely low number of patients with FMF. New findings in pathophysiology of FMF are showing that mutations in *MEFV* gene are actually a gain of function mutations and not a loss of function as was considered before. A gene dosage effect can explain that as many as 30% of the patients diagnosed with FMF have only single mutation in *MEFV* genomic region [5].

In the present study, there was no statistically significant difference for clinical or laboratory data among patients with and without MEFV mutations. However, our group of patients with mutations was too small to asses the impact of individual mutations on the clinical picture of HSP. Among our HSP patients only one was a carrier of p.M694V which seems to be the most frequent mutation in HSP patients in Turkey and was found to have effects on clinical and laboratory findings in children with HSP [11]. The results of previously published studies of *MEFV* mutations in Turkish children with HSP also suggested that *MEFV* gene mutations affect the clinical picture of HSP. Among 80 HSP patients in study by Özçakar et al., carriers of *MEFV* mutations were younger, had edema more often and were found to have elevated levels of ESR and CRP more frequently [10]. Recently published study in 107 HSP Turkish patients also showed that scrotal involvement was statistically more frequent in patients with mutations [11]. In the study by Gershoni-Baruch et al. including 52 Israeli HSP patients, no difference in the mean age at the disease onset and clinical findings of patients with or without mutations was found [9].

In conclusion, our study demonstrated that *MEFV* mutations are present also in Slovenian children with HSP but there was no significant difference in the carrier rate of *MEFV* mutations comparing to apparently healthy Slovenian population. These results are in contrast to previously published studies in populations with a high carrier rate of *MEFV* mutations, where the presence of *MEFV* mutation presents a risk factor for development of HSP.

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