MEFV gene mutations and their clinical significance in Korean patients with adult-onset Still's disease

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Received on January 18, 2013; accepted in revised form on May 31, 2013.

Clin Exp Rheumatol 2013; 31 (Suppl. 77): S60-S63.

Key words: MEFV, E148Q, adult-onset Still's disease, Korea

Funding: this work was supported by the research fund of Hanyang University (HY-2009-MC).

Competing interests: none declared.

ABSTRACT

Objective. *Adult-onset Still's disease (AOSD) and periodic fever syndrome share clinical features in some aspects. Familial Mediterranean fever (MEFV) is a typical periodic fever syndrome and MEFV gene mutations may contribute to the clinical features of certain rheumatic diseases. The purpose of this study is to research the incidence and clinical utility of MEFV gene mutations in Korean AOSD patients.*

Methods. *The study included 96 AOSD patients and 165 healthy controls. In both groups, genomic DNA was isolated and genotyped using restriction fragment length polymorphism for 5 MEFV gene mutations (E148Q, P369S, M680I, V726A and M694V). In the AOSD patients, the clinical significance of MEFV mutation was assessed by the laboratory and clinical features.*

Result. *M680I, V726A and M694V were not found in both groups. P369S was detected in 7 (7.3%) AOSD patients and 10 (6.1%) healthy controls. E148Q mutation was found in 77 (46.7%) among healthy controls with 6 QQ and 44 (45.8%) of AOSD patients with 5 QQ, respectively. The allele frequency of E148Q was 0.25 in AOSD patients, and that of P369S was 0.04. However, there was no significant difference in most clinical manifestations and laboratory findings by the presence and absence of E148Q mutation.*

Conclusion. *MEFV mutations including E148Q mutation were not associated with the development of AOSD patients in Korea. Although high incidence of E148Q mutation was found, E148Q mutation did not show major effect on the clinical features of AOSD. But we need to look for association with clinical response to certain treatments and long-term prognosis.*

Introduction

Adult-onset Still's disease (AOSD) is a rare, systemic inflammatory disorder characterised by high spiking fever, rash, arthritis and leukocytosis (1). To date, pro-inflammatory cytokines may play important roles in the pathogenesis (2). Dysregulated production of interleukin (IL)-1 played a crucial role in the pathogenesis of systemic onset juvenile idiopathic arthritis (JIA), which shares clinical features and pathogenesis with AOSD (3). However, genetic studies, including our own on IL-1 could not identify significant genetic association in AOSD patients (4). But, there is a possibility that certain mutations and/or polymorphisms of genes within the IL-1 pathway may contribute to pathogenesis of AOSD.

Familial Mediterranean fever (FMF) is a hereditary auto-inflammatory disorder characterised by periodic episodes of fever, peritonitis, pleuritis, arthritis or erysipelas-like erythema. It occurs mainly in Mediterranean and Middle Eastern populations (5). FMF is associated with a single gene named *MEFV* encoding pyrin, a major regulator of inflammation, and mutations of this gene result in uncontrolled inflammatory cascade, probably by dysregulated inflammasome function and excessive IL-1β activation (6). Among more than 220 *MEFV* mutations reported to date (7), M694V, M680I, V726A and M694I are major mutations associated with disease (5).

The *MEFV* gene may play as an independent modifier of the clinical manifestations of rheumatoid arthritis, and in systemic onset JIA (8, 9). It is still uncertain whether *MEFV* mutation is associated with clinical manifestations of chronic inflammatory disease, especially in the area where the incidence of FMF is very low alike Korea, even though three FMF cases, lacking major

MEFV mutations, were reported very recently (10-12).

To date, there has been no previous study about association between *MEFV* gene mutations and AOSD. In this study, we investigate the incidence of *MEFV* gene mutations and whether *MEFV* gene mutations have an effect on disease manifestations in a cohort of AOSD patients in Korea.

Patients and methods

This study included 96 patients with AOSD meeting the Yamaguchi's criteria (1). The data collected were: complete blood cell count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver function test, albumin, lactic dehydrogenase (LDH) and serum ferritin at first flare in our hospital. A total of 165 otherwise healthy controls were recruited. The protocol of this study was approved by the Institutional Review Board of Hanyang University Hospital. All patients and healthy subjects gave informed consent prior to the study. Medical history, clinical findings and laboratory findings including Pouchot's clinical score were collected through a review of the electronic medical records in the patient group.

Mutational analysis of both patients and healthy controls was carried at the Institute of Rheumatology at Hanyang University. Total genomic DNA was extracted from peripheral blood leukocytes using standard protocols of QIAamp DNA Investigator kit (Qiagen, Duesseldorf, Germany). Each sample was tested for the five mutations: E148Q, M694V, M680I, V726Aand P369S by using polymerase chain reaction (PCR) and restriction fragment length polymorphism methods as described previously (13). All of the PCR primers were produced by Bioneer (Daejeon, Korea), and restriction enzymes (AvaI, Sac I, Hinf I, Hph I, Alu I) were purchased from Thermo Fisher (Waltham MA, USA).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 20.0, Chicago, Illinois, USA). The χ^2 test or Fisher's exact test and Mann-Whitney U-test were used as appropriate. A probability (p) value of <0.05 was considered significant.

Table I. Demographic and clinical features of the patients with AOSD according to E148Q alleles.

Demographic/Clinical features	EQ or QQ (n=44)	EE (n=52)	Total (n=96)
Onset age (years)	33.6 ± 11.8	34.1 ± 11.8	39.1 ± 12.2
Female (%)	81.8	82.7	82.3
Fever (%)	97.7	100	98.9
Rash (%)	81.4	78.4	79.8
Sore throat (%)*	34.4	65.5	58.5
Arthritis or arthralgia (%)	92.2	83.7	89.3
Myalgia (%)	62.8	54.2	62.8
Hepatomegaly (%)	37.2	40.4	38.9
Serositis (%)	9.3	5.8	7.4
Rheumatoid factor (%)	2.3	1.9	2.1
Antinuclear antibody (%)	4.4	21.6	13.5
Clinical progression type			
Monocyclic pattern (%)	25.0	15.3	19.8
Polycyclic systemic pattern (%)	40.9	51.9	46.9
Chronic articular pattern (%)	29.5	30.7	30.2
Undetermined pattern (%)	4.5	1.9	3.1
Modified Pouchot's score	5.8 ± 1.7	6.2 ± 1.6	6.0 ± 1.7
Steroid mean dosage (prednisolone equivalent dose, mg/day)	9.6 ± 5.7	17.2 ± 46.5	13.7 ± 34.5
Response rate to steroid treatment (%)	79.5	82.7	81.3
Macrophage activation syndrome (%)	0	1.9	1.0

Steroid dosage was estimated total used cumulative steroid divided by durations of follow-up. *p-value 0.012.

Table II. Laboratory findings of the patients with AOSD according to E148Q allele.

Laboratory findings (normal range)	EQ or QQ (n=44)	EE (n=52)	Total (n=96)
WBC (4,000-10,000/mm^3)	15285.7 ± 7704.9	14223.1 ± 8107.9	14710.1 ± 7902.2
Hemoglobin (12.0-16.0mg/dL)	10.3 ± 1.6	11.8 ± 1.5	10.6 ± 1.6
Platelet (150-450x10^3/mm^3)	282.8 ± 112.4	313.9 ± 135.4	299.7 ± 125.7
CRP (<0.3 mg/dL)	10.2 ± 6.2	8.4 ± 7.0	9.2 ± 6.7
ESR (≤20 mm/hour)*	82.9 ± 26.1	66.5 ± 35.5	74 ± 36.5
AST (5-40 U/L)	63.4 ± 85.9	112.1 ± 254.1	89.8 ± 196.4
ALT (5-45 U/L)	65.5 ± 113.1	117.7 ± 226.2	98.8 ± 184.2
Albumin (3.2-5.5 g/dL)	3.3 ± 0.5	3.5 ± 0.5	3.4 ± 0.5
LDH (60-200 U/L)	383.9 ± 319.2	501.5 ± 648.1	447.7 ± 524.7
Ferritin (13-150 ng/mL)	6548.3 ± 10038.0	7907.9 ± 15785.7	7284.7 ± 13410.7

WBC: white blood cell; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactic dehydrogenase. *p-value 0.021.

Results

The 96 patients were composed of 17 males and 79 females, and mean age was 39.1 years. The healthy groups were composed of 21 males, and 144 females, and mean age was 31.4 years. The main clinical manifestations of the AOSD patients were as follows; fever (98.9%), arthritis or arthralgia (89.3%) and rash (79.8%) (Table I). The laboratory findings were described in Table II. Among 5 mutations tested, M680I, V726A and M694V were not found among AOSD patients and healthy controls. Heterozygous P369S mutation was found in 7 AOSD patients (7.3%) and 10 healthy controls (6.1%). In patients with AOSD, 44 patients (45.8%) had E148Q, composed of 39 patients with heterozygous E148Q (EQ) and 5 female patients with homozygous E148Q mutation (QQ). Among healthy controls, 77 had E148Q mutation (46.7%) with 6 QQ. The allelic frequencies of E148Q in AOSD patients and controls were 0.25 and 0.25, respectively, and those of P369S were 0.04 and 0.05, respectively. Four AOSD patients and 7 healthy controls had E148Q + P369S mutations. The

Table III. The frequency and allelic frequency of the MEFV mutations in AOSD patients and healthy controls in Korea.

Mutation types	Allele frequency (95% confidence interval)		
	AOSD patients	Healthy controls	Total
E148Q	0.25 (0.19-0.32)	0.04 (0.01-0.06)	0.25 (0.21-0.30)
P369S	0.05 (0.02-0.07)	0.25 (0.21-0.28)	0.04 (0.03-0.06)
M680I	<0.005	<0.003	<0.002
V706A	<0.005	<0.003	<0.002
M694V	<0.005	<0.003	<0.002

frequencies of all five *MEFV* gene mutations, and allelic frequency were not statistically different between AOSD patients and healthy controls (Table III).

The symptom of sore throat was less frequently developed (Table I) and ESR levels were higher (Table II) in group with E148Q mutation compared with patients without E148Q mutation. But, other clinical, laboratory findings and mean dosage of steroid used were not statistically different according to presence or absence of E148Q mutation. There was no significant difference between patients with EQ and QQ (data not shown). The one patient, who had no E148Q mutation, developed macrophage activation syndrome. Total three patients without E148Q mutation died of infection (n=2) or multiple organ failure (n=1).

Discussion

The carrier frequency of *MEFV* mutations is highly various depending on area and ethnic population (5, 14). Since most important *MEFV* mutations were identified in exon 10, M694V was the most. M680I and V726A were also important variations associated with FMF. In contrast to Mediterranean or Arabian region, M694V was not responsible mutation in Japanese patients with FMF. Instead, M694I was most associated for the clinical expression of FMF (14). These major mutations were also not found in Korean FMF cases (10-12). A few studies reported that systemic onset JIA had *MEFV* gene mutation in 4.2%–25.4% of patients. They reported FMF and systemic onset JIA have some common pathogenic features and systemic onset JIA with *MEFV* mutations may have a severe and resistant disease course. M694V mutations had more common and severe disease courses than E148Q mutations in systemic onset JIA (9). E148Q mutation results in the substitution of glutamine for glutamic acid at codone 148 in exon 2. Although there is some ambiguity whether E148Q is a true mutation, E148Q mutation has been known as a milder phenotype with reduced penetrance or usually required another additional *MEFV* mutation to cause the classical manifestation of FMF (15, 16). The E148Q mutations were found in various ranges from 1.4 to 20.2% in Turkish patients with FMF and population screening (7, 17). E148Q mutations were also identified in 27.2% in Chinese and 23.7% of Japanese population (18, 19). In this study, Korean cohort showed high incidence of E148Q mutation among *MEFV* gene mutations in both AOSD patients (45.8%) and healthy controls (46.7%) compared with Turkey, other Mediterranean and other Far East group. However, allelic frequencies of *MEFV* mutations were highly similar to those of Japanese. In one report from Japan, allelic frequencies of E148Q and P369S were 0.23 and 0.057, respectively. However, the allelic frequencies of M694V, M694I, M680I were less than 0.001 (14). In this Korean study, allelic frequencies of E148Q and P369S were also 0.25 and 0.04, and the allelic frequencies of M694V, M694I and M680I were less than 0.002.

Even though we could not find a distinct association of E148Q and P369S mutations in Korean patients with AOSD, sore throat and serum ESR were found to be lower in AOSD patients with E148Q mutation compared to patients without mutation. In a report from Israel, an *MEFV* gene mutation, especially M694V, was regarded as a modifier gene for an attenuated disease activity in periodic fever, aphthous stomatitis, pharyngitis and adenopathy syndrome (PFAPA syndrome) (20). However, the relationship of *MEFV* mutation and clinical feature could not be completely explained, because the M694V mutation was not detected in this study. Therefore, the rarity of these important mutations in this Korean population suggests that *MEFV* mutations may have a minor role in the development and clinical features of AOSD, if other *MEFV* genes participate in the pathogenesis.

This study has some limitations such as small sample size of total 261 cases, cross sectional collection of data, and no *MEFV* gene results in relatives of patients. In addition, a further challenge of colchicine to AOSD patients with *MEFV* mutations will be required. This study is the first attempt to analyse the association of AOSD with *MEFV* gene mutations. We found a high incidence of E148Q mutation and no significant association with the development of AOSD in this Korean cohort. Our results suggest that this E148Q mutation may have a minor role in the clinical expression of Korean AOSD population and not associated with severity. Taken together, the clinical utility of screening for *MEFV* gene mutation seems to be quite different depending on the area, and ethnic populations, and may not provide significant information especially where FMF is infrequent.

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