MEFV mutations in systemic onset juvenile idiopathic arthritis

N. A. Ayaz1, S. Özen1, Y. Bilginer1, M. Ergüven2, E. Taşkıran3, E. Yılmaz3, N. Beşbaş1, R. Topaloğlu1 and A. Bakkaloglu1

Objectives. Autoinflammatory diseases constitute a large spectrum of monogenic diseases like FMF or cryopyrin-associated periodic syndromes (CAPS) and complex genetic trait diseases such as systemic onset juvenile idiopathic arthritis (SoJIA). An increased rate of MEFV mutations has been shown among patients with PAN and HSP, in populations where FMF is frequent. The aim of the study is to search for MEFV mutations in our patients with SoJIA and see whether these mutations had an effect on disease course or complications.

Methods. Thirty-five children with the diagnosis of SoJIA were screened for 12 MEFV mutations. The control data were obtained from a previous study of our centre determining the carrier frequency in Turkish population.

Results. Two patients were homozygous and three patients were heterozygous for the M694V mutation. One patient was a compound heterozygote for the M680I/V726A mutations. Heterozygous V726A mutation was found in one patient. The overall mutation frequency of patients was 14.28%. This figure had been compared with the previously published rate of disease-causing mutations in this country, which is 5%. Disease-causing mutations were found to be significantly more frequent in the SoJIA patients than the population (P < 0.01). Among these, M694V was the leading mutation with a frequency of 10% in SoJIA. Six patients carrying MEFV mutations were among the most resistant cases requiring biological therapy.

Conclusion. SoJIA patients had a significantly higher frequency of MEFV mutations but clinical studies with large number of patients are needed to confirm the association of MEFV mutations with SoJIA and its course.

Key words: Familial Mediterranean fever, Systemic onset juvenile idiopathic arthritis, Mediterranean fever, Mutation.

Introduction

The autoinflammatory diseases constitute a large spectrum from monogenic diseases, such as Mediterranean fever (FMF) or cryopyrin-associated periodic syndromes (CAPS), to complex genetic trait diseases such as Behçet’s disease or systemic onset juvenile idiopathic arthritis (SoJIA) [1]. Autoinflammatory diseases are defined as a group of disorders characterized by episodes of seemingly unprovoked inflammation that, in contrast to the traditionally defined autoimmune diseases, lack high-titre autoantibodies or antigen-specific T cells [2]. FMF is the prototype of the ‘autoinflammatory diseases’ and it is associated with mutations in the MEFV gene. Mutations in this MEFV gene are associated with activation in the IL-1β pathway, which results in attacks of severe inflammation [3]. Mutations and polymorphisms in the MEFV gene even in one allele are associated with subclinical inflammation [4, 5]. MEFV gene has been shown as an independent modifier of the clinical manifestations of RA [6]. An increased rate of MEFV mutations has been shown among patients with PAN and HSP in populations where FMF is frequent [7–9]. On the other hand, a MEFV polymorphism (E158Q) has been associated with severe disease complications in British and Indian patients although this polymorphism is not encountered in the British population [10].

SoJIA is characterized by a chronic inflammation and a lack of autoimmunity. IL-1 has been shown to be a critical cytokine in the pathogenesis of this disease. JIA is a complex genetic trait disease and has been associated with a number of polymorphisms in the gene coding for cytokines and polymorphisms in the MHC region.

We hypothesized that MEFV mutations/polymorphisms may be one of the many genetic factors associated with SoJIA. We thus searched for MEFV mutations in our patients with systemic JIA and determine whether it had an effect on disease course or complications. This is the first attempt to analyse the association of mutations of a monogenic autoinflammatory disease with SoJIA, which is accepted as an autoinflammatory disease with complex genetic trait.

Methods

Thirty-five paediatric patients diagnosed and followed up at Hacettepe Medical Faculty Pediatric Nephrology and Rheumatology Unit as SoJIA according to the Durban criteria were enrolled in this study. Patients were excluded from the study if they had an established or suspected diagnosis of FMF according to the Tel Hashomer criteria [11]. Demographic and clinical data of the patients were reviewed from their follow-up charts.

MEFV gene mutation analysis

All the patients were screened for 12 common MEFV mutations. For all patients, EDTA blood was sampled and DNA was extracted from lymphocytes by standard methods. For the simultaneous detection of 12 Mediterranean fever (MEFV) mutations a reverse hybridization assay (FMF StripAssay, ViennaLab, Vienna, Austria) was used according to the instructions provided by the manufacturer. In a first step, exons 2, 3, 5 and 10 were amplified for each patient in a single, multiplex PCR. A thermocycling programme of 35 cycles (94°C for 15 s, 58°C for 30 s and 72°C for 30 s) with a final extension at 72°C for 3 min was performed, leading to four biotinylated DNA fragments (206, 236, 295 and 318 bp). Biotinylated PCR products were selectively hybridized to a test strip presenting a parallel array of allele-specific oligonucleotide probes, and detected by enzymatic colour reaction. The following MEFV1 mutations were investigated: E148Q in exon 2, P369S in exon 3, F479L in exon 5 and M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H in exon 10.
The control data were obtained from a previous study of our centre determining the carrier frequency in Turkish population [12]. The study was approved by the university ethical committee and informed consent was obtained from the parents.

Statistics

The results were analysed using the Social Package for Statistical Sciences (SPSS, Vienna, Austria) 11.0 and expressed as median (minimum–maximum) for data not showing normal distribution and as mean ± s.d. for data showing normal distribution. The comparison of the frequency of MEFV mutations between the SoJIA patients and the control data of the Turkish population was made by chi-square test.

Results

A total of 35 children (17 females/18 males) with SoJIA were enrolled in the study. The mean age of the patients was 5.79 yrs (range 1–22 yrs). The mean disease duration was 47.97 months (range 3–145 months). All patients received DMARDs and 12 were on biologic therapy. Two patients were homozygous and 3 patients were heterozygous for the M694V mutation. One patient was a compound heterozygote for the M680I/V726A mutations. Heterozygote V726A mutation was found in one patient. The overall mutation frequency of patients was 14.28%. This figure has been compared with the previously published rate of disease-causing mutations in the country which is 5% (excluding E148Q). Disease-causing mutations were found to be significantly more frequent in the SoJIA patients than the population (P < 0.01). However, when the frequency of E148Q was included in the analysis the significance was lost.

Among these three mutations M694V was the leading mutation with a frequency of 10% in SoJIA (P < 0.01).

Six of the patients carrying MEFV mutations were among the patients with the most resistant disease courses, polyarticular involvement and frequent disease flares. They did not respond to the conventional therapy and had disease courses requiring biological therapy due to uncontrolled inflammation with persistently high acute-phase reactants. Among mutation carriers, only one patient with a V726A heterozygous mutation had a mild disease course and proper control of inflammation.

Although not expected as a disease-causing mutation, E148Q was found only in two patients; one of them was homozygous and the other was heterozygous for this low-penetrance mutation.

Discussion

A diagnosis of FMF was ruled out according to Tel Hashomer criteria in all children with SoJIA in this study. We showed that this group of Turkish children with SoJIA had a significantly higher frequency of disease-causing MEFV mutations compared with the healthy control population. The leading mutation was M694V, which is widely accepted as a severe mutation.

FMF is a recessively inherited periodic inflammatory syndrome, characterized by recurrent fever, synovitis and serositis episodes. The disease is common among people coming from eastern Mediterranean ancestry. MEFV gene encodes a protein named pyrin or marenostrin, which is expressed in neutrophils and monocytes. The function of pyrin is still unknown and remains to be determined. Pyrin inhibits the processing of the IL-1β to active form and nuclear factor-xB activation. In the presence of MEFV mutations, these actions of pyrin are deficient and there is uncontrolled production of active IL-1β. This ongoing inflammation leads to recurrent febrile inflammatory episodes and appearance of the classical clinical features of FMF.

SoJIA is a subtype of JIA, and constitutes 10–20% of the JIA patients. It has long been known that it might be an autoinflammatory disease rather than a subtype of JIA. IL-1 is found to be the major cytokine responsible for the pathogenesis of SoJIA.

Ten alleles (14.28%) of MEFV mutations (discarding E148Q) were present in the patients when the data were analysed without E148Q mutation (Table 1). Two patients with homozygous M694V mutations had a severe course and needed biological therapy.

Two patients with homozygous M694V mutations had a very severe disease course and required biological therapy. Only one patient carrying V726A mutation had a mild course and controlled disease without requiring the biological therapy. In a previous study, it was shown that patients with RA carrying one MEFV mutation seem to develop a more severe disease and they hypothesized that MEFV mutations may be a prognostic factor for autoimmune disease outcome [6]. Clinical studies with larger number of patients are required to further comment on the effect of MEFV mutations on the severity of SoJIA.

The higher frequency of mutations in the MEFV gene was further shown in a couple of inflammatory diseases. Giaglis et al. [13] evaluated the frequency of MEFV mutations in a cohort of patients with ulcerative colitis and found an increased prevalence. In ARF [14] and Henoch-Schönlein purpura (HSP) [8] increased frequencies of MEFV mutations were also shown. It was suggested that these patients may have a tendency to develop certain manifestations due to an increased baseline of inflammation, and the presence of these mutations may affect their disease course when they develop rheumatic disease [15]. On the other hand Rabinovich et al. [6] found that MEFV mutations were comparable both in healthy subjects and RA patients. Although the frequencies were comparable, the disease complications were more pronounced in patients with RA.

FMF and SoJIA have some common pathogenic features. Presence of a defective MEFV gene may potentiate the inflammatory response and thus act as a predisposing genetic factor in SoJIA or may cause the disease to have a more aggressive course. The association with MEFV mutations may be one of the genetic determinants in this disease, with a complex genetic trait. Mutations may be a triggering factor for the development of inflammatory state in systemic JIA that is an autoinflammatory disease in itself. Further studies will enlighten the mechanisms and possible epigenetic relationships.

<table>
<thead>
<tr>
<th>MEFV mutations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V, n (%)</td>
<td>7 (10)</td>
</tr>
<tr>
<td>M680I, n (%)</td>
<td>1 (1.43)</td>
</tr>
<tr>
<td>V726A, n (%)</td>
<td>2 (2.85)</td>
</tr>
<tr>
<td>E148Q, n (%)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Total, n (%)</td>
<td>13 (18.48)</td>
</tr>
</tbody>
</table>

Rheumatology key messages

- There is an association of mutations of FMF, which is a monogenic autoinflammatory disease, with SoJIA that is accepted as an autoinflammatory disease with complex genetic trait.
- SoJIA patients with MEFV mutations may have a severe and resistant disease course but clinical studies with large number of patients are needed for confirmation.

Disclosure statement: The authors have declared no conflicts of interest.

References


