# MEFV *Mutations in Egyptian Children with Systemic-Onset Juvenile Idiopathic Arthritis*

**Hala M. Lotfy, Manal E. Kandil, Marianne Samir Makboul Issac, Samia Salah, Nagwa Abdallah Ismail & Mohamed A. Abdel Mawla**

**Molecular Diagnosis & Therapy**

ISSN 1177-1062

Mol Diagn Ther
DOI 10.1007/s40291-014-0105-4


Springer

Mol Diagn Ther
DOI 10.1007/s40291-014-0105-4

ORIGINAL RESEARCH ARTICLE

# *MEFV* Mutations in Egyptian Children with Systemic-Onset Juvenile Idiopathic Arthritis

**Hala M. Lotfy · Manal E. Kandil · Marianne Samir Makboul Issac · Samia Salah · Nagwa Abdallah Ismail · Mohamed A. Abdel Mawla**



**Abstract**

*Background and Objectives* Systemic-onset juvenile idiopathic arthritis (SoJIA) is a chronic auto-inflammatory disease of childhood, with a complex genetic trait, which is characterized by arthritis associated with systemic manifestations. Familial Mediterranean fever (FMF) is another auto-inflammatory disorder that is monogenic. There are speculations as to whether Mediterranean fever (*MEFV*) mutations are among the genetic determinants of SoJIA. Our aim was to explore the frequency and clinical significance of *MEFV* mutations in Egyptian SoJIA patients. A group of healthy children were assigned to the control group in an attempt to estimate the carrier rate of *MEFV* mutations in Egypt.

*Methods* Eighty-four children were recruited in this study; 54 children, age (mean ± standard deviation; 8.31 ± 2.85 years), diagnosed as having SoJIA with no typical symptoms of FMF; 30 healthy age- and gender-matched children served as the control group. All recruited children were screened for 12 common *MEFV* mutations using a reverse hybridization assay of biotinylated PCR products.

*Results* SoJIA patients had a significantly higher frequency of *MEFV* mutations (66.7 %) than in the healthy control population (16.7 %). V726A was the leading mutation in SoJIA patients, with an allelic frequency of 15.74 %, followed by E148Q, with an allelic frequency of 7.4 %. Children who were carriers of *MEFV* mutations had an 18 times higher risk of developing SoJIA than wild-type carriers [odds ratio 18.0 (95 % CI 5–69), $P < 0.01$]. E148Q was the leading mutation, present in 13.3 % of healthy controls.

*Conclusion* These findings suggest that *MEFV* mutations may be responsible for auto-inflammatory diseases other than FMF, and patients with SoJIA, especially those with a positive family history of FMF or SoJIA, should be screened for *MEFV* mutations in countries where FMF is frequent.

**Key Points**

Systemic-onset juvenile idiopathic arthritis (SoJIA) patients had a higher frequency of Mediterranean fever (*MEFV*) mutations than did healthy controls.

V726A was the leading mutation in SoJIA patients, followed by E148Q.

Children who were carriers of *MEFV* mutations had an 18 times higher risk of developing SoJIA than wild-type carriers.

H. M. Lotfy, M. E. Kandil and M. S. M. Issac contributed equally to this article.

**Electronic supplementary material** The online version of this article (doi:10.1007/s40291-014-0105-4) contains supplementary material, which is available to authorized users.

H. M. Lotfy · S. Salah
Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

M. E. Kandil · N. A. Ismail · M. A. Abdel Mawla
Pediatrics Department, National Research Center, Cairo, Egypt

M. S. M. Issac (✉)
Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, El Saray St., El Manial, 11956 Cairo, Egypt
e-mail: mariannesamir@kasralainy.edu.eg; mar_path@hotmail.com

Published online: 27 May 2014

## 1 Introduction

Systemic-onset juvenile idiopathic arthritis (SoJIA) is a chronic auto-inflammatory disease of childhood characterized by quotidian fever, evanescent rash, serositis, hepatosplenomegaly, lymphadenopathy, and inflammation [1]. SoJIA as a subtype constitutes about 10–15 % of all juvenile idiopathic arthritis (JIA) patients [2]. At onset, it is clinically well-distinguished from other forms of JIA by the prominence of extra-articular features such as spiking fevers, a salmon-colored skin rash, lymphadenopathy, and serositis [3]. The multisystem inflammation characteristic of SoJIA is a consequence of being an auto-inflammatory disease with abnormality in the innate immune system. A loss of control of the alternative secretory pathway, leading to aberrant activation of phagocytes including monocytes, macrophages, and neutrophils, seems to be involved in the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-18, and pro-inflammatory S100 proteins [4]. Many patients need combined treatment with glucocorticoids and disease-modifying anti-rheumatic drugs (DMARDs) for years [5]. Treatment-resistant patients with SoJIA show frequent flares or persistent disease activity with significant morbidity and may develop serious complications. Reactive amyloidosis complicates between 1 and 2 % of JIA and is typically associated with active SoJIA [6], although its frequency is dramatically decreasing, probably in relation with a more active DMARD treatment policy [7]. The new insights into the pathogenesis of SoJIA lead to new promising treatment approaches, such as recombinant anti-IL-1 receptor antagonist [8] or anti-IL-6 receptor antibodies [9].

SoJIA is known to be of a complex genetic trait and there are many genes implicated in its pathogenesis [10]. The most consistently reported genetic effects have been the cytokine/chemokine gene polymorphisms and genes encoding IL-6 and macrophage inhibitory factor [11, 12], as well as some Mediterranean fever (*MEFV*) mutations [13, 14].

*MEFV*, the gene responsible for familial Mediterranean fever (FMF), has been mapped to chromosome 16p13.3 [15]. FMF is an autosomal recessive disease with a high prevalence among Mediterranean populations such as Sephardic Jews, Armenians, Middle Eastern Arabs, and Turks. FMF is the most prevalent monogenic auto-inflammatory disease worldwide [16, 17]. Associations have been shown with polygenic auto-inflammatory diseases, as evidenced by an increased carrier rate for *MEFV* mutations in SoJIA [17].

*MEFV* consists of ten exons and encodes pyrin/marenostrin, which is expressed in polymorphonuclear cells, cytokine activated monocytes, dendritic cells, and synovial fibroblasts [18]. Pyrin is a 781 amino acid protein that modulates the production of the potent pro-inflammatory cytokine IL-1β through the regulation of nuclear factor-κB and caspase-1, serving as a negative regulatory protein of inflammation. In the presence of *MEFV* mutations, these actions of pyrin are deficient and there is uncontrolled production of active IL-1β [19]. Therefore, *MEFV* mutations might have a modifying effect on the down-regulation of inflammation mediated by granulocytes, and leads to clinical symptoms by increased migration of leukocytes to serosal/synovial membranes [20, 21].

More than 285 variations have been found in the *MEFV* gene [22], with a significant number located in exon 10. It harbors the four most frequent mutations—M694V, V726A, M680I, and M694I—whose pathogenic significance is well-established and widely accepted [23]. E148Q is a frequent sequence variation situated in exon 2, but its involvement in the development of the disease remains controversial [24].

The aim of this study was to explore the frequency and clinical significance of *MEFV* mutations in Egyptian SoJIA patients and to study the hypothesis that the phenotypic expression of the disease may be attributable to the existence of a particular mutation. A group of healthy children were assigned to the control group in an attempt to estimate the carrier rate of *MEFV* mutations in Egypt.

## 2 Subjects and Methods

### 2.1 Subjects

The study included 54 Egyptian children with SoJIA, diagnosed according to the International League of Associations for Rheumatology (ILAR) criteria [25] for classification of JIA. Those patients were followed up at the Pediatric Rheumatology Clinic, Abo El-Reesh Hospital, Cairo University, Cairo, Egypt, during the period from July 2004 to August 2013, for a minimum period of 6 months. Thirty age- and gender-matched apparently healthy controls, with no family history or clinical manifestations suggestive of SoJIA or FMF, were assigned to the control group.

Inclusion criteria for patients included age of disease onset from 6 months to 16 years and disease duration $\geq$1 year. Exclusion criteria included age of disease onset $>$16 years and children diagnosed as FMF prior to the onset of SoJIA. The study was approved by the Cairo University Clinical Research Ethics Committee, and informed consents were obtained from parents of all participants. All included children were subjected to thorough history taking, including demographic data and disease duration, together with full physical examination.

*MEFV* mutation analysis was performed for all recruited children. The results of laboratory investigations, including complete blood count, C-reactive protein (CRP), rheumatoid factor, anti-nuclear antibody, and erythrocyte sedimentation rate (ESR), performed during the same week of *MEFV* mutation analysis, were collected from the follow-up data in patients' files. Results of the last abdominal ultrasonography and slit lamp examination were also obtained from patients' files.

### 2.2 Methods

#### *2.2.1 MEFV Mutation Analysis*

A 2 mL venous blood sample was collected from all recruited children, in a sterile EDTA vacutainer. All children were screened for 12 *MEFV* mutations (E148Q in exon 2, P369S in exon 3, F479L in exon 5, M680I (G/C), M680I (G/A), I692del (2076–2078), M694V, M694I, K695R, V726A, A744S, R761H in exon 10) using the FMF Strip Assay® (Vienna Lab Diagnostics GmbH, Vienna, Austria) [26]. The assay is based on PCR and reverse hybridization and includes three steps: (1) DNA isolation from anticoagulated blood; (2) PCR amplification using biotinylated primers; and (3) hybridization of amplification products to a test strip containing both wild-type and mutant allele-specific oligonucleotide probes immobilized as an array of parallel lines.

According to the manufacturer's instructions, exons 2, 3, 5, and 10 were amplified for each subject in a single, multiplex PCR. A thermocycling program of denaturation at 94 °C for 2 min, followed by 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 in a Hybaid (express) thermal cycler (Promega Corporation, Fitchburg, WI, USA). Four biotinylated DNA fragments (206, 236, 295, and 318 bp) were produced and visualized by agarose gel electrophoresis stained with ethidium bromide. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. For each polymorphic position, one of three possible staining patterns were obtained either wild-type probe only (normal genotype), wild-type and mutant probe (heterozygous genotype), or mutant probe only (homozygous mutant genotype).

#### *2.2.2 Statistical Analysis*

Data management and analysis were performed using SPSS® version 17 (SPSS Inc., Chicago, IL, USA). Quantitative data were statistically described in terms of mean ± standard deviation, while qualitative data were statistically described as frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between more than two groups was done using the one-way analysis of variance (ANOVA) test with post hoc multiple two-group comparisons in normal data. Association between qualitative data was done using the Chi-square test. To study the independent effect of the different predictors of SoJIA, a stepwise logistic regression analysis was performed, with arthritis as the dependent variable. All $P$ values are two-sided. $P$ values $<0.05$ were considered significant.

## 3 Results

### 3.1 Demographic Data of Patients and Controls

The study included 54 Egyptian children with SoJIA, 55.6 % females and 44.4 % males. Their mean age at diagnosis was 5.57 ± 2.9 years, and the mean age at time of examination was 8.31 ± 2.8 years. The parents of 20 % of the patients were consanguineous, and 18.5 % of patients had family history of JIA, rheumatoid arthritis (RA) or FMF. The control group included 30 children whose mean age was 8.63 ± 3.10 years. The demographic and clinical characteristics of these patients, together with the medications used throughout the disease course; according to the disease activity, were summarized in Tables 1 and 2, while investigations are presented in Table 3.

### 3.2 The Frequency of *MEFV* Mutations in Systemic-Onset Juvenile Idiopathic Arthritis (SoJIA) Patients and Controls

Carriers of *MEFV* mutations constituted 16.70 % of the controls and 66.70 % of SoJIA patients. V726A mutation was present in 31.48 % of patients, while codon 148 mutations were present in 20.37 % of patients as shown in Table 1. The *MEFV* genotypic and allelic frequencies are summarized in Table 4. One test strip showed negative staining for both wild-type codon 148 and E148Q bands, which denotes a homozygous mutation (other than E148Q) in codon 148, so it was named as unspecified codon 148 mutation, which needs further identification by sequencing. The same pattern was present in another two test strips, in addition to the presence of M694 heterozygous mutation, so these patients were denoted as unspecified codon 148 homozygous/M694 heterozygous.

### 3.3 Characteristics of SoJIA Patients Stratified by the Presence of *MEFV* Mutations

A positive family history of JIA or FMF was present in 27.8 % of SoJIA patients who were *MEFV* mutation carriers, while 100 % of the wild-type SoJIA patients showed a negative family history, with a statistically significant

**Table 1** Characteristics of systemic-onset juvenile idiopathic arthritis patients and controls

| | SoJIA patients ($n = 54$) | Controls ($n = 30$) | $P$ value |
|---|---|---|---|
| Age (years) | 8.31 ± 2.85 | 8.63 ± 3.10 | 0.640 |
| Gender | | | |
| Male | 24 (44.40 %) | 11 (36.7 %) | 0.748 |
| Female | 30 (55.60 %) | 19 (63.3 %) | |
| Presence of *MEFV* mutations | | | |
| Wild-type (no mutation) | 18 (33.30 %) | 25 (83.3 %) | <0.01 |
| Mutation | 36 (66.70 %) | 5 (16.7 %) | |
| Presence of V726A mutation | | | |
| Yes | 17 (31.48 %) | 0 (0 %) | <0.01 |
| No | 37 (68.52 %) | 30 (100 %) | |
| Presence of codon 148 mutation[a] | | | |
| Yes | 11 (20.37 %) | 4 (13.3 %) | 0.190 |
| No | 43 (79.63 %) | 26 (86.7 %) | |

Quantitative data are represented as mean ± standard deviation, while qualitative data are represented as frequency (%)

*SoJIA* systemic-onset juvenile idiopathic arthritis

[a] Codon 148 mutations include E148Q and unspecified codon 148 mutations

difference ($P = 0.02$). Oral corticosteroids intake showed a statistically significant difference when compared between the two groups ($P = 0.04$), where 100 % of wild-type SoJIA patients and 77.80 % of *MEFV* mutation carrier SoJIA patients were on oral corticosteroid treatment. SoJIA patients who were carriers of *MEFV* mutations showed a statistically significant decrease in hemoglobin level and platelets count ($P = 0.03$ and 0.01, respectively) when compared with wild-type carriers. Fifty percent of wild-type SoJIA patients and 80.60 % of SoJIA patients who were carriers of *MEFV* mutations showed positive CRP with a statistically significant difference ($P = 0.02$), as shown in Tables 2 and 3.

### 3.4 Characteristics of SoJIA Patients with *MEFV* Mutations Stratified According to Type of Mutation

SoJIA patients who were carriers of *MEFV* mutations were divided into three groups according to the type of mutation: patients with V726A mutation ($n = 17$); patients with codon 148 mutation [E148Q + unspecified codon 148 mutation) ($n = 11$)]; and patients with other mutations [M680I, M694V, A744S ($n = 8$)]. We aimed to examine whether there was an association between the type of *MEFV* mutation in SoJIA patients and the demographic data, clinical characteristics, investigations and intake of medications, as shown in Tables 1 and 2 in the Electronic Supplementary Material. There was no statistically significant difference in the variables examined between the three studied groups.

### 3.5 Multivariate Logistic Regression Analysis

Multivariate logistic regression analysis was performed to examine the contribution of age in years and presence of *MEFV* mutation to the development of SoJIA. Children who were carriers of *MEFV* mutation had an 18 times higher risk of developing SoJIA than wild-type carriers [odds ratio 18.0 (95 % CI 5–69), $P < 0.01$].

## 4 Discussion

The frequency of *MEFV* mutations in our group of Egyptian SoJIA children was significantly higher than in the healthy control population. Moreover, carriers of *MEFV* mutations had an 18 times higher risk of developing SoJIA than wild-type carriers. V726A and E148Q were the most frequently detected *MEFV* mutations.

It was previously reported that there was an association between mutations of FMF, which is a monogenic auto-inflammatory disease, and SoJIA, which is accepted as an auto-inflammatory disease with a complex genetic trait [13]. *MEFV* mutations are associated with activation in the IL-1β pathway [19]; IL-1 is also one of the major cytokines responsible for the pathogenesis of SoJIA [27]. Therefore, the association of *MEFV* mutations with SoJIA may be recognized as a triggering factor for the development of an inflammatory state in systemic JIA [28]. The association between *MEFV* mutations and arthritic diseases was reported in previous studies [13, 14, 28–30]. To our knowledge, the present work provides the first study investigating the frequency of *MEFV* mutations in Egyptian SoJIA children.

Our results are consistent with most of the studies that reported a high frequency of *MEFV* mutations among children with JIA. Ayaz et al. [13] reported 14.28 % as an overall *MEFV* mutation frequency in Turkish SoJIA patients, with M694V being the leading mutation, while Comak et al. [28] reported an overall *MEFV* mutation allele frequency of 19.2 % in Turkish children with JIA.

**Table 2** Characteristics of systemic-onset juvenile idiopathic arthritis patients stratified by the presence of Mediterranean fever (*MEFV*) mutations

| Variable | SoJIA patients ($n = 54$) | | *P* value |
|---|---|---|---|
| | Wild-type ($n = 18$) | Presence of *MEFV* mutations ($n = 36$) | |
| Age (years) | 9 ± 3.4 | 7.97 ± 2.5 | 0.210 |
| Gender | | | |
| Male | 10 (55.6 %) | 14 (38.9 %) | 0.250 |
| Female | 8 (44.4 %) | 22 (61.1 %) | |
| Age at diagnosis (years) | 5.33 ± 1.4 | 5.81 ± 1.8 | 0.340 |
| History of consanguinity | | | |
| Yes | 2 (11.1 %) | 9 (25 %) | 0.300 |
| No | 16 (88.9 %) | 27 (75 %) | |
| Family history of JIA or FMF | | | |
| Yes | 0 (0 %) | 10 (27.8 %) | 0.020 |
| No | 18 (100 %) | 26 (72.2 %) | |
| Presence of fever | | | |
| Yes | 18 (100 %) | 36 (100 %) | 1.000 |
| No | 0 (0 %) | 0 (0 %) | |
| Presence of rash | | | |
| Yes | 16 (88.9 %) | 31 (86.1 %) | 1.000 |
| No | 2 (11.1 %) | 5 (13.9 %) | |
| Presence of abdominal pain | | | |
| Yes | 13 (72.7 %) | 17 (47.2 %) | 0.080 |
| No | 5 (27.8 %) | 19 (52.8 %) | |
| Presence of abdominal enlargement | | | |
| Yes | 2 (11.1 %) | 9 (25 %) | 0.300 |
| No | 16 (88.9 %) | 27 (75 %) | |
| Presence of lymphadenopathy | | | |
| Yes | 0 (0 %) | 4 (11.1 %) | 0.290 |
| No | 18 (100 %) | 32 (88.9 %) | |
| Presence of chest pain | | | |
| Yes | 0 (0 %) | 3 (8.3 %) | 1.000 |
| No | 18 (100 %) | 33 (91.7 %) | |
| Intake of medications | | | |
| NSAIDs | | | |
| Yes | 15 (83.3 %) | 34 (94.4 %) | 0.380 |
| No | 3 (16.7 %) | 2 (5.6 %) | |
| Oral corticosteroids | | | |
| Yes | 18 (100 %) | 28 (77.8 %) | 0.040 |
| No | 0 (0 %) | 8 (22.2 %) | |
| Intravenous corticosteroids | | | |
| Yes | 8 (44.4 %) | 18 (50 %) | 0.700 |
| No | 10 (55.6 %) | 18 (50 %) | |
| DMARDs | | | |
| Yes | 14 (77.8 %) | 33 (91.7 %) | 0.210 |
| No | 4 (22.8 %) | 3 (8.3 %) | |
| Biological treatment | | | |
| Yes | 2 (11.1 %) | 6 (16.7 %) | 0.700 |
| No | 16 (88.9 %) | 30 (83.3 %) | |

Quantitative data are represented as mean ± standard deviation, while qualitative data are represented as frequency (%)

*DMARDs* disease-modifying anti-rheumatic drugs, *FMF* familial Mediterranean fever, *JIA* juvenile idiopathic arthritis, *NSAIDs* non-steroidal anti-inflammatory drugs, *SoJIA* systemic-onset juvenile idiopathic arthritis

An *MEFV* G196W mutation has been recently reported in a 9-year-old Palestinian girl diagnosed with SoJIA and early-onset renal amyloidosis [14]. Moreover, compound *MEFV* E148Q heterozygous/V726A homozygous mutations were reported in a 14-year-old Turkish girl affected with juvenile psoriatic arthritis [29]. An association between the

**Table 3** Investigations of systemic-onset juvenile idiopathic arthritis patients stratified by the presence of Mediterranean fever (*MEFV*) mutations

| Variable | SoJIA patients ($n = 54$) | | *P* value |
|---|---|---|---|
| | Wild-type ($n = 18$) | Presence of *MEFV* mutations ($n = 36$) | |
| Hemoglobin level (g/dL) | 11.28 ± 1.32 | 10.16 ± 1.99 | 0.03 |
| Total leukocytic count (×1,000/μL) | 13.22 ± 4.44 | 11.08 ± 5.36 | 0.15 |
| Platelet count (×1,000/μL) | 580.17 ± 297.34 | 378.22 ± 183.21 | 0.01 |
| C-reactive protein | | | |
| Positive | 9 (50 %) | 29 (80.6 %) | 0.02 |
| Negative | 9 (50 %) | 7 (19.4 %) | |
| Rheumatoid factor | | | |
| Positive | 0 (0 %) | 3 (8.3 %) | 0.54 |
| Negative | 18 (100 %) | 33 (91.7 %) | |
| Anti-nuclear antibody | | | |
| Positive | 0 (0 %) | 3 (8.3 %) | 0.80 |
| Negative | 18 (100 %) | 33 (91.7 %) | |
| ESR | | | |
| Elevated | 11 (61.1 %) | 26 (72.2 %) | 0.41 |
| Normal | 7 (38.9 %) | 10 (27.8 %) | |
| Abdominal ultrasound | | | |
| Normal | 13 (72.7 %) | 24 (66.7 %) | 0.68 |
| Abnormal | 5 (27.8 %) | 12 (33.3 %) | |
| Slit lamp | | | |
| Normal | 16 (88.9 %) | 36 (100 %) | 0.11 |
| Abnormal | 2 (11.1 %) | 0 (0 %) | |

Quantitative data are represented as mean ± standard deviation, while qualitative data are represented as frequency (%)

*ESR* erythrocyte sedimentation rate, *SoJIA* systemic-onset juvenile idiopathic arthritis

presence of certain *MEFV* mutations in FMF patients with chronic arthritis was reported in some studies targeting adults [31, 32]. On the other hand, our results are inconsistent with those of Rabinovich et al. [33], which concluded that *MEFV* mutations were comparable both in healthy subjects and RA adult patients in Israel.

There are conflicting results as to whether *MEFV* mutations were associated with JIA disease severity. Our findings showed that the SoJIA patients did not show statistically significant differences in their demographic and clinical data, treatment modalities, and investigations when stratified according to the type of mutation. Similarly, Migita et al. [34] have reported that *MEFV* mutations do not alter the susceptibility and/or severity of RA in Japan. This is in discordance with results of previous studies showing that patients carrying *MEFV* mutations had a severe disease course [13, 30, 33].

Our results regarding the type of *MEFV* mutation encountered in SoJIA patients are inconsistent with those of previous studies [13, 30], where M694V was the commonest mutation detected in both SoJIA and JIA patients. There are several reasons for the discrepancy in findings between the various studies: the ethnic background, differences in the number of recruited subjects, as well as differences in study design and clinical stratification of the type of arthritis.

The V726A genetic mutation, which is the commonest *MEFV* mutation encountered in our work, is different from the commonest *MEFV* mutations detected in some studies investigating *MEFV* mutations in Egyptian FMF children, where M694V was reported in one study [35], while M694I [36], E148Q [37], and V726A [38] mutations were mentioned in three other studies. These differences in the allelic frequencies of *MEFV* mutations found among Egyptian patients could indicate the mutational heterogeneity of *MEFV* in the Egyptian population. This heterogeneity has been the consequence of the strategic position of Egypt as a crossroads between Asia, Africa, and Europe, which has resulted in considerable human movements (i.e., migration and trade) throughout history in this area [39]. This mutational heterogeneity appears to be less obvious among other ethnic populations.

In our study, none of the *MEFV* mutations P369S, F479L, I692del, M694I, K695R, and R761H were detected in the enrolled subjects. These findings may suggest that the analysis of the main five founder mutations might be enough and that it is more cost effective to screen for *MEFV* mutations in clinical practice rather than conducting an analysis on a wider number of gene mutations.

Mutations and polymorphisms in the *MEFV* gene, even in one allele, are associated with subclinical inflammation [40]. Thus, it might be speculated that once SoJIA

**Table 4** Mediterranean fever (*MEFV*) mutations in systemic-onset juvenile idiopathic arthritis patients and controls

| | SoJIA patients ($n = 54$) | Controls ($n = 30$) |
|---|---|---|
| Wild-type, [mutation (–)] | 18 (33.33 %) | 25 (83.3 %) |
| Presence of *MEFV* mutations | 36 (66.67 %) | 5 (16.7 %) |
| Heterozygous for one mutation | | |
| p.V726A/– | 17 (31.48 %) | |
| p.E148Q/– | 8 (14.81 %) | 4 (13.3 %) |
| p.M680I (G/A)/– | 5 (9.26 %) | 1 (3.4 %) |
| p.M694V/– | 2 (3.70 %) | |
| p.A744S/– | 1 (1.86 %) | |
| Homozygous for one mutation | | |
| Unspecified codon 148 mutation/unspecified codon 148 mutation | 1 (1.86 %) | |
| Compound two mutations | | |
| Unspecified codon 148 mutation homozygous/M694V heterozygous | 2 (3.70 %) | |
| Allelic frequency of *MEFV* mutations | | |
| | SoJIA patients alleles ($n = 108$) | Controls alleles ($n = 60$) |
| V726A | 17 (15.74 %) | |
| E148Q | 8 (7.4 %) | 4 (6.66 %) |
| Unspecified codon 148 mutation | 6 (5.6 %) | |
| M680I (G/A) | 5 (4.6 %) | 1 (1.66 %) |
| M694V | 4 (3.7 %) | |
| A744S | 1 (0.9 %) | |
| Total | 41 (37.96 %) | 5 (8.3 %) |

Unspecified codon 148 mutation: cases showing negative staining for wild-type codon 148 and E148Q bands. Qualitative data are represented as frequency (%)

*SoJIA* systemic-onset juvenile idiopathic arthritis

develops, the presence of a *MEFV* mutation may increase the baseline level of inflammation, altering the disease course. The results of the present study showed a statistically significant higher percentage of *MEFV* mutation carriers showing positivity for CRP and a decreased hemoglobin level than did wild-type carriers. These findings could be explained by an association with chronic inflammation, though there were no significant differences in total leukocytic counts (TLC) and percentages of elevated ESR between mutation carriers and non-carriers at first presentation. Comak et al. [28] reported a non-significant difference in CRP, TLC, and ESR between carriers of *MEFV* mutation and wild-type carriers. The difference in study design, ethnic background, and groups with different disease subtypes may explain the discrepancy in findings.

A positive family history of JIA or FMF was present in 27.8 % of SoJIA patients who were *MEFV* mutation carriers, with a statistically significant difference when compared with patients who were wild-type carriers, suggesting that patients with SoJIA with positive family history of FMF or JIA should be screened for *MEFV* mutations. Female preponderance was noted in the SoJIA patients, with an overall female:male ratio of 1.25:1, which is concordant with the JIA male:female ratio of some previous studies [27, 28]. However, they are discordant with those of other studies [13, 41] where males were more affected than females. Mean age at diagnosis was $5.57 \pm 2.9$ years, which is consistent with a previous study investigating JIA in Egypt [42].

Our results show that the *MEFV* mutant allele frequency was 8.3 % in our recruited healthy controls, similar to the previously reported carrier rate of *MEFV* mutations in Egyptians (9.3 %) [43]. It is evident that examining 60 chromosomes would not give a precise estimate of the carrier rate of *MEFV* mutations in the Egyptian general population; however, it gives an idea about the presence of *MEFV* mutations in healthy individuals. In concordance with our findings, the E148Q mutation was the leading mutation present in our group of healthy controls and in other neighboring Mediterranean countries [44–46]. This is probably due to the reduced penetrance of this mutation and explains the fact that a considerable proportion of the genetically affected individuals remain asymptomatic [46].

Oberkanins et al. [47] stated that one of the known limitations of reverse hybridization is the difficulty to

correctly identify and distinguish between two closely spaced mutations, as in the case of the common E148Q and the rare E148V; the FMF Strip Assay® will correctly identify heterozygous and homozygous E148Q but will fail to identify heterozygous E148V, showing a wild-type staining pattern. In three of our patients, we experienced negative signals for both wild-type codon 148 and E148Q, so these three patients were described as having unspecified codon 148 mutation where a homozygous mutation at or close to codon 148 and interfering with hybridization is likely to be present. Whether this is E148V or another mutation can be determined by sequencing, which will be performed in a future study.

Our study is not without limitations; primarily, the relatively small sample size of both SoJIA patients and healthy controls. A larger number of patients is needed in future studies to study the effect of *MEFV* mutations on the clinical presentation and prognosis of SoJIA patients. Large cohort studies involving healthy controls are warranted to give a more precise estimate of the *MEFV* carrier rate in Egypt. Detection of the unspecified codon 148 mutations in the three SoJIA patients by sequencing is highly indicated and will be performed in a future study. These unspecified codon 148 mutations constituted an *MEFV* allelic frequency of 5.6 %, a significant percentage not to be ignored. The strength in the present work lies in being the first study examining the frequency of *MEFV* mutations in SoJIA patients in Egypt, incorporating highly detailed patient data involving the demographic and clinical data, medications, and investigations.

## 5 Conclusion

Our group of Egyptian children with SoJIA had a significantly higher frequency of *MEFV* mutations than in the healthy control population. V726A was the leading mutation in our studied cohort of SoJIA patients, with an allelic frequency of 15.74 %, followed by E148Q with an allelic frequency of 7.4 %. Children who were carriers of *MEFV* mutations had an 18 times higher risk of developing SoJIA than did wild-type carriers. Positivity for CRP was present in a higher percentage of SoJIA patients who were *MEFV* mutation carriers. Of our studied healthy controls, 16.7 % were carriers for *MEFV* mutations, mostly E148Q. These findings suggest that mutations of the *MEFV* gene may be responsible for auto-inflammatory diseases other than FMF, and patients with SoJIA should be screened for *MEFV* gene mutations in countries where FMF is frequent.

**Acknowledgments and Disclosures** Special thanks to all Rheumatology Clinic members, who helped us in our work, and to our dear patients, who participated in conducting the study. The authors have no conflicts of interest that are directly relevant to the content of this article.

## References

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet. 2007;369(9563):767–78.
2. Woo P. Systemic juvenile idiopathic arthritis: diagnosis, management, and outcome. Nat Clin Pract Rheumatol. 2006;2:28–34.
3. Behrens EM, Beukelman T, Gallo L, et al. Evaluation of the presentation of systemic onset juvenile rheumatoid arthritis: data from the Pennsylvania systemic onset juvenile arthritis registry (Pasojar). J Rheumatol. 2008;35:343–8.
4. Lin YT, Wang CT, Gershwin ME, et al. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. Autoimmun Rev. 2011;10:482–9.
5. Lovell DJ. Update on treatment of arthritis in children—new treatments, new goals. Bull Hosp Jt Dis. 2006;64:72–6.
6. David J. Amyloidosis in juvenile chronic arthritis. Clin Exp Rheumatol. 1991;9:73–8.
7. Immonen K, Savolainen HA, Hakala M. Why can we no longer find juvenile idiopathic arthritis-associated amyloidosis in childhood or in adolescence in Finland? Scand J Rheumatol. 2007;36:402–3.
8. Pascual V, Allantaz F, Arce E, et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med. 2005;201:1479–86.
9. Yokota S, Imagawa T, Mori M, et al. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. Lancet. 2008;371:998–1006.
10. Ozen S, Hoffman HM, Frenkel J, Kastner D. Familial Mediterranean fever (FMF) and beyond: a new horizon. Fourth International Congress on the Systemic Autoinflammatory Diseases held in Bethesda, USA, 6–10 November 2005. Ann Rheum Dis. 2006;65:961–4.
11. de Benedetti F, Meazza C, Vivarelli M, et al. Functional and prognostic relevance of the −173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2003;48:1398–407.
12. Ogilvie EM, Fife MS, Thompson SD, et al. The −174 G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. Arthritis Rheum. 2003;48:3202–6.
13. Ayaz NA, Özen S, Bilginer Y, et al. MEFV mutations in systemic onset juvenile idiopathic arthritis. Rheumatology. 2009;48:23–5.
14. Cantarini L, Lucherini OM, Simonini G, et al. Systemic-onset juvenile idiopathic arthritis complicated by early onset amyloidosis in a patient carrying a mutation in the MEFV gene. Rheumatol Int. 2012;32:465–7. doi:10.1007/s00296-009-1331-7.
15. Touitou I, Notarnicola C, Grandemange S. Identifying mutations in autoinflammatory diseases: towards novel genetic tests and therapies? Am J Pharmacogenomics. 2004;4(2):109–18.
16. de Jesus AA, Goldbach-Mansky R. Monogenic autoinflammatory diseases: concept and clinical manifestations. Clin Immunol. 2013;147:155–74.
17. Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. Nat Rev Rheumatol. 2014;10:135–47. doi:10.1038/nrrheum.2013.174.
18. Centola M, Wood G, Frucht DM, et al. The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte

development and is regulated in response to inflammatory mediators. Blood. 2000;95:3223–31.
19. Onen F. Familial Mediterranean fever. Rheumatol Int. 2006;26:489–96.
20. Samuels J, Aksentijevich I, Torosyan Y, et al. Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. Medicine (Baltimore). 1998;77:268–97.
21. Ben-Chetrit E, Levy M. Familial Mediterranean fever. Lancet. 1998;351:659–64.
22. Infevers: an online database for autoinflammatory mutations. http://fmf.igh.cnrs.fr/ISSAID/infevers/. Accessed 11 Jan 2014. Milhavet F, Cuisset L, Hoffman HM, et al. The infevers autoinflammatory mutation online registry: update with new genes and functions. Hum Mutat. 2008;29(6):803–8. doi:10.1002/humu.20720.PMID:18409191.
23. Hershko AY, Ben-Chetrit E. The *MEFV* E148Q allele: a deleterious mutation or harmless variation? Clin Exp Rheumatol. 2006;24(Suppl. 42):S51–2.
24. Ben-Chetrit E, Lerer I, Malamud E, et al. The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? Hum Mutat. 2000;15:385–6.
25. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. International League of Associations for Rheumatology. J Rheumatol. 2004;31(2):390–2.
26. Tchernitchko D, Legendre M, Delahaye A, et al. Clinical evaluation of a reverse hybridization assay for the molecular detection of twelve MEFV gene mutations. Clin Chem. 2003;49:1942–5.
27. Petty R, Cassidy JY, Kastner D. Chronic arthritis in childhood. In: Cassidy JT, Petty RE, Laxer RM, Lindsley CB, editors. Textbook of pediatric rheumatology. 6th ed. Philadelphia: Saunders; 2011. p. 272–86.
28. Comak E, Dogan CS, Akman S, et al. MEFV gene mutations in Turkish children with juvenile idiopathic arthritis. Eur J Pediatr. 2013;172:1061–7. doi:10.1007/s00431-013-2003-x.
29. Yeniay BS, Karaca NE, Yuksel SE, et al. Juvenile psoriatic arthritis carrying familial Mediterranean fever gene mutations in a 14-year-old Turkish girl. J Dermatol. 2007;34:344–8.
30. Ozen S, Bakkaloglu A, Yilmaz E, et al. Mutations in the gene for familial Mediterranean fever: do they predispose to inflammation? J Rheumatol. 2003;30:2014–8.
31. Booth DR, Lachmann HJ, Gillmore JD, et al. Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148. QJM. 2001;94:527–31.
32. Olgun A, Akman S, Kurt I, et al. MEFV mutations in familial Mediterranean fever: association of M694V homozygosity with arthritis. Rheumatol Int. 2005;25:255–9. doi:10.1007/s00296-003-0433-x.
33. Rabinovich E, Livneh A, Langevitz P, et al. Severe disease in patients with rheumatoid arthritis carrying a mutation in the Mediterranean fever gene. Ann Rheum Dis. 2005;64:1009–14.
34. Migita K, Nakamura T, Maeda Y, et al. MEFV mutations in Japanese rheumatoid arthritis patients. Clin Exp Rheumatol. 2008;26:1091–4.
35. Settin A, El-Baz R, Abd Rasool M, et al. Clinical and molecular diagnosis of familial Mediterranean fever in Egyptian children. J Gastrointest Liver Dis. 2007;16(2):141–5.
36. El Gezery DA, Abou-Zeid AA, Hashad DI, et al. MEFV gene mutations in Egyptian patients with familial Mediterranean fever. Genet Test Mol Biomarkers. 2010;14(2):263–8. doi:10.1089/gtmb.2009.0180.
37. Talaat HS, Mohamed MF, El Rifai NM, et al. The expanded clinical profile and the efficacy of colchicine therapy in Egyptian children suffering from familial Mediterranean fever: a descriptive study. Ital J Pediatr. 2012;38:66.
38. El-Garf A, Salah S, Iskander I, et al. MEFV mutations in Egyptian patients suffering from familial Mediterranean fever: analysis of 12 gene mutations. Rheumatol Int. 2010;30:1293–8. doi:10.1007/s00296-009-1140-z.
39. Temtamy SA, Aglan MS, Meguid NA, Teebi AS. Chapter 8: genetic disorders in Egypt. In: Teebi AS, editor. Genetic disorders among Arab populations. 2nd ed. Heidelberg: Springer; 2010. doi:10.1007/978-3-642-05080-0_8.
40. Lachmann HJ, Sengul B, Yavuzs TU, et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford). 2006;45:746–50.
41. Aggarwal A, Agarwal V, Danda D, et al. Outcome in juvenile rheumatoid arthritis in India. Indian Pediatr. 2004;41:180–4.
42. Salah S, Hamshary A, Lotfy H, et al. Juvenile idiopathic arthritis, the Egyptian experience. J Med Sci. 2009;9(2):98–102.
43. Al-Alami JR, Tayeh MK, Najib DA, et al. Familial Mediterranean fever mutation frequencies and carrier rates among a mixed Arabic population. Saudi Med J. 2003;24(10):1055–9.
44. Ait-Idir D, Khilan A, Djerdjouri B, et al. Spectrum of mutations and carrier frequency of familial Mediterranean fever gene in the Algerian population. Rheumatology. 2011;50:2306–10. doi:10.1093/rheumatology/ker328.
45. Medlej-Hashim M, Serre JL, Corbani S, et al. Familial Mediterranean fever (FMF) in Lebanon and Jordan: a population genetics study and report of three novel mutations. Eur J Med Genet. 2005;48:412–20.
46. Mattit H, Joma M, Al-Cheikh S, et al. Familial Mediterranean fever in the Syrian population: gene mutation frequencies, carrier rates and phenotype–genotype correlation. Eur J Med Genet. 2006;49:481–6.
47. Oberkanins C, Weinhäusel A, Kriegshäuser G, et al. Genetic testing for familial Mediterranean fever in Austria by means of reverse-hybridization teststrips. Clin Chem. 2003;49(11):1948–50.