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ARTICLE

The detection of a novel insertion mutation in exon 2 of the *MEFV* gene associated with familial mediterranean fever in a moroccan family

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Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease that is inherited in an autosomal recessive manner and is caused by mutations in the *MEFV* gene. As the name indicates, FMF occurs within families and is more common in individuals of Mediterranean descent than in persons of any other ethnicity. To date, 314 mutations have been reported. We studied a Moroccan family with a total of five members, including a mother who was presenting with symptoms of FMF, while her four children remained asymptomatic. The five patients were screened by DNA sequencing of exon 2 and exon 10 of the *MEFV* gene. Then, complete exome sequencing analysis of the *MEFV* gene was done for the patients in whom a novel mutation was detected. This analysis identified a novel single base Cytosine (C) insertion mutation in the coding region of the *MEFV* gene, named c.441dupC (p. Glu148Argfs*5 or E148RfsX5), which resulted in a mutated Pyrin/Marenostrin protein. This is the first report of a new mutation in exon 2 of the *MEFV* gene in a Moroccan family. This novel insertion mutation may provide important information for further studies of FMF pathogenesis.

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INTRODUCTION

Familial Mediterranean fever (FMF), which belongs to a family of autoinflammatory diseases, remains a major health threat to the Mediterranean population, especially Sephardic Jews, North African Arabs, Armenians, Turks, Greeks, and Italians.

However, in recent years, an increasing number of cases have been reported in countries not near this area, such as the United States and Japan. Clinically, FMF is characterized by recurrent attacks of fever with pleuritis, skin lesions, abdominal pain, peritonitis, and amyloidosis (Amyloid A 'SAA').[1,2]

The symptoms and severity vary among affected individuals, sometimes even among members of the same family. FMF is usually inherited in an autosomal recessive manner[1,3] and is caused by mutations of the *MEFV* gene on the short arm of chromosome 16 at position p13.3. *MEFV* comprises 10 exons and normally encodes a 781-amino acid protein, named pyrin or marenostrin.[2,4] Gene mutations result in defective pyrin molecules; it is hypothesized that altered pyrin cannot suppress minor and unknown triggers to inflammation that are normally inhibited by intact pyrin. To date, more than 314 gene mutations and polymorphisms have been discovered in the *MEFV* gene.[5]

In this study, we examined five related patients. The mother of the family was homozygous for this mutation and was presenting with FMF symptoms. However, her four children were heterozygous for this mutation and did not show any clinical features of FMF.

MATERIALS AND METHODS

Subjects

Approximately 300 clinically pre-diagnosed FMF cases are referred to our laboratory for the detection of *MEFV* mutations each year. Among those patients with clinical evidence of FMF, five individuals from a Moroccan family were studied.

PCR amplification and sequencing

After obtaining informed consent, peripheral blood was collected from each patient and genomic DNA samples were extracted from blood lymphocytes using the *Gene Cather Magnetic Beats kit* (Invitrogen, Carlsbad, CA, USA). For each patient, both *MEFV* exon 2 and 10, which are considered as mutation hot spots,[6] were individually amplified byPCR using 2 pairs of corresponding primers:

Exon 2: F: 5′-GCCTGAAGACTCCAGACCACCCCG-3′, R: 5′-AGGCCCTCCGAG-GCCTTCTCTCTG-3′

Exon 10: F: 5′-GAGGTGGAGGTTGGAGACAA-3′, R: 5′-TGACCACCCACT-GGACAGAT-3′. PCR was performed in a 25 ml reaction volume containing 60 ng of genomic DNA, 5 U of Taq (Invitrogen), 20 pmol of each primer, 50 mM $MgCl_2$, 10 mM d NTP, and 10× PCR buffer (Invitrogen) in the Veriti 96-well Thermal Cycler 9902 (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s and 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. Bidirectional direct sequencing of purified PCR products was performed using the BigDye Terminator V1.1 Cycle Sequencing kit (ABI prism, Foster City, CA, USA) and an Applied Biosystems 3500DX Genetic analyzer.

The resulting chromatogram was analyzed using the Sequencing Analysis SeqA V5.4 (Applied Biosystems) program. The obtained sequences underwent bioinformatics analysis using the 'Nucleotide Blast' Alignment Program at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

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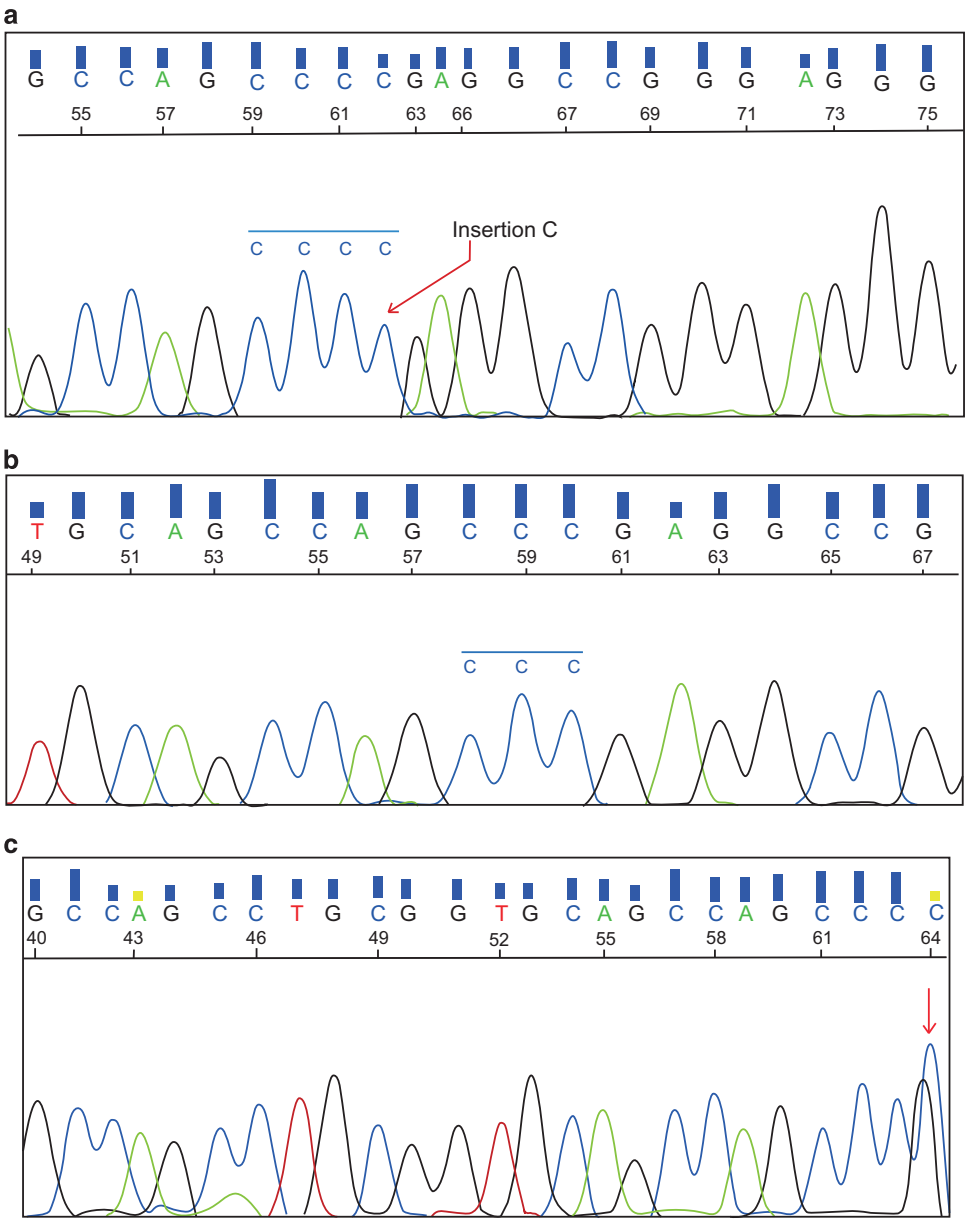

Figure 1. Electrophoregrams of the new homozygous and heterozygous insertion at the 441st nucleotide of MEFV as well as the wild type. The insertion resulted in a frameshift in codon 148. (**a**) Forward electrophoregram for the homozygote (Mother); (**b**) Forward electrophoregram for the wild type (Father); (**c**) Forward electrophoregram for the heterozygotes (Children).

Table 1. Clinical symptoms and results of mutation analysis of a Moroccan family

Relation	*Gender, age (years)*	*Age of symptoms onset*	*Exon 2 mutation type*	*Common FMF symptoms*					
				Abd.pain	*Fever*	*Chest pain*	*Arthritis*	*Skin lesions*	*Amyloidosis*
Father	M, 38	No, Sx	Wt	–	–	–	–	–	–
Mother	F, 35	30 y	E148R/E148R	+	+	+	–	–	–
Child 1	M, 15	No, Sx	E148R/0	–	–	–	–	–	–
Child 2	F, 10	No, Sx	E148R/0	–	–	–	–	–	–
Child 3	F, 8	No, Sx	E148R/0	–	–	–	–	–	–
Child 4	F, 3	No, Sx	E148R/0	–	–	–	–	–	–

Abbreviations: +, present; –, absent; No,Sx, No Symptoms; Wt, Wild type; y, years.

RESULTS

Our analysis of five related Moroccan patients revealed that the mother of this family (age 35 years) presented with the clinical features of FMF, including recurrent attacks of fever, abdominal pain, and skin lesions.

Indeed, the routine analysis of *MEFV* mutations in this family conducted in our laboratory clearly showed a single C nucleotide duplication, resulting in a frameshift mutation in exon 2 at the 148th codon (between the 441st and 442th nucleotides). The frameshift mutation was confirmed by DNA sequencing of the children as well as their parents, and the results indicated the existence of this mutation in the mother and her children (Figures 1a and c), but the absence of this mutation in the father (Figure 1b).

Our findings also showed that the four children of this Moroccan family were mutation c.441dupC (p. Glu148Argfs*5 or E148RfsX5) carriers. It is well documented that mutations of the *MEFV* gene cause FMF in an autosomal recessive manner; therefore, the heterozygous mutation carriers should not become symptomatic at any point in their lives (Table 1). The family tree of these five individuals and the detected mutations are illustrated in Figure 2. The C nucleotide insertion and frameshift mutation between the 441st and 442th nucleotides caused the conversion of the nucleotide sequence from 5′-CCCGAGGCC … CGACCTA-GA-3′ to 5′-CCCCGAGGC … GCGACCTAG-3′ and this conversion resulted in a stop at codon 241.

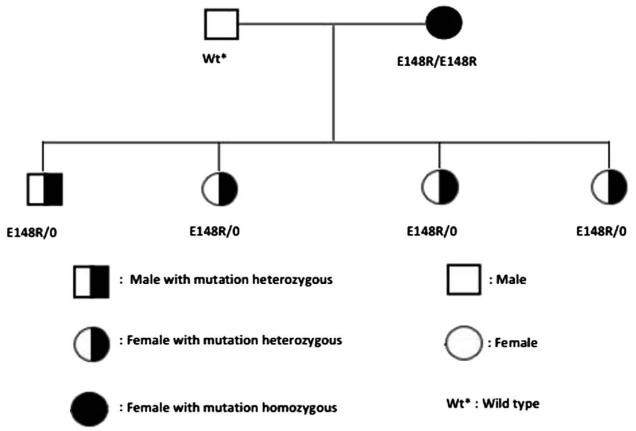

Figure 2. Genealogical tree of the studied patients.

The protein sequence converted from Glu-Ala-Gly … Met-Arg-Pro-Arg to Arg-Gly-Arg … Asp-Ala-Thr-STOP (E148RfSX5 or Glu148Argfs*5). The c.441dupC mutation was searched for using six different web-based mutation analysis tools. MutationTaster, PolyPhen2 (HumDiv prediction version), and Ensembl.org indicated that the c.441dupC mutation might have pathogenic effects (Table 2).[7–10]

Children

A 15-year-old male (child (1) a 10-year-old female (child (2) an 8-year-old female (child (3) and a 3-year-old female (child (4) all without FMF symptoms; c.441dupC was detected by sequencing of the 4 children (Figure 1c).

DISCUSSION

The main findings of this investigation have shown for the first time a novel frameshift mutation in the *MEFV* gene (c.441dupC) that was detected in 5 FMF patients from one Moroccan family. Analysis of the clinical and genetic features of the mother showed that she was homozygous for the c.441dupC mutation and presented with the clinical symptoms that are associated with FMF. In addition, the children were heterozygous for the c.441dupC mutation and were asymptomatic because, in autosomal recessive inheritance, two copies of a disease allele are required for an individual to be susceptible to expressing the phenotype. The four asymptomatic children had one copy of a disease allele and one copy of a normal allele and, as a result, they are carriers but do not show symptoms of the disease.

This novel mutation was detected in exon 2 of the *MEFV* gene, where major mutations that have been identified and described as being responsible for FMF are located. Moreover, each functional region of *MEFV* encodes part of the pyrin protein. Exon 1 encodes a domain named the pyrin domain (PYD). The bZIP is encoded by exon 2, an alpha helical region is encoded by exons 3–8, and a domain called B303/SPRY is encoded by exon 10.[11]

In addition, each domain of the pyrin protein is responsible for many protein-protein interactions: the bZIP domain, which is encoded by exon 2, interacts with p65 and Ikβ-α.[12]

Table 2. Outcome of mutation analyzer web-based tools

	Computational pathogenicity predictions			
	Prediction	*Score*	*Score description*	*Coding consequences*
(A) http://genetics.bwh.harvard.edu/pph2/ (HumVar Prediction)[7]	P: possibly damaging	0.714		Frameshift
(B) http://genetics.bwh.harvard.edu/pph2/ (HumDiv Prediction)[8]	D: probably damaging	0.981		
(C) http://www.mutationtaster.org/[8]	D	1.83	A: disease Causing automatic, D: disease causing, N: polymorphism, P: polymorphism automatic	Frameshift
(D) http://Mutationtassessor	Low		High or medium: predicted functional, low or neutral: predicted non-functional	
(E) http://sift.jcvi.org/[9]	Damaging	0.03	SIFT score. Ranges from 0 to 1. The amino acid substitution is predicted damaging is the score is $< = 0.05$, and tolerated if the score is > 0.05.	
(F) http://www.ensembl.org[10]	Coding sequence variant: 100%			Frameshift

(A) Predicted as possibly damaging to the pyrin protein with a score = 0.714, (B) predicted as probably damaging to the pyrin protein with a score = 0.981, (C) predicted as disease causing with a score = 1.83, (D) predicted non-functional protein, (E) SIFT score = 0.03, the amino acid substitution is predicted as damaging, (F) predicted as a coding sequence variant at 100%.

The existence of a bZIP transcription factor basic domain and two nuclear localization signals, which are encoded by a basic residue cluster PLESKREF beginning at 150-amino acid residue and a bipartite NLS motif at residues 420–437, suggests that pyrin acts as a nuclear factor.[13]

However, in transfected cells, full-length pyrin exclusively localizes to the cytoplasm, while isoforms lacking exon 2 can enter the nucleus.[14] Nevertheless, immunostaining of various pyrin-expressing cells showed that endogenous pyrin is predominately localized to the nucleus in synovial fibroblasts, dendritic cells, and polymorphonuclear cells, but to the cytoplasm in monocytes.[15] To date, 101 mutations have been detected in exon 2, the most recent of which was identified in 2016 and was a deletion called C.382-390del (P. Glu-128-As 130del).[5]

The mother was homozygous for the mutation since she carried two copies of the mutated allele, while her children were heterozygous. This novel frameshift mutation is located in exon 2 of the *MEFV* gene and causes the replacement of amino acids after the 148th amino acid, resulting in a new amino acid sequence of Glu148Arg—Ala149Gly— …… -Pro240Thr—Arg241STOP. Previously identified FMF causing mutations are also located in exon 2 of the *MEFV* gene. The insertion described in this article is significant because it is the only insertion and frameshift mutation reported so far in exon 2 of the *MEFV* gene.

CONCLUSIONS

Our study is the first to show a novel insertion in exon 2 of the *MEFV* gene through the examination of the clinical and genetic features of five related Moroccan patients who were clinically suspected of having FMF. Thus, we suggest that this novel insertion mutation could provide important information for further studies on FMF pathogenesis.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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