Unique spectrum of MEFV mutations in Iranian Jewish FMF patients—clinical and demographic significance

Y. Shinar, I. Kuchuk, S. Menasherow, M. Kolet, M. Lidar, P. Langevitz and A. Livneh

Objectives. To determine the spectrum of mutations in the Mediterranean fever gene (MEFV) of Iranian Jews with familial Mediterranean fever (FMF) and to analyse their clinical manifestations.

Methods. FMF patients with both parents of Iranian-Jewish (IJ) extraction or with one IJ parent (IJ–other, 10 of each) were characterized for clinical manifestations, and the B30.2 (PRYSPRY) domain of their MEFV was sequenced for mutations.

Results. Only one rare mutation, R653H, and one new mutation, G632S were present in the IJ group (in 2/10 patients), whereas the new, and common mutations were present in the IJ–other patients (8/10 patients). The new mutation was traced thrice to an IJ ancestor, and although carried asymptotically by family members, it was over-represented in the patients (3/28 unrelated IJ alleles) compared with non-affected IJ subjects (1/126 alleles, \(P = 0.03\)) or with non-Jewish Iranians (0/108 alleles, \(P = 0.001\)). The mutation was associated with a distinct phenotype regarding sites involved in the attack (\(P = 0.001\)), mild severity, sole expression of febrile episodes (\(P = 0.01\)) and a male bias (\(P = 0.01\)). In two 3D PRYSPRY models the G632S mutation was localized to a surface loop and close to a putative binding site.

Conclusions. Iranian Jews with FMF have a unique spectrum of mutations including a newly described mutation with a non-typical phenotype.

KEY WORDS: Familial Mediterranean fever, Mediterranean fever gene, Pyrin, Mutation, G632S, B30.2 domain, PRYSPRY, Tertiary (3D) structure, Haplotype, Iranian Jew.
Common FMF mutations, M694V (rs28940577), V726A (rs28940579) and E148Q (rs3743930) were screened for by enzyme restriction-site analysis of PCR amplified fragments of DNA, as previously described [11]. For sequencing, we amplified ME芙 exons 1–10 with primers and reaction conditions that were previously specified [5]. Amplified products were purified using a commercial kit (PCR product purification kit, Roche, ID, USA), and the Big Dye fluorescent terminator method was used for the sequencing reaction. The fluorescent sequences were analysed by an ABI 3130xl automated sequencer (Abbot, USA). The sequence of the PCR product was compared with that of ME芙 by blast software.

Screening for the G632S mutation

The G632S mutation abolishes a Sau96 I enzyme restriction site (G’GNCC, New England Biolabs, MA, USA) in exon 10 of ME芙. For screening, DNA was PCR amplified with the exon 10 primers and the product was subjected to the Sau96 I digestion. All the alleles that lacked the Sau96 I site were sequenced to confirm the presence of the G632S mutation.

Haplotype analysis

Polymorphic nucleotide substitutions on ME芙 exon 2, exon 3, exon 5 and exon 9 were identified by direct sequencing, and heterozygous genotypes were separated to haplotypes by their intra-familial segregation. The haplotype nomenclature was assigned according to Aldea et al. [12].

The inferred position of the G632S mutation in structure models of the B30.2 domain

We localized the new mutation on the newly deciphered models of the B30.2 domain tertiary structure, to evaluate a possible mutation effect [13–15]. This domain is present on several diverse protein families and contains two independent and sequential subdomains, PRY (IPR003879, 49 amino acids in average and a 94% overlap score with the B30.2 domain) and SPRY (IPR03877, average of 106 amino acids, score overlap = 78%) [16]. The position of the new mutation in the PRY domain of Drosoophilua GUSTAVUS protein and human PRYSPRY was extracted from sequence alignments [13, 14], and located on the 3D structures of the domain (PDB 2HIS, [13]; PDB 2FBE, [14]) with Cn3D software.

Statistical analysis

The χ² and Fisher’s exact tests were used to determine the probability that the differences in frequency of G632S mutation rate, between the patients and two healthy cohorts, could have occurred by chance. After Bonferroni adjustment for two comparisons, differences were considered significant when \( P \leq 0.05 \). The Student’s \( t \)-test evaluated the significance of differences in continuous disease variables between G632S mutation carriers and non-carriers. Differences were significant when \( P \leq 0.05 \).

Results

FMF expression and ME芙 mutations in the IJ patients

The group recruited for this study consisted of 10 IJ patients with both parents of IJ extraction and 10 patients with one IJ parent (IJ–other), three of which were of the same family. Both subgroups had similar clinical manifestations and demographic characters, except that the IJ–other group showed a trend toward a younger age at disease onset (15.6 ± 8.7 vs 29.6 ± 17.8, \( P = 0.04 \)). Most patients had a mild-to-moderate disease, when assessed by the Tel Hashomer score, or a mild disease when assessed by the Mor score (Table 1).

The distribution of common ME芙 mutations differed significantly between the IJ and IJ–other subgroups. Whereas the IJ group had no common mutations, six were found in the IJ–other group, two of which could be assigned with certainty to the non-IJ parent (Table 2). Exon 10 was sequenced in 19 patients, and this identified a known, rare mutation, R653H (rs28940581) in one IJ patient and a novel mutation, c.1894G>A/G632S (g.13001G>A, Fig. 1) in five patients, belonging to three unrelated families. Family A, of a mixed IJ–Ashkenazi origin, had three FMF patients with the G632S mutation and three asymptomatic carriers (Fig. 2A). The new mutation was the sole mutation in the entire coding region of the two brothers with FMF of family A, whereas the father, who inherited the G632S mutation from an IJ grandmother, carried an additional mutation, V726A, traced to an Ashkenazi grandparent. Family B had one patient, typed G632S/E148Q, who received the new mutation from his asymptomatic IJ mother and the E148Q mutation from his Turkish-Jewish father (Fig. 2B). The patient of family C, typed G632S/0, had two IJ parents (Fig. 2C). Analyses of single nucleotide polymorphisms (SNPs) within the ME芙 coding region of the IJ–Ashkenazi Jewish family (family A) revealed a known haplotype at linkage with G632S, termed M6, which is scarcely reported in Jewish patients (Fig. 2) [12, 17].

The three unrelated G632S alleles described above, comprised 11% of the unrelated IJ ME芙 alleles in the 20 FMF patients (3 of 28 unrelated alleles, 20 from 10 IJ patients and 8 from 10 mixed-IJ/ME芙 patients where 3 are members of one family and therefore counted only once). In comparison, only one subject was identified as a carrier of the G632S mutation among 63 unaffected IJ subjects (1/126, 0.8%, \( P = 0.03 \)), and none was discovered in a cohort of 54 non-Jewish Iranians from the Bahai’ community in Haifa, Israel (\( P = 0.001 \)). The mutation was also not found in 93

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<th>Table 2. MEFV genotypes of the IJ FMF patients</th>
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<td>Genotype</td>
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<tr>
<td>G632S/V726A</td>
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<td>E148Q/0</td>
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*Exon 10 of ME芙 was sequenced in 19 patients. In addition, the sequence of the entire coding region was completed for five patients, and of exons 2, 3, 5 and 9 for two additional patients. One patient was typed only for the M694V, V726A, E148Q and G632S mutations.
Jewish and 60 Arab subjects, who sought diagnosis by sequence of exon 10 in our laboratory. These analyses establish a statistically significant association between the G632S mutation and FMF patients of IJ origin. However, the total mutation frequency remained considerably low in the IJ patients group (2/20 unrelated alleles, 0.1), compared with that of the IJ–other patients group (8/18 unrelated alleles when considering the G632S allele that runs in family A only once, 0.44, $P = 0.025$, Table 2).

The inferred position of the G632S mutation in SPRY domain models

When the B30.2 domain of pyrin is aligned with the Drosophila B30.2/SPRY domain of the SPRY-SOCS box protein, GUSTAVUS, the G632 residue is positioned within a six amino acid insertion that is inferred on to loop 3–4 in the GUS tertiary structure model (Fig. 3A). This loop is located on surface A of the structure. Six of ten pyrin mutations, including M694V, were reported to localize to this surface [13]. On the structure model based on the human PRYSPRY domain of a human protein (PRY-SPRY-19q13.4.1) [14], the G632 residue is homologous to the Q44 residue of PRY, and is inferred to localize on loop 2, the end of which (namely residues 50–53) is implicated to comprise the putative acceptor binding site of the PRYSPRY dimer (Fig. 3B).

Clinical manifestations in patients with the G632S mutation

The five FMF patients carrying the G632S mutation had a mild-to-moderate disease when assessed by the Tel Hashomer severity score (grades 3–5 in the severity score), or mild severity when assessed by the Mor score (Table 3). Peritonitis was undermanifested in the G632S carrier patients in comparison with the remaining patients ($n = 15$, $P = 0.001$), while febrile episodes alone were over-represented ($P = 0.01$). All five G632S patients were males ($P = 0.01$). Only two of these patients complied with colchicine treatment: a 25-yr-old heterozygous subject with peritoneal attacks in family A and a 10-yr-old child with two mutations in family B, who suffered from recurrent fever attacks.

Discussion

Low rate and unique MEFV mutation spectrum—significance and bio-history considered

The present study had set out to identify the molecular basis for the rare and relatively mild FMF morbidity in IJ patients. Our results show that there were only 4 mutated alleles in 28 unrelated IJ FMF-alleles. These were the rare R653H allele (found once), or the unique G632S allele (found thrice). FMF in IJ patients is therefore associated with low frequency, unusual MEFV.
mutations. The remaining large number of MEFV alleles may have mutations in other parts of the gene that were not sequenced, such as other exons, introns or the promoter region, or even be dominated by a mutation on one allele. Alternatively, an additional gene may be operating in some patients to cause the typical FMF manifestations [18].

The absence of common mutations in IJ FMF patients is another intriguing finding of this study, especially in comparison to the high rate of common mutations in Iraqi FMF patients [19] reaching 50% (unpublished data). Since according to historical records, both the IJ and Iraqi-Jewish populations date back to the expulsion of Jews from the Kingdoms of Israel and Judea by the Assyrians in 730 bc and by the Babylonians in 650 bc, especially to the region of Media, a genetic drift or dilution of these common alleles may have dropped specifically in Iranian Jews. Such admixture could explain how the frequency of common MEFV mutations may have dropped specifically in Iranian Jews.

Another finding of this study is a novel IJ mutation, G632S. Its ethnic confinement can be explained either by being a very recent mutation or as a consequence of the isolation of the Iranian Jews in the last few hundred years. Historical records indicate that during the domination of Persia by Shiite dynasties, the Jewish minority in central and eastern Iran was oppressed, especially since the 16th century, and became relatively isolated from other Jewish communities. This seclusion may explain other distinct recessive IJ diseases [22–25], and unique DNA markers, compared with other Jews in Israel and in comparison with indigenous Iranians [26].

**Mutation vs a polymorphism**

Is there enough evidence to determine that the new mutation is indeed a disease causing mutation and not a polymorphism? The basic population genetics performed on 20 patients showed that this substitution was over represented in unrelated FMF patients, compared with non-affected subjects (0.11 vs 0.01, P = 0.03). Second, analysis of clinical manifestations showed a distinct, mild disease phenotype, with sole expression of febrile episodes, and a male-biased penetrance. It is also recalled that most mild FMF mutations have incomplete penetrance [5], and that mild mutations are over-represented by 1.5 fold in male compared with female patients in Israeli FMF cohorts [6].

Third, the putative spatial structure of the B30.2 domain of pyrin suggests that the G632S mutation is placed in a loop at the surface of the molecule [15]. This position was suggested to be functionally important recently by two models [13, 14]. Therefore a change in an amino acid in this site may impair the function of pyrin.

To conclude, G632S is a new mutation with confined ethnic distribution, mild clinical phenotype and incomplete penetrance. All in all, the study of the molecular basis of FMF in Iranian Jews revealed a divergent mutation spectrum, associated mainly with mild disease and only a weak association with the MEFV. The data also offer an insight into the ethnic makeup of Iranian Jews, and perhaps to the epidemiology of FMF in the outskirts the Mesopotamian region.

### Rheumatology key messages

- FMF is relatively rare among Iranian Jews.
- Most patients have a mild disease, with a weak association to the MEFV that is limited to uncommon or new mutations.

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References