

RESEARCH ARTICLE

Prevalence of common *MEFV* mutations and carrier frequencies in a large cohort of Iranian populations

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Abstract

Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disorder caused by mutations in the *MEFV* gene. The disease is especially common among Armenian, Turkish, Jewish and Middle East Arab populations. To identify the frequency and the spectrum of common *MEFV* mutations in different Iranian populations, we investigated a cohort of 208 unselected asymptomatic individuals and 743 FMF patients. Nine hundred and fifty-one samples were analysed for the presence of 12 *MEFV* mutations by PCR and reverse-hybridization (FMF StripAssay, ViennaLab, Vienna, Austria). Confirmatory dideoxy sequencing of all *MEFV* gene exons was performed for 39 patients. Fifty-seven (27.4%) healthy individual carried mutant *MEFV* alleles. Three hundred and ninety-one (52.6%) FMF patients were found positive for either one (172/743; 23.1%), two or three *MEFV* mutations. Using dideoxy sequencing, three novel variants, A66P, R202W and H300Q, could be identified. Our analysis revealed an allele frequency and carrier rate of 15.6 and 27.4%, respectively, among healthy Iranians. Still moderate compared to neighbouring Armenia, but higher than in Turkey or Iraq, these data suggest that FMF is remarkably common among Iranian populations. E148Q was most frequent in the group of healthy individuals, whereas M694V was the most common mutation among FMF patients, thereby corroborating previous studies on *MEFV* mutational spectra in the Middle East. Accordingly, *MEFV* mutations are frequent in healthy Iranian individuals across different ethnic groups. Based on this finding, the awareness for FMF and the implementation of augmented carrier screening programmes considering the multiethnic nature of the Iranian population should be promoted.

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Introduction

Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease with short, self-limited and recurrent attacks of fever and painful episodes of inflammation. It is particularly common in people with Mediterranean ancestry, comprising Armenians, Arabs, Jews and Turks (Sohar *et al.*

1967; Pras *et al.* 1992; Touitou 2001). FMF is caused by mutations in the Mediterranean fever (*MEFV*, *pyrin*, *marenosttrin*) gene, which differently affect the severity of the disease. Although, dominant transmissions have been identified in rare cases, it is classified as a disorder with autosomal recessive mode of inheritance (Booth *et al.* 2000). Until now, more than 304 sequence variants have been recorded (<http://fmf.igh.cnrs.fr/infevers>, 2015), including missense mutations, nonsense mutations and small deletions (Touitou 2001). Mutational hot spots in exon 2 (codon 148)

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and exon 10 (codons 680 and 694) have been described (Bernot *et al.* 1998; Magal *et al.* 1998).

The carrier frequency for *MEFV* mutations in classically affected populations range from 20% for Turks, North African, Ashkenazi Jews and Arabs, up to 39% for Iraqi Jews and Armenians. The high prevalence of carriers supports the idea that heterozygosity may confer a selective advantage. Nevertheless, the number of actual FMF cases is less than expected, indicating that the disease is either underdiagnosed or that reduced penetrance of mutations might play a role (Booty *et al.* 2009).

Further, the high prevalence of five FMF mutations (M694V, M694I, M680I, V726A and E148Q) may support a founder effect, descending from common ancestors in affected populations with spreading very diverse mutational patterns, especially from the Middle East (Touitou 2001).

E148Q (40.2%) has also been reported as the most common mutation among Japanese patients followed by M694I (21.0%) and L110P (18.8%) (Kishida *et al.* 2014). Although, the number of genetically detected patients with periodic fever syndromes is estimated very low in eastern and central European countries (Toplak *et al.* 2010) Sediva *et al.* (2014) indicated that FMF may not be so rare in the Czech Republic. Moreover, La Regina *et al.* (2003) showed that FMF exists in Italy and is often undiagnosed or diagnosed with a long delay. M694V, V726A, M680I, M694I and E148Q were found as the five most frequent FMF-associated mutations in Italian patients.

Iran being a highly multiethnic country (Najmabadi *et al.* 2001), surrounded by Armenia, Azerbaijan and Turkmenistan in the north, Persian Gulf and Gulf of Oman in the south, Afghanistan and Pakistan in the east and, Turkey and Iraq in the west, seems particularly interesting for studying FMF population genetics.

Therefore, the aim of our study was to identify the prevalence and frequency of common *MEFV* mutations in a large cohort of Iranian populations, including both healthy individuals as well as FMF patients.

Materials and methods

DNA samples

We collected blood samples from 743 Iranian patients (322 females, 421 males; age range 1–73 years) fulfilling international criteria for FMF (Livneh *et al.* 1997), who were referred to the Kariminejad-Najmabadi Pathology and Genetics Center by their physicians for counselling and genetic analysis between September 2007 and February 2015.

In addition, 208 samples from apparently healthy and knowingly unrelated individuals (105 females, 103 males) were collected from the Iranian Normal DNA bank, at the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran (Najmabadi *et al.* 2003). The cohort consisted of 36% Persians, 14% Azeris, 12% Gilakis/Mazandarani, 11% Balochs, 9% Kurds, 9% Lurs and 9% Arabs, thus resembling the proportions of these ethnic groups within

Iran (51, 24, 8, 2, 3, 7, 2%, respectively). For evaluation of the geographical distribution, we recorded the place of residence and subdivided the country into eight regions as previously described (Najmabadi *et al.* 2001): central (Tehran, Qom, Markazi, Semnan, Isfahan and Yazd), north (Gilan, Golestan and Mazandaran), northeast (Khorasan), northwest (Ardebil, east and west Azerbaijan and Zanjan), south (Bushehr and Hormozgan), southeast (Kerman, Sistan and Balochestan), southwest (Khuzestan, Chaharmahal and Bakhtiari, Kohgiluyeh and Boyer-Ahmad, Fars), west (Kurdistan, Kermanshah, Hamedan, Lorestan and Ilam).

Our study is in accordance with the declaration of Helsinki, and we obtained a written informed consent from all individuals investigated.

DNA extraction was done according to standard salting-out protocols using fresh or frozen EDTA blood (Miller *et al.* 1988).

Mutation analysis

All 951 participants of the study were tested for 12 common *MEFV* mutations using a reverse-hybridization teststrip-based assay (FMF StripAssay, ViennaLab Diagnostics, Vienna, Austria). Exons 2, 3, 5 and 10 were amplified in a single multiplex PCR, and biotinylated products were selectively hybridized to a teststrip presenting a parallel array of allele-specific oligonucleotide probes and detected by enzymatic colour reaction. The assay covers the following *MEFV* mutations: E148Q (c.442G>C), P369S (c.1105C>T), F479L (c.1437C>G), M680I (c.2040G>C), M680I (c.2040G>A), I692del (c.2076_2078del), M694V (c.2080A>G), M694I (c.2082G>A), K695R (c.2084A>G), V726A (c.2177T>C), A744S (c.2230G>T) and R761H (c.2282G>A) an automated hybridization device was used (profiBlot II T30, TECAN, Grödig, Austria) for parallel processing up to 48 samples.

Dideoxy sequencing of the entire *MEFV* gene was performed on a subset of 39 FMF patient samples using an ABI 3130 Automated Capillary DNA Sequencer in conjunction with the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA). Sequencing results were analysed by Codon Code Aligner software ver. 4.2.4 (32 bit version) (CodonCode Corporation, Centerville, USA).

In silico and statistical analyses

To determine the pathogenicity of three novel variants identified by dideoxy sequencing, we performed an *in silico* analysis using PolyPhen (<http://genetics.bwh.harvard.edu/pph2>) and SIFT (<http://sift.jcvi.org>) software algorithms.

Data management and statistical analysis were done using SPSS® software package 11.0.1 (SPSS, Chicago, USA). Associations between genotype frequencies and ethnic groups within the cohort of healthy individuals were done using Pearson's chi-square and likelihood ratio testing. $P < 0.05$ were considered statistically significant.

Table 1. *MEFV* allele-frequencies among 208 healthy Iranians.

Mutation	According to geographic area								According to ethnic origin							Total	Allele frequency
	C	N	NE	NW	S	SE	SW	W	Arab	Azeri	Baloch	Gilak	Kurd	Lur	Persian		
E148Q	7	4	1	7	1	9	6	5	3	5	8	3	2	3	16	40	0.0962
P369S	–	2	–	1	–	1	3	–	2	–	1	2	–	–	2	7	0.0168
M694V	–	2	–	–	–	–	1	–	–	–	–	1	–	–	2	3	0.0072
V726A	2	–	–	1	–	–	1	–	1	1	–	–	–	–	2	4	0.0096
A744S	–	1	–	–	–	1	2	–	1	–	–	1	–	1	1	4	0.0096
R761H	1	5	–	–	–	–	1	–	1	–	–	5	–	–	1	7	0.0168
Total mutant alleles	10	14	1	9	1	11	14	5	8	6	9	12	2	4	24	65	–
Total analysed	62	58	26	68	10	56	78	58	38	58	46	52	36	36	150	416	–
Percentage mutant	16.1	24.1	3.8	13.2	10.0	19.6	17.9	8.6	21.1	10.3	19.6	23.1	5.6	11.1	16.0	15.6	–

C, central; N, north; NE, northeast; NW northwest; S, south; SE, southeast; SW, southwest; W, west.

Results

A total of 65 mutant *MEFV* alleles could be identified in 57/208 (27.4%) reportedly asymptomatic individuals by FMF StripAssay analysis (table 1). Eight of the samples were either homozygous, compound heterozygous, or complex alleles, comprising the following multiple mutation genotypes: E148Q/E148Q and E148Q/M694V in two samples each, E148Q/P369S, E148Q/A744S, E148Q/R761H and P369S/A744S, each in a single sample. The overall frequency of mutant *MEFV* alleles in the healthy control population was 15.6%. E148Q was most prevalent, accounting for 9.6% of *MEFV* alleles, while five other mutations were found with distinct lower frequencies (P369S, 1.7%; R761H, 1.7%; V726A, 1.0%; A744S, 1.0%; M694V, 0.7%). With respect to ethnicity and geographical origin, above-average *MEFV* mutation rates were noted within the Gilak (North), Arab (SouthWest) and Baloch (SouthEast) population samples, but the observed differences failed to reach statistical significance.

Six hundred and seventeen mutant *MEFV* alleles could be identified in 391/743 (52.6%) FMF patients by FMF

StripAssay analysis (table 2). M694V, E148Q, M680 (G/C) and R761H were the most prevalent variants.

Two or more alleles were found to be mutated in 219 (29.5%) FMF patients. Sixty-two (8.3%) and 151 (20.3%) patients were identified as homozygotes and compound heterozygotes, respectively. Additionally, in six (0.8%) patients complex alleles were identified (table 3). In 172 (23.1%) patients, only one mutant allele could be detected with E148Q being most common.

Due to requests by their physicians, dideoxy sequencing was performed on 39 samples that were either found wild-type or heterozygous by FMF StripAssay analysis (table 4). Results were fully concordant in 11 (28.2%) wild, type and 12 (30.8%) heterozygous patients, while dideoxy sequencing led to the identification of one or more mutations in five (12.8%) previously wild, type, as well as a second or third mutation in 11 (28.2%) previously heterozygous samples, the latter contained mutations not covered by the FMF StripAssay: R202Q (c.605G>A) in exon 2, R408Q (c.1223G>A) in exon 3 and c.1588-69G>A in intron 5.

Interestingly, three novel variants were also identified: R202W (c.604C>T) in exon 2 (heterozygous), A66P (c.196G>C) in exon 1 (compound heterozygous with E148Q) and H300Q (c.900T>G) in exon 2 (compound heterozygous with A744S) (table 5). Our *in silico* analysis suggested a disease-causing potential for these new mutations.

Table 2. *MEFV* mutations in 743 Iranian FMF patients using the FMF StripAssay.

Mutation	Total mutant allele	Allele frequency
M694V	169 (11.4%)	0.1137
E148Q	158 (10.6%)	0.1063
M680I (G/C)	81 (5.5%)	0.0545
R761H	81 (5.5%)	0.0545
V726A	72 (4.8%)	0.0484
M694I	20 (1.3%)	0.0135
P369S	18 (1.2%)	0.0121
A744S	13 (0.9%)	0.0087
F479L	5 (0.3%)	0.0034
M680I (G/A)	–	–
I692del	–	–
K695R	–	–
Total mutant allele	617	–
Total analysed	1486	1486

Discussion

Our study examined the prevalence and frequency of *MEFV* mutations in a large cohort of different Iranian populations using reverse-hybridization teststrips for first-line screening of 743 FMF patients and 208 healthy individuals plus dideoxy sequencing to extend screening results in 39 patients.

We found a high frequency of *MEFV* mutation carriers (27.4%) among our healthy populations (table 1 in appendix). Earlier work including 200 healthy subjects from Iranian Azeri Turkish population reported a carrier rate of 25.5% (Bonyadi *et al.* 2010). In Armenia, even higher carrier rates

Table 3. Spectrum of *MEFV* genotypes in Iranian FMF patients using the FMF StripAssay.

Mutation	Genotype	Number	% (of 743)
Homozygotes <i>n</i> = 62 (8.3%)	M694V	31	4.2
	M680I (G/C)	12	1.6
	R761H	7	0.9
	E148Q	5	0.7
	V726A	4	0.5
	M694I	2	0.3
	F479L	1	0.1
Compound heterozygotes <i>n</i> = 151 (20.3%)	M694V/V726A	23	3.1
	M694V/R761H	22	3.0
	E148Q/M694V	21	2.8
	M680I (G/C)/R761H	15	2.0
	M680I (G/C)/M694V	13	1.7
	M680I (G/C)/V726A	10	1.3
	E148Q/P369S	8	1.1
	E148Q/M680I (G/C)	7	0.9
	E148Q/V726A	7	0.9
	M694I/R761H	6	0.8
	V726A/R761H	6	0.8
	M694I/V726A	4	0.5
	E148Q/M694I	2	0.3
	E148Q/R761H	2	0.3
	Others (E148Q/A744S, E148Q/F479L, F479L/V726A, M694V/A744S, M694V/M694I)	1 each	0.1 each
Complex alleles <i>n</i> = 6 (0.81%)	E148Q/R761H/R761H	4	0.5
	E148Q/M694V/V726A	1	0.1
	E148Q/E148Q/R761H/R761H	1	0.1
Heterozygotes <i>n</i> = 172 (23.1%)	E148Q	92	12.4
	M694V	25	3.4
	M680I (G/C)	12	1.6
	V726A	12	1.6
	A744S	11	1.5
	P369S	10	1.3
	R761H	6	0.8
	M694I	3	0.4
	F479L	1	0.1

could be demonstrated with 1/3 of inhabitants being FMF carrier or patient (Touitou 2001). The percentage of mutant *MEFV* alleles (15.6%) in our asymptomatic Iranian cohort was higher than what had previously been reported from Turkey (12%) and Iraq (11%). However, to some extent, this may be influenced by the fact that the majority of prior studies tested for the 4–5 most common mutations only (Touitou 2001; Yilmaz *et al.* 2001; Tunca *et al.* 2002; Al-Alami *et al.* 2003). Moreover, this heterogeneity may

support the consequence of the situation of Iran comprising seven different ethnic groups.

Nearly two-thirds of mutant *MEFV* alleles in our healthy population comprised E148Q, a mutation which is controversially discussed as perhaps being a benign polymorphic variant (Akar *et al.* 2001; Booth *et al.* 2001; Tchernitchko *et al.* 2003). The prevalence of E148Q in this study is comparable with Iraq, but exceeds values reported for Armenia, Turkey and several Arab countries (Yilmaz *et al.* 2001; Tunca *et al.* 2002; Al-Alami *et al.* 2003). Interestingly, Booth *et al.* (2001) reported extraordinary high frequencies of mutant E148Q alleles among Indians from Punjab (21%), a region in northern India and eastern Pakistan. Eventually, the increased presence of E148Q that we observed among Balochs from the south-eastern part of Iran may reflect historic connections and migration between this part of the country and the Punjab area.

With respect to FMF patients, our data indicate that M694V is the most common mutation, accounting for 11.4%

Table 4. FMF StripAssay analysis versus dideoxy sequencing in 39 FMF patients.

Mutation	StripAssay	Dideoxy sequencing
Negative	16	11
Heterozygous	23	15
Compound heterozygous	0	11
Complex	0	2

Table 5. Spectrum of mutant *MEFV* genotypes in a subset of patient samples analysed by dideoxy sequencing.

Mutation	Genotype	Number	Per cent
Compound heterozygotes <i>n</i> = 11 (28.2%)	E148Q/R202Q	3	7.7
	P369S/R408Q	2	5.1
	R202Q/c.1588-69G>A	2	5.1
	H300Q*/A744S	1	2.6
	c.1588-69G>A/A744S	1	2.6
	A66P**/E148Q	1	2.6
	R202Q/M694V	1	2.6
Complex alleles <i>n</i> = 2 (5.1%)	R202Q/R202Q/M694V	2	5.1
Heterozygotes <i>n</i> = 15 (38.5%)	E148Q	8	20.5
	R202Q	2	5.1
	R761H	2	5.1
	A744S	1	2.6
	R202W***	1	2.6
	V726A	1	2.6

*Bioinformatic predictions: polyphen, possibly damaging; SIFT, tolerated. Not found in 285 inhouse Iranian control database; neither heterozygous nor homozygous states.

**Bioinformatic predictions: polyphen, probably damaging; SIFT, not tolerated. Not found in 285 inhouse Iranian control database; neither heterozygous nor homozygous states.

***Bioinformatic predictions: polyphen, benign; SIFT, not tolerated. Not found in 285 inhouse Iranian control database; neither heterozygous nor homozygous states.

of *MEFV* alleles. Likewise, M694V has been reported in other studies as the most common mutation, responsible for 40–70% of all *MEFV* mutations in different populations (see table 1 in appendix). In patients with Iranian origin, however, this mutation seems to be less frequent than in Jews, Turks and Armenians (Cazeneuve *et al.* 1999; Medlej-Hashim *et al.* 2000; Yalcinkaya *et al.* 2000; Mansour *et al.* 2001; Al-Alami *et al.* 2003; Padeh *et al.* 2003; Tunca *et al.* 2005), ranging between 21 and 42.4% among Iran Azeri Turkish with FMF.

Although, discussed as being a benign polymorphism, we found E148Q in 10.6% of the FMF patient group, supporting the role of allelic variability in FMF expression (Gershoni-Baruch *et al.* 2002). Eighty-one (5.4%) of FMF alleles identified in this study carried M680I, a mutation which is known to be common in Armenians. Interestingly, we did not detect this mutation in our healthy individuals cohort. V726A was reported to be second most common in Arabs and Armenians (Touitou 2001; Majeed *et al.* 2005). Moreover, it has been demonstrated that V726A is more frequent in Oriental Arabs, mainly living in Syria, Lebanon, Palestine, Jordan and Iraq, and North African Arabs living in North Africa (Touitou 2001; Majeed *et al.* 2005). In contrast, V726A was fifth most common (4.8%) in our FMF patients and present only 1% in our healthy subjects.

Among Arab FMF patients, M694I appears to be the third most frequent disease-causing mutation. A study including 75 north African Arab FMF patients demonstrated M694I to be responsible for 61% of all detected mutations (Majeed *et al.* 2005). Here, 1.3% of FMF patients and no healthy individual carried M694I. R761H, which does not belong to the

five most prevalent mutations in the Middle East was found in 5.4 and 1.7% of all FMF patients and healthy subjects, respectively. These results corroborate an earlier study that found R761H rather prevalent in Iran Azeri Turks suffering from FMF (Bonyadi *et al.* 2009). Although, known to be frequent among Turks and Armenians (Touitou 2001), R761H was significantly more common among the Gilaki healthy population ($P = 0.001$) and samples obtained from healthy individuals in the north of Iran ($P = 0.004$).

Dideoxy sequencing revealed four relatively rare and three novel mutations in a subgroup of our FMF patients and confirmed the presence of disease-causing *MEFV* genotypes in 13 of 39 cases. The R202Q mutation had been found in heterozygous, homozygous and compound heterozygous state in symptomatic patients with a mild phenotype (Comak *et al.* 2014). R408Q had been reported in complex state (Cazeneuve *et al.* 1999). The c.1588-69G>A mutation in intron 5 had been observed in symptomatic patients with Arab origin (<http://fmf.igh.cnrs.fr/infervers>, 2015). Three novel *MEFV* variants (A66P, R202W and H300Q) were detected in patients presenting clinical symptoms of FMF. A66P and H300Q were found in compound heterozygous state with E148Q and A744S. With respect to R202W in a symptomatic patient, sequence analysis of the remaining exons failed to detect a second *MEFV* mutation. All three may be classified as potentially disease-causing mutations, but further studies are warranted to clarify their pathogenicity.

In conclusion, our study demonstrates that six *MEFV* mutations (E148Q, M680I, M694V, M694I, V726A and R761H) are widely distributed among Iranian FMF patients.

To the best of our knowledge, it is the first study which showed the influence of ethnic background on FMF in Iran. While we have the highest allele-frequency in Gilak in the north of Iran (23.1%), we just detected 5.6% of allele mutated in Kurdish population. Together with a high *MEFV* mutation carrier rate of 27.4% across different Iranian populations, our data suggest the need for augmented counselling and molecular diagnostic programmes considering the multiethnic nature of the Iranian population. In this regard, the FMF StripAssay demonstrated to be a reliable and cost-effective

first-line screening tool, which in combination with more comprehensive diagnostic methods such as dideoxy sequencing may render a very suitable strategy for population-based FMF genotyping in Iran.

Acknowledgement

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Appendix

Table 1. Overview of published *MEFV* mutation frequencies.

Healthy individuals	M694V	E148Q	V726A	M694I	M680I	Overall carrier rate	Reference
Arabs	0.0031	0.0654–0.0820	0.0260–0.0380	NA	0.0128	0.233	Al-Alami <i>et al.</i> (2003); Papadopoulos <i>et al.</i> (2008)
Armenians	0.0235–0.0470	0.0170–0.0340	0.0230–0.0460	0.0000–0.0020	0.0090–0.0180	0.210–0.390	Touitou (2001); Papadopoulos <i>et al.</i> (2008); Booty <i>et al.</i> (2009)
Jews (nonAshkenazi)	0.0193	0.0304	0.0339	NA	0	0.235 (up to 0.390 in Iraqi Jews)	Papadopoulos <i>et al.</i> (2008)
Syrians	0.0083	0.0661	0.0227	0	0	0.175	Booty <i>et al.</i> (2009); Mattit <i>et al.</i> (2006); Papadopoulos <i>et al.</i> (2008)
Turks	0.0150–0.0310	0.0600–0.0610	0.0100–0.0200	0	0.0250	0.200–0.224	Touitou (2001); Yilmaz <i>et al.</i> (2001); Tunca <i>et al.</i> (2002); Papadopoulos <i>et al.</i> (2008)
Iranians	0.0072	0.0962	0.0096	0	0	0.274	This study
Patients	M694V	E148Q	V726A	M694I	M680I	Reference	
Turks	15.6–52.2%	2–32.7%	1.5–14.1%	0.5–7%	1.5–15.5%	Tunca <i>et al.</i> (2005); Papadopoulos <i>et al.</i> (2008); Akin <i>et al.</i> (2010); Papadopoulos <i>et al.</i> (2010); Ozdemir <i>et al.</i> (2011); Dogan <i>et al.</i> (2012); Ece <i>et al.</i> (2014); Gunesacar <i>et al.</i> (2014)	
Egyptians	7.8–32.4%	17.5–25%	15.6–41.2%	20.6–42.5%	12.1–29.4%	el-Garf <i>et al.</i> (2010); El Gezery <i>et al.</i> (2010); Ibrahim <i>et al.</i> (2010)	
Jewish	65–77%	5–10.2%	3–12.3%	0	0.6–1%	Papadopoulos <i>et al.</i> (2008); Mohammadnejad and Farajnia (2013); Salehzadeh <i>et al.</i> (2015)	
Armenians	37–52%	1.8–3%	19–26%	0.2–2%	15.9–20%	Papadopoulos <i>et al.</i> (2008); Mohammadnejad and Farajnia (2013); Salehzadeh <i>et al.</i> (2015)	

Table 1 (contd)

Patients	M694V	E148Q	V726A	M694I	M680I	Reference
Syrians	36.5–45.8%	6–14.5%	13.8–15.2%	4.8–10.2%	9.6–13.2%	Papadopoulos <i>et al.</i> (2008); Jarjour (2010)
Arabs	20–42.5%	6–13%	14–23.1%	12–14.1%	7–9.6%	Al-Alami <i>et al.</i> (2003); Papadopoulos <i>et al.</i> (2008); Mohammadnejad and Farajnia (2013); Salehzadeh <i>et al.</i> (2015)
Iranians	21–42.4%	7–21%	4–19%	1–11%	6–17%	Esmacili <i>et al.</i> (2008); Bonyadi <i>et al.</i> (2009); Bidari <i>et al.</i> (2010); Mohammadnejad and Farajnia (2013); Sabokbar <i>et al.</i> (2014); Jabbarpour Bonyadi <i>et al.</i> (2015)
Current study	26.4%	24.5%	11.1%	3.1%	12.5%	This study

NA, not available.

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