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The M694V Mutation in Armenian-Americans: A Ten-Year Retrospective Study of *MEFV* Mutation Testing for Familial Mediterranean Fever at UCLA

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Abstract

Familial Mediterranean Fever (FMF), inherited in an autosomal recessive manner, is a systemic auto-inflammatory disorder characterized by recurrent attacks of fever with peritonitis, pleuritis, synovitis and erysipeloid rash. The marenostin-encoding fever gene (*MEFV*), located on chromosome 16p13.3, is the only gene in which mutations are currently known to cause FMF. To correlate specific genotypes with adverse phenotypes of affected populations residing in the Western United States, a retrospective case series review was conducted of all *MEFV* gene mutation testing completed at UCLA Clinical Molecular Diagnostic Laboratory between February 2002 and February 2012, followed by clinical chart review of all subjects who either have a single or double mutation. All 12 common mutations in the *MEFV* gene were analyzed and the M694V variant was found to be associated with an adverse FMF clinical outcome in the Armenian-American population, manifested by earlier onset of disease, increased severity of disease, and renal amyloidosis.

Introduction

Familial Mediterranean Fever (FMF), inherited in an autosomal recessive manner, is a systemic auto-inflammatory disorder.¹ FMF Type I is characterized by recurrent short episodes of inflammation and serositis, including fever, peritonitis, synovitis, pleuritis, with

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amyloidosis being the most severe complication. FMF Type II is characterized by amyloidosis as the first clinical manifestation of the disease in an otherwise asymptomatic individual.² The marenstrin-encoding fever gene (*MEFV*, MIM#: 608107), located on chromosome 16p13.3, is the only gene in which mutations are currently known to cause FMF.^{3–8} Five founder mutations, namely p.Met694Val, p.Met694Ile, p.Val726Ala, p.Met680Ile in exon 10 and p.Glu148Gln in exon 2, are responsible for the majority of mutations in the Mediterranean population of patients with classic presentation of FMF.⁹ FMF predominantly affects populations from the Mediterranean region, such as Armenians, non-Ashkenazi Jews, Turks, Persians and Arabs, with carrier rates as high as 1 in 7 in particular populations.^{1, 10–12} However, occurrence of FMF has also been observed in individuals with non-Mediterranean ancestries, such as Japanese, Chinese, European, and South American populations.^{13, 14}

Molecular genetic diagnostic testing is usually used to provide a confirmation of the FMF diagnosis when the clinical presentation of FMF is less typical. In most individuals with symptoms of FMF, targeted mutation analysis of the common *MEFV* mutations identifies double (homozygous or compound heterozygous) mutations, confirming the diagnosis. It is not uncommon to detect no mutant allele or only a single-mutant allele, particularly in patients with non-classic FMF or patients with mild, variable or atypical clinical presentations. Sequence analysis of the entire coding region may then be warranted; however, even that approach will not always yield the second mutation.¹⁵ Clinicians have accepted the molecular test as supportive of FMF and presume that the second mutation may not be detected possibly due to rarity or location outside the analyzed gene regions.¹⁶ There had been previous reports of certain genotypes correlating with adverse phenotypes in the populations around the Mediterranean region^{10, 12, 14}; however, it was not known whether this was true for affected populations residing in the Western United States. Thus, a retrospective case series review was conducted of all *MEFV* gene mutation testing completed at UCLA Clinical Molecular Diagnostic Laboratory between February 2002 and February 2012, followed by clinical chart review of all subjects who either have a single or double mutation.

Materials & Methods

All data were collected after approval by the University of California, Los Angeles Institutional Review Board (IRB# 11-00517).

MEFV mutation testing

The detection of the 12 *MEFV* mutations in exons 2, 5, and 10 – E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H—was conducted using the ViennaLab FMF StripAssay kit¹. Detection involves multiplex polymerase chain reaction amplifying four products with biotinylated primers, reverse hybridization of PCR products to test strips containing oligonucleotide probes (wild-type and mutant-specific), and visualization of bound products with streptavidin-alkaline phosphatase and chromogenic substrates.

Subjects and clinical history data

We conducted a retrospective case series chart review of patients that tested positive for mutations in the *MEFV* gene at the UCLA Clinical Molecular Diagnostic Laboratory between February 2002 and February 2012. A total of 476 non-consanguineous subjects were reviewed. We followed the OHRPP Data Security in Research guidance and procedures, coded all data during the study, and destroyed the coded data after the study. A total of 69 patient reviews were completed for positive double mutations and 83 patient

reviews were completed for positive single mutation. Data were collected for: 1) Ethnicity or ancestry, 2) FMF Type I or Type II, 4) Presence or history of amyloidosis, 5) Early onset (< 18 years of age), 6) Time between chief complaint and testing, and 7) Patient follow-up after testing. A proband of Armenian ethnicity was defined as having one or both parents of Armenian descent.

Statistical analysis

Genetic mutations that were positive in less than 5 subjects are reported as descriptive statistics. Mutations that were seen in more than 5 subjects were grouped and analyzed by ethnicity, Type I or Type II, and early versus later onset. The genetic differences between the two groups were then compared using Pearson's chi-squared test or Fisher's exact test. Genotypes were also grouped as no mutation, compound heterozygous mutations, or homozygous mutation, and analyzed by ethnicity using the Cochran-Armitage trend test. All statistical analyses were performed with JMP (JMP Pro 7.0, SAS Institute Inc., Cary, NC, USA), and a p-value of less than 0.05 was considered statistically significant.

Results

Armenian ancestry

During the ten years from February 2002 to February 2012, the UCLA Clinical Molecular Diagnostic Laboratory tested a total of 476 cases for suspected Familial Mediterranean Fever by *MEFV* mutation testing. There were 69 double positive mutation cases (14.5%) and 83 single mutation cases (17.4%). Of the 69 cases of double positive mutations, there were 34 cases of Armenian ethnicity (49.3%) and 35 cases of non-Armenian descent (50.7%). Of the 83 single mutation cases, there were 26 cases of Armenian ethnicity (31.3%) and 57 cases of non-Armenian ethnicity (68.7%).

M694V Double Mutations—Of the 34 cases of double positive mutation cases of Armenian ethnicity, 12 (35.3%) were M694V homozygotes and 22 (64.7%) compound heterozygotes, with 17 of the 22 (77.3%) having an M694V mutation as one of the two mutations. In contrast, out of the 35 cases of double mutation of non-Armenian ethnicity, there was only one (2.9%) M694V homozygote, while 34 (97.1%) were compound heterozygotes, with 16 of the 34 (47.1%) having an M694V mutation as one of the two mutations (Table 1). The difference in frequencies of the M694V homozygous mutation is highly significant between the two groups ($p=0.0006$ by Fisher's exact test, Fig. 1). The ethnicities were compared by analyzing for trend by genetic contribution of M694V (zero, one, or two copies). There was a significant trend of increasing genetic contribution of M694V mutation in Armenians by the Cochran-Armitage trend test ($P_{\text{trend}} < 0.0001$, Fig. 2). Conversely, in the non-Armenians, there was a significant trend of decreasing genetic contribution of M694V mutation ($P_{\text{trend}} < 0.0001$, Fig. 2).

M694V Single Mutation—Of the 83 single mutation cases, 26 cases were *M694V* single mutation (31.3%), 16 of which were of Armenian ethnicity (61.5%). In contrast, of the 57 cases without *M694V* mutation (68.7%), only 10 were of Armenian ethnicity (17.5%) and 47 were not (82.5%). Collectively, these data indicate a higher prevalence rate of *M694V* mutation in Armenians ($p=0.0001$ by Pearson's chi-squared test, Fig. 1).

Far East and South Asian ancestries

Interestingly, *MEFV* gene mutations were also found in Asians with ancestry from the Far East, the Indian subcontinent, and in admixed populations from the Philippines. There was one case of confirmed Type I FMF with E148Q homozygous mutation of Chinese ancestry (parents from Hong Kong), three cases of suspected FMF with E148Q single mutation of

Filipino/Chinese ancestry, and one case of confirmed FMF diagnosis with E148Q single mutation of Thai ancestry. There were two cases of E148Q/P369S compound heterozygote – one proband had a mother with Filipino ancestry and a father with English and Polish ancestry. The other proband has Hispanic parents. There were two cases of E148Q single mutation – one proband was from the Indian subcontinent and the other had Scottish and Irish ancestry.

FMF Type I vs. Type II

In an attempt to correlate FMF Type I or Type II to specific mutations, a comprehensive chart review for each patient that tested positive was undertaken. Out of the 69 cases of double positive mutations, FMF Type I or Type II data was available for 30 cases (43.5%). There were only three cases of FMF Type II documented (10%), and the remaining 27 cases were FMF Type I (90%). Of the three FMF Type II cases, two were M694V homozygotes, and one was M694V/M680I (G/C) compound heterozygote. All three FMF Type II cases were of Armenian ethnicity. Of the 27 FMF Type I cases, 18 were of Armenian ethnicity (66.6%). M694V homozygotes accounted for six cases (22.2%), of which all were of Armenian ethnicity.

Early onset of disease

Data were available for 41 of the 69 double positive mutations cases (59.4%) for early onset (defined as <18 years of age) of disease. Of these 41 cases, there were 31 cases of early onset disease (75.6%), 17 of whom were Armenians (54.8%). M694V homozygotes accounted for seven of the 31 cases (22.6%) where data were available, and all seven were Armenians. M694V/V726A compound heterozygotes accounted for nine of the 31 cases (29.0%), five of whom were Armenians (55.6%). M694V/M680I (G/C) compound heterozygotes accounted for three additional cases, all of whom were Armenians. M694V/R761H, M694V/M694I, M694V/E148Q compound heterozygous mutations each accounted for one case, and all three of these cases were Armenians.

Renal amyloidosis

Among the double positive mutations, there were four cases of documented renal amyloidosis, three of which had documented end stage renal disease and were status post kidney transplant at the time of data collection. The mutations for the renal amyloidosis cases were M694V homozygous (three cases) and M694V/M680I (G/C) compound heterozygous (one case). All three cases were of Armenian descent.

Discussion

In this study, we found that the M694V mutation is associated with an adverse FMF clinical outcome in the Armenian-American population, manifested by earlier onset of disease, severity of disease, and renal amyloidosis sequelae. Prior to 1989, the mode of FMF inheritance was unclear in Armenians, and a prospective study in families of 64 Armenian index cases from the UCLA FMF clinic was conducted.¹² When the gene for FMF was found to be linked to the alpha-globin complex on chromosome 16p in both Armenians and non-Ashkenazi Jews by studying 14 Armenian and 9 non-Ashkenazi Jewish families with FMF, it was concluded that there was no evidence for locus heterogeneity.³ By sampling 65 Jewish, Armenian and Arab families and genotyping for eight markers from chromosome 16p, it was discovered that the FMF susceptibility gene was located within 0.305 cM of the D16S246 locus.⁴ It was observed through high-resolution mapping that there was significant allelic association for D16S2617 among Armenians and that a sizable minority of Armenian carriers appeared to be derived from the North African Jewish ancestral haplotype.⁶ By studying 56 families of different ethnicities (14 of which were Armenian), Shohat et al.

identified 14 different *MEFV* core haplotypes and found significant association between amyloidosis and a specific haplotype, 153bp:104bp, at markers D16S3370 and D16S2617.¹⁷ In a later study of slightly larger size (83 families), Shohat et al. identified the M694V mutation as the particular mutation associated with amyloidosis.² In our dataset of Armenian-Americans, we also found that the cases of documented renal amyloidosis, often associated with end-stage renal disease and status post kidney transplantation, were associated with the M694V mutation. In fact, in our population sample, we did not observe a single case of amyloidosis without an M694V variant. Not surprisingly, in every FMF Type II case, the M694V variant was present. This M694V variant was also notable in FMF Type I cases in the Armenians. Even though our sample size for affected East and South Asians was not large, it is interesting to note that the E148Q mutation was common throughout all cases identified, suggesting a historic founder mutation probably originating several centuries ago at the height of maritime trade between the Middle East and the Far East via the Indian subcontinent. It is also interesting to note that the time between initial chief complaint and date of testing is approximately ten years. This implies that physicians are still missing this diagnosis frequently and patients with FMF are still suffering needlessly for lengthy periods of time. The advent of next-generation sequencing technologies and the gradual incorporation of this methodology into the clinical setting could benefit patients not only by potentially arriving at a definitive diagnosis sooner, but also by identifying other causative variants or modifier genes that may contribute to FMF.

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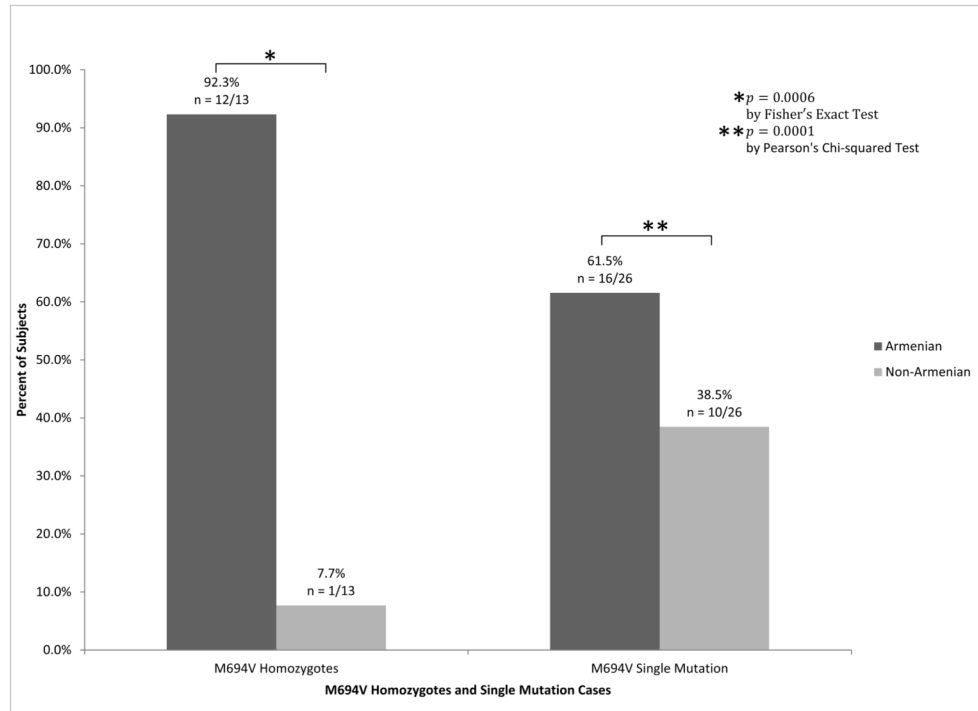


Fig. 1. Difference in M694V Mutations between Armenians and Non-Armenians. Of the 34 cases of double positive mutation cases of Armenian ethnicity, 12 (35.3%) while out of the 35 cases of double mutation of non-Armenian ethnicity, there was only one (2.9%) M694V homozygote. The difference in frequencies of the M694V homozygous mutation is highly significant between the two groups by Fisher’s exact test ($p=0.0006$). Of the 83 single mutation cases, 26 cases were M694V single mutation (31.3%), 16 of which were of Armenian ethnicity (61.5%) and 10 of which were of non-Armenian ethnicity (38.5%). The data indicate a higher prevalence rate of M694 single mutation in Armenians by Pearson’s chi-squared test ($p=0.0001$).

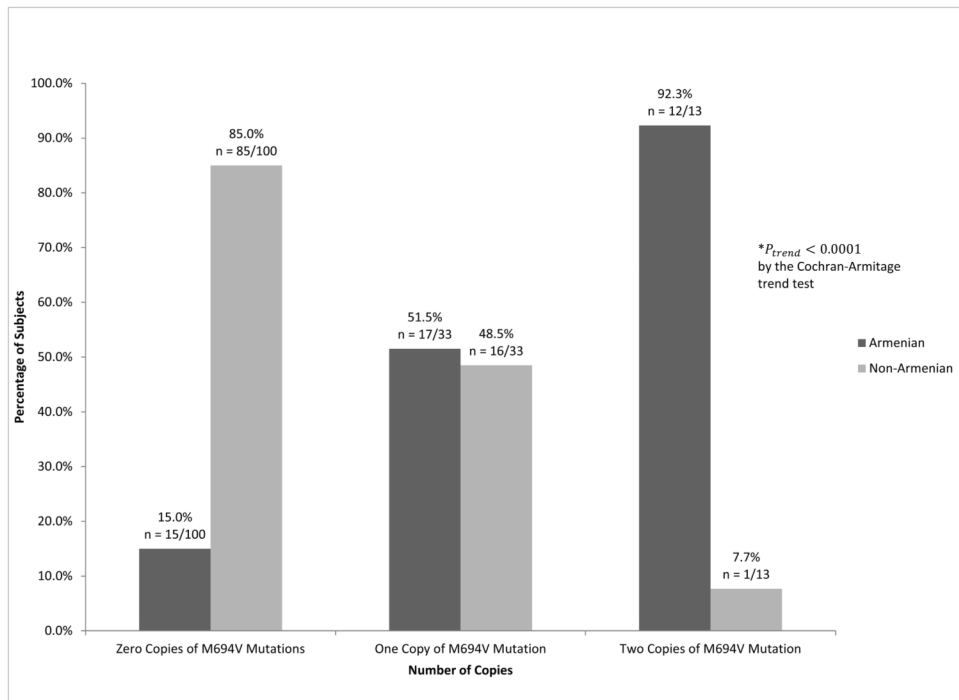


Fig. 2. Ethnicities Compared by Genetic Contribution of M694V. The ethnicities were compared by analyzing for trend by genetic contribution of M694V (zero, one, or two copies). There was a significant trend of increasing genetic contribution of M694V mutation in Armenians by the Cochran-Armitage trend test ($P_{\text{trend}} < 0.0001$). Conversely, in the non-Armenians, there was a significant trend of decreasing genetic contribution of M694V mutation ($P_{\text{trend}} < 0.0001$).

Table 1

Double and Single Mutations

	Armenian		Non-Armenian	
	n	%	n	%
Double Mutations Cases*				
M694V Double Mutations				
Homozygotes	12	35.3	1	2.9
Compound Heterozygotes	17	50.0	16	45.7
V726A Double Mutations				
Homozygotes	0	0.0	3	8.6
Compound Heterozygotes	10	29.4	13	37.1
E148Q Double Mutations				
Homozygotes	0	0.0	1	2.9
Compound Heterozygotes	2	5.9	10	29.4
M680I (G/C) Double Mutations				
Homozygotes	0	0.0	0	0.0
Compound Heterozygotes	13	38.2	7	20.0
Single Mutation Cases**				
Positive for Mutation				
M694V	16	61.5	10	38.5
E148Q	2	9.1	20	90.9
Negative for Mutation				
M694V	10	17.5	47	82.5
E148Q	24	39.3	37	60.7

* Percentages calculated out of 34 Armenian and 35 Non-Armenian double mutation cases respectively.

** Percentages calculated out of total number of cases for each mutation.