Association between *serum amyloid A1* genotype and age of onset restricts to M694 homozygote familial Mediterranean fever patients in Armenia

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ABSTRACT

Objective. *Familial Mediterranean fever (FMF) is an autosomal-recessive, inflammatory disorder characterised by short, recurrent attacks of fever, accompanied by pain in the abdomen, chest, or joints and complications of amyloidosis. Recently, we observed a significant association between the serum amyloid A1 (SAA1) β/β genotype and a delayed disease onset in 386 M694V homozygous FMF patients. This follow-up study was conducted to additionally analyse MEFV genotypes other than M694V/M694V for a possible influence of the SAA1 genotype on the age of disease onset.*

Methods. *A total of 700 Armenian patients diagnosed with FMF based on the Tel-Hashomer criteria and carrying two MEFV mutant alleles were included in this study. Patients were divided into three MEFV genotypic subgroups: M694V homozygotes (M694V/M694V), M694V compound heterozygotes (M694V/Other), and patients with genotypes excluding M694V (Other/Other). MEFV and SAA1 analyses were performed by a commercial reverse-hybridisation assay, and resulting genotypes were matched against the demographic and clinical characteristics of the patients.*

Results. *Within the subgroup of M694/M694 homozygotes, SAA1 genotype β/β could be identified in 115 (34.43%) and 32 (61.54%) patients with an age of onset <20 and ≥20 years, respectively (p<0.001). However, no such relationship could be observed for MEFV genotypic subgroups M694V/Other (p=0.465) and Other/Other (p=0.697).*

Conclusion. *Our data suggest, that the influence of SAA1 genotypic variation on the age of disease onset restricts to FMF patients homozygous for MEFV mutation M694V.*

Introduction

Familial Mediterranean fever (FMF, OMIM 249100) is an autosomal-recessive, inflammatory disorder characterised by short, recurrent attacks of fever, accompanied by pain in the abdomen, chest, or joints and complications of amyloidosis (1). FMF predominantly affects Turks, Arabs, Armenians and Sephardic Jews, but it has been observed in lower frequencies throughout the Mediterranean area (2). It is caused by numerous mutations in the pyrin-encoding Mediterranean fever (*MEFV*) gene (OMIM 608107; GenBank NM_000243.2) on chromosome 16p13.3, that differently affect the disease phenotype and the risk of developing renal amyloidosis (3, 4). Very recently, the *MEFV* gene was found duplicated in a typical FMF patient compound heterozygous for *MEFV* mutations E148Q/M694, with the authors discussing the potential role of a gene dosage effect or *MEFV* deregulation in the patient's disease phenotype (5).

The age of onset of FMF varies with about 60% and 90% of patients experiencing their first attack before the age of 10 and 20 years, respectively (6). Moreover, recent research suggests that adult-onset FMF (*i.e.* first FMF attack at the age ≥ 20 years) is associated with a milder disease phenotype (6, 7).

FMF shows considerable variability in severity and type of clinical manifestations by geographic region which are attributed to *MEFV* allelic heterogeneity, additional genetic modifiers and environmental factors (8). Of these, 2 single nucleotide polymorphisms (SNPs) in exon 3 at positions 2995C/T (rs1136743) and 3010C/T (rs1136747) of the *serum amyloid A1* (*SAA1*) locus were shown to differentially influence susceptibility to renal amyloidosis in patients with FMF (8-10). While the *SAA1*α/α (2995T-3010C/2995T-3010C)

genotype was found associated with a higher risk of amyloidosis (8-10), the *SAA1*β/β (2995C-3010T/2995C-3010T) genotype seemed to be protective of this devastating FMF complication (10).

Recently, we observed a significant association between the *SAA1*β/β genotype and a delayed disease onset in 386 Armenian M694V homozygous FMF patients (11), a finding that had not been reported by former studies (8-10). At that time, we speculated that the *SAA1* genotype might exert its influence on the age of disease onset in the subgroup of M694V homozygotes only (11). Therefore, this follow-up study was conducted to additionally analyse *MEFV* genotypes other than M694V/M694V for a possible association between *SAA1* genotype and the age of onset in 700 Armenian FMF patients.

Table I. Clinico-demographic characteristics, *MEFV* and *SAA1* genotypes for FMF patients with age of onset <20 and ≥20 years.

	<20 years n=555	≥20 years n=145	*p*-value
Mean age, years	18.29 ± 13.49	41.48 ± 12.05	**< 0.001****
Mean age of onset, years	6.05 ± 5.13	28.55 ± 8.64	-
Gender			
Male	267 (48.11%)	79 (54.48%)	
Female	288 (51.89%)	66 (45.52%)	0.192
Family history of FMF	219 (39.46%)	45 (31.03%)	0.068
Fever (≥ 38°C)	486 (87.57%)	122 (84.14%)	0.272
Arthralgia	304 (54.77%)	89 (61.38%)	0.160
Skin rash	49 (8.83%)	4 (2.76%)	0.158
Abdominal pain	464 (83.60%)	129 (88.97%)	0.121
Chest pain	209 (37.66%)	62 (42.76%)	0.292
Myalgia	124 (22.34%)	28 (19.31%)	0.498
Pras			
Mild	199 (35.86%)	103 (71.03%)	
Moderate	326 (58.74%)	42 (28.97%)	
Severe	30 (5.41%)	0 (0.00%)	**<0.001****
Attacks per month			
<1	178 (32.07%)	63 (43.45%)	
>1 and <2	246 (44.32%)	53 (36.55%)	
≥2	131 (23.60%)	29 (20.00%)	**0.027*****
MEFV			
M694V/M694V	334 (60.18%)	52 (35.86%)	
M694V/Other*	140 (25.23%)	52 (35.86%)	
Other*/Other*	81 (14.59%)	41 (28.28%)	**<0.001****
SAA1			
α/α	70 (12.61%)	21 (14.48%)	
α/β	280 (50.45%)	62 (42.76%)	
β/β	205 (36.94%)	62 (42.76%)	0.256

*Refers to chromosomes with either one of the 12 *MEFV* mutations covered by the FMF-SAA1-StripAssay except for mutation M694V.
**$p<0.01$, ***$p<0.05$.

Patients and methods

Study participants

A cohort of 700 Armenian patients diagnosed with FMF based on the Tel-Hashomer criteria and carrying two *MEFV* mutant alleles who visited the Centre of Medical Genetics and Primary Health Care in Yerevan were included in this study. Patients were divided into three groups according to *MEFV* genotype: M694V homozygotes (M694V/M694V), M694V compound heterozygotes (M694V/Other) and patients with genotypes excluding M694V (Other/Other). Personal and medical data, including age, sex, age of disease onset, fever, abdominal pain, chest pain, arthralgia, skin involvement (erysipelas-like erythema), myalgia and frequency of attacks were recorded. Disease severity was determined by the use of a scoring system suggested by Pras *et al*. (12). Patients also suffering from non-FMF related inflammation or chronic disease were excluded from the study. The study was approved by the local ethics committee of the Yerevan State Medical University, Yerevan, Armenia and is in accordance with the latest version of the Declaration of Helsinki. All patients provided written informed consent.

Molecular genetic analysis

DNA was isolated from anticoagulated blood using the GenXtract DNA extraction system (ViennaLab Diagnostics GmbH, Vienna, Austria). Twelve common *MEFV* mutations, including E148Q (c.442G>C) in exon 2; P369S (c.1105C>T) in exon 3; F479L (c.1437C>G) in exon 5; M680I G/C (c.2040G>C), M680I G/A (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; ref.seq. NG_007871.1) in exon 10, and two polymorphic loci, 209 T>C and 244 T>C, in the *SAA1* gene were simultaneously analysed using a test strip-based reverse-hybridisation assay (FMF-SAA1 StripAssay, ViennaLab Diagnostics GmbH). The operators were blinded to the patient's disease status.

Statistical analysis

All parameters were recorded, tabulated and evaluated in a descriptive statistical manner. Comparisons between *MEFV* genotypes for normally distributed continuous variables and non-normality (verified by the Kolmogorov-Smirnov test with Lilliefors significance correction) relied on the independent two-sample t-test and the Mann-Whitney U test, respectively. For subgroup comparison of categorical variables, the Chi-square test (n x k table) and the Fisher's exact test (2 x 2 tables) were calculated. The type I error was set to 5% (two-sided) without adjustment for multiple testing. All statistical analyses were performed using the open-source statistical program package R Version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

In total, 700 individuals with a clinical diagnosis of FMF were included, of whom 354 (50.57%) were female, and 346 (49.43%) were male. The

mean age was 23.09±16.20 years. Of all participants, 264 (37.71%) could be identified with a family history of FMF. Concerning the age of disease onset, 555 (79.30%) and 145 (20.70%) patients experienced their first attack before the age of 20 years and at or after the age of 20 years (*i.e.* adult-onset FMF), respectively.

Table I shows the clinico-demographic characteristics and *MEFV*/*SAA1* genotypic distributions for FMF patients with an age of disease onset <20 and ≥20 years. In the adult-onset FMF group (female: 66 [45.52%], male: 79 [54.48%]) and the control group (female: 288 [51.89%], male: 267 [48.11%]) the mean age of disease onset was 28.55±8.64 and 6.05±5.13 years, respectively.

The distribution of *MEFV* genotypes was significantly different between patients with an age of disease onset <20 and ≥20 years. M694V homozygotes (M694V/M694V), M694V compound heterozygotes (M694V/Other) and patients with genotypes excluding M694V (Other/Other) could be identified in 52 (35.86%), 52 (35.86%) and 41 (28.28%) adult-onset patients, while this was the case for 334 (60.18%), 140 (25.23%), and 81 (14.59%) patients with a disease onset <20 years, respectively ($p<0.001$). Furthermore, adult-onset disease was associated with a less severe FMF phenotype ($p<0.001$) and a lower attack frequency ($p=0.027$).

No overall association between the patients' *SAA1* genotype and the age of disease onset was observed (Table I). However, when *MEFV* genotypic subgroubs were analysed, the genotypic distribution of *SAA1* alleles was significantly different between patients with an age of disease onset <20 and ≥20 years in M694V homozygous individuals only (Table II).

Within the subgroup of M694/M694 homozygotes, *SAA1* genotype β/β could be identified in 115 (34.43%) and 32 (61.54%) patients with an age of onset <20 and ≥20 years, respectively ($p<0.001$). However, no such relationship, could be observed for *MEFV* genotypic subgroups M694V/Other ($p=0.465$) and Other/Other ($p=0.697$) (Table II).

Table II. *MEFV* genotypic subgroup analysis with respect to *SAA1* genotype in FMF patients with age of onset <20 and ≥20 years.

		<20 years n=555	≥20 years n=145	*p*-value
M694V/M6949V				
SAA1	α/α	47 (14.07%)	8 (15.38%)	
	α/β	172 (51.50%)	12 (23.08%)	
	β/β	115 (34.43%)	32 (61.54%)	**<0.001***
M694V/Other**				
SAA1	α/α	13 (9.29%)	7 (13.46%)	
	α/β	69 (49.29%)	28 (53.85%)	
	β/β	58 (41.43%)	17 (32.69%)	0.465
Other/Other****				
SAA1	α/α	10 (12.35%)	6 (14.63%)	
	α/β	39 (48.15%)	22 (53.66%)	
	β/β	32 (39.51%)	13 (31.71%)	0.697

*$p<0.01$.
**Refers to chromosomes with either one of the 12 *MEFV* mutations covered by the FMF-SAA1-StripAssay except for mutation M694V.

Discussion

Genotype-phenotype correlations have identified a variable risk for FMF patients with the same *MEFV* genotype to develop renal amyloidosis, suggesting a disease modifying role either for genetic or environmental factors (8). For example, an increased susceptibility to amyloidosis was observed to be coupled with male sex and articular manifestations in Jewish FMF patients homozygous for M694V (13). Another study found the SAA1 genotype α/α to be associated with a 7-fold increased risk of developing renal amyloidosis, a finding which was extremely marked in M694V homozygous FMF patients (8). Recently, we observed a significant association between the *SAA1* β/β genotype and a delayed disease onset in 386 Armenian M694V homozygous FMF patients (11). To further substantiate this observation, the current study investigated 700 Armenian FMF patients with various *MEFV* genotypes including 145 (20.70%) individuals with adult-onset disease.

MEFV genotypes M694V/M694V, M694V/Other and Other/Other could be identified in 386 (55.14%), 192 (27.43%) and 122 (17.43%) patients, respectively. Mutation M694V was most prevalent representing 964/1400 (68.85%) alleles analysed here. A previous Armenian study comprising 10,370 FMF patients covering the same mutational spectrum found and M694V allelic frequency of 41.34% (6). This discrepancy obviously reflects our sampling bias towards mutation M694V caused by including a preselected cohort of 386 M694V homozygous FMF patients (11).

In the current study, genotype M694V/M694V was significantly associated with an age of disease onset <20 years ($p>0.001$). This finding is in line with several former studies that related M694V homozygosity to an earlier age of onset (9, 14, 15). Also corroborating previous data (7, 10, 16), we found the adult-onset disease to be associated with a less severe disease phenotype ($p<0.001$) and a lower attack frequency ($p=0.027$).

Recently, we observed a significant association between the *SAA1* β/β genotype and a delayed disease onset in 386 Armenian M694V homozygous FMF patients (11), a finding that had not been reported by former studies on the topic (8-10). Here, the *SAA1* genotype β/β was significantly associated with the adult-onset variant ($p<0.001$), however, this relationship was exclusively found in FMF patients homozygous for *MEFV* mutation M694V. Therefore, our novel data suggest an explanation why other studies, not conducting *MEFV* genotypic subgroup analysis, have failed to demonstrate such an association (8-10).

The main strength of this retrospective study is the availability of clinically and genetically well-defined cases that enabled the analysis of 145 patients with adult-onset FMF. However, some

bias may have been introduced through the sample selection based on *MEFV* genotype and age of disease onset.

In conclusion, our data suggest, that the influence of *SAA1* genotypic variation on the age of disease onset restricts to FMF patients homozygous for *MEFV* mutation M694V.

References

1. 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: 1948-50.
2. DRENTH JP, VAN DER MEER JW: Hereditary periodic fever. *N Engl J Med* 2001; 345: 1748-57.
3. FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 7: 25-31.
4. TOUITOU I: The spectrum of familial Mediterranean fever (FMF) mutations. *Eur J Hum Genet* 2001; 9: 473-83.
5. MECHIN D, DURINIKOVA A, BOURSIER G *et al*.: First report of *MEFV* duplication in a patient with familial Mediterranean fever. *Clin Exp Rheumatol* 2020; 38 (Suppl. 127): S129-30.
6. KRIEGSHÄUSER G, ENKO D, HAYRAPETYAN H *et al*.: Clinical and genetic heterogeneity in a large cohort of Armenian patients with late-onset familial Mediterranean fever. *Genet Med* 2018; 20: 1583-8.
7. YASAR BILGE NS, SARI I, SOLMAZ D *et al*.: Comparison of early versus late onset familial Mediterranean fever. *Int J Rheum Dis* 2018; 21: 880-5.
8. CAZENEUVE C, AJRAPETYAN H, PAPIN S *et al*.: Identification of MEFV-Independent Modifying Genetic Factors for Familial Mediterranean Fever. *Am J Hum Genet* 2000; 67: 1136-43.
9. GERSHONI-BARUCH R, BRIK R, ZACKS N *et al*.: The Contribution of Genotypes at the MEFV and SAA1 Loci to Amyloidosis and Disease Severity in Patients With Familial Mediterranen Fever. *Arthritis Rheum* 2003; 48: 1149-55.
10. ATOYAN S, HAYRAPETYAN H, SARKISIAN T *et al*.: MEFV and SAA1 genotype associations with clinical features of familial Mediterranean fever and amyloidosis in Armenia. *Clin Exp Rheumatol* 2016; 34: 72-6.
11. KRIEGSHÄUSER G, HAYRAPETYAN H, ATOYAN S *et al*.: Serum amyloid A1 genotype associates with adult-onset familial Mediterranean fever in patients homozygous for mutation M694V. *Rheumatology* 2020; doi: 10.1093/rheumatology/keaa452.
12. PRAS E, LIVNEH A, BALOW JE JR *et al*.: Clinical differences between North African and Iraqui Jews with familial Mediterranean fever. *Am J Med Genet* 1998; 13: 216-9.
13. GERSHONI-BARUCH R, BRIK R, LIDAR M *et al*.: Male Sex Coupled with Articular Manifestations Cause a 4-fold increase in Suceptiobility to Amyloidoisis in Patients with Familial Mediterranean Fever Homozygous for the M694V-MEFV Mutation. *J Rheumatol* 2003; 30: 308-12.
14. CAZENEUVE C, SARKISIAN T, PECHEUX C *et al*.: MEFV-Gene analysis in Armenian patients with familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype – genetic and therapeutic implications. *Am J Hum Genet* 1999; 65: 88-97.
15. LIDAR M, YONATH H, SHECHTER N *et al*.: Incomplete response to colchicine in M694V homozygote FMF patients. *Autoimmun Rev* 2012; 12: 72-6.
16. SAYARLIOGLU M, CEFLE A, INANC M *et al*.: Characteristics of patients with adult-onset familial Mediterranean fever in Turkey: analysis of 401 cases. *Int J Clin Pract* 2005; 59: 202-5.