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Common MEFV mutations and polymorphisms in an elderly population: an association with E148Q polymorphism and rheumatoid factor levels

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ABSTRACT

Objectives. *To analyse the most common MEFV (Mediterranean fever gene) mutations and polymorphisms in an elderly population free of chronic inflammatory disease (n=164), and explore possible associations between hsCRP (high sensitive C-reactive protein) and RF (rheumatoid factor) levels with MEFV mutations and polymorphisms.*

Methods. *An elderly group free of chronic inflammatory disease was chosen among the outpatients of the division of geriatric medicine. Total genomic DNA was isolated from blood, and PCR-RFLP analysis was performed using established protocols. Sera were analyzed for hsCRP and RF levels.*

Results. *The frequencies for 694V (1.8%), 694I (1.8%), 680I (0.6%), 726A (2.1%) and 148Q (5%) alleles were found to be similar to Turkish historic controls, with a carrier frequency of 1/4. Further analyses with rheumatoid factor (RF) levels and mutations revealed a significant association between the presence of the E148Q polymorphism with increased RF levels (>15 mg/dl) (χ^2= 7.358, p=0.007, OR=5.41 95% CI 1.41-20.64).*

Conclusions. *Common MEFV mutations and polymorphisms were similarly represented among the elderly population compared to historic controls. On the other hand, a significant association was found between the presence of E148Q polymorphism and increased RF levels. This suggests that the previously noted increased RF levels in elderly populations may somehow be related to the now described association of RF with MEFV E148Q polymorphism.*

Introduction

Familial Mediterranean fever (FMF; OMIM no: 249100) is the most common periodic fever syndrome. It is typically characterized by attacks of serositis usually in the abdomen, chest and joints accompanied by fever and an elevated acute-phase response (1-3).

Since the cloning of the Mediterranean fever associated gene (MEFV) in 1997 (4, 5), about 64 disease-associated mutations have been identified (6, 7). Five mutations in the MEFV gene, E148Q, M680I, M694V, M694I, and V726A, make up for at least 70-80% of the disease associated alleles (8). FMF is especially predominant in the Turkish, Non-Ashkenazi Jew and Armenian populations. The frequency of FMF in the Turkish population had been estimated as 1 in 1073; however, this increases to 1 in 395 in central Turkey (9) where a gene carrier frequency of 1/5 has been described (10).

Observation of the increased MEFV mutation frequencies in chronic inflammatory diseases such as Behçet's disease (11-15), ulcerative colitis (16), rheumatoid arthritis (17, 18) and polyarteritis nodosa (19), suggest that the MEFV gene is involved in the inflammatory pathway in general. We therefore hypothesized that the frequency of MEFV mutations in the elderly population who have remained free of disorders with chronic inflammation, like FMF or rheumatoid arthritis (RA) would be lower than that which has been described in the general population. To this end, we have analyzed the frequency of the most common MEFV mutations in an elderly population. We also wanted to explore if there was any association between the common inflammatory markers such as hsCRP (high sensitive C-reactive protein) and RF (rheumatoid factor) levels with MEFV mutations.

Patients and methods

Patients

An elderly group free of chronic inflammatory disease was chosen among the patients from the outpatient clinics of the division of geriatrics of the Cerrahpasa Medical Faculty (CMF). A rheumatologist and a geriatrician selected the group, who were patients over 60 and who did not have a history of any chronic inflammatory disease. They were chosen among those who attended the clinic for routine health control examinations or follow-up visits for hypertension, osteoporosis, osteoarthritis, hyperlipidemia or hypercholesterolemia (n=170). The hospital ethics committee approved the study protocol. An oral informed consent was obtained from all participants.

RF and hsCRP determinations

Serum RF and hsCRP levels were quantified using an immunonephelometric method (Dade Behring BNII Analyzer).

MEFV mutation genotyping

Genomic DNA isolation was done by Magtration System 8Lx Instrument, with the kit provided by the supplier (Precision System Science). Mutations and polymorphisms were genotyped using established protocols based on PCR-RFLP analysis (20-21).

Statistical analysis

Allele frequencies were compared with those historic controls from the Turkish population (10, 23, 24) using chi-squared tests. RF and hsCRP levels were categorized into two groups; low group hsCRP included people with hsCRP levels <3.0 IU/mL and high group >3.0 IU/mL (21); low group RF included those with <15mg/dl and high group RF >15mg/dl. Subgroup analyses were done between MEFV genotypes and RF and hsCRP groups using non-parametric analysis including chi-squared tests. Correction for multiple testing was used when appropriate. All statistical analyses were done using SPSS Ver 15.0 software (SPSS Inc., Chicago, USA).

Table I. Characteristics of elderly population without chronic inflammatory disorders.

Elderly group n=170	Number (~%)
Female / male	128/42 (75%/25%)
Age	Mean=74±0.5 (61-92) (median=74, mode=67)
Hypertension	128 (75 %)
Osteoporosis	87 (51%)
Osteoarthrosis	49 (29%)
Hypercholesterolemia	8 (5%)
Hyperlipidemia	4 (2%)
hsCRP (IU/mL) (n=154)	Median=1.8 (0.16-43.5)
hsCRP low group (hsCRP <3 IU/mL)	Median=1.2 (0.1-2.9); 106 (69 %)
RF (mg/dL) (n=157)	Median=27.6 (15.20-154); 13 (8%)
RF low group (RF <15mg/dL)	Median=<10.3 (<10.3-14.90); 144 (92%)
RF high group (RF >15mg/dL)	Median=27.6 (15.20-154); 13 (8%)

Table II. Frequency of five common MEFV mutations and polymorphism in the elderly population (n=164).

Mutation	Number of alleles	Carrier frequency (mutant genotypes)	Allele frequency
694V	6	3.7 %	1.8%
694I	6	3.7 %	1.8%
680I	2	1.2%	0.6%
726A	7	4.3%	2.1%
148Q	17	10%	5%
Total	38	23%	11.3%

Table III. MEFV mutation analysis: comparisons with historic controls.

Alleles (percentages)	M694V	M694I	M680I	V726A	E148Q
Elderly (n=164)	6/322 (1.8%)	6/328 (1.8%)	2/324 (0.6%)	7/324 (2.1%)	17/328 (5%)
Turkish controls [10] (n=100)	3/200 (1.5%)	0/200	5/200 (2.5%)	2/200 (1 %)	12/200 (6%)
Turkish controls [23] (n=100)	8/200 (4%)	0/200	5/200 (2,5%)	4/200 (2%)	7/200 (3.5%)
Turkish controls [24] (n=49)	4 (4%)	0	0	2 (2%)	2 (2%)

Table IV. E148Q Association with RF levels in elderly population.

		E148Q genotype		
		EE	EQ	Total
RF (mg/dL)	Low(<15)	130	12	142
	High (>15)	8	4	12
Total		138	16	154
χ^2= 7.358, df(1), *p*=0.007		Odds ratio = 5.41 95% CI 1.41-20.64		

Results

The demographic and clinical characteristics of the elderly group (n=170) are given in Table I. Majority of the group had normal of hsCRP (91%) and RF (88%) determinations. The most common clinical condition was hypertension (74%), followed by osteoporosis (51%), osteoarthrosis (29%), hypercholesterolemia (5%) and hyperlipidemia (2%).

The allele and genotype frequencies of the common MEFV mutations and polymorphisms are shown in Table II. There were no significant differences in the frequencies of the MEFV mutations and polymorphisms in the elderly population (M694V=0.018, M694I=0.018, M680I=0.006, V726A=0.021, E148Q=0.050) when compared to the frequencies in the published historic controls from our region (Table III).

Table IV analyzes the association of hsCRP and/or RF levels with MEFV mutations and polymorphisms. A significant correlation was found between the E148Q variant and increased RF levels (χ^2= 7.358, *p*=0.007, OR=5.41 95% CI 1.41-20.64). The significance remained robust (*p*=0.035) after correction for multiple testing.

Discussion

A landmark study analyzing the biological results of the absence rather

than the presence of a particular trait has been the comparison of the risk of dementia in the relatives of healthy elderly people to those observed in the general population and those of patients with Alzheimer's disease (25). The study had shown that the risk of dementia is minimum in the relatives of healthy elderly people, higher in the randomly chosen general population and highest in the relatives of patients with Alzheimer's disease. In a more recent study Grimaldi MP *et al*. has observed that decreased MEFV M694V mutation frequency in centenarians compared to other people with acute myocardial infarction and age matched controls, which led them to suggest that wild type MEFV genotype may predispose to a greater chance of living longer (26).

In our study, similarly designed to look for the less frequent presence of a trait in a peculiar population, the hypothesis tested was the less frequent occurence of MEFV mutations and polymorphisms in the elderly who have lived a healthy life without inflammatory disease as compared to the general population. However, we found that common MEFV mutations and polymorphisms were similarly represented among the elderly population compared to the Turkish historic controls from different regions (10, 23, 24). In fact there was a slight increase in the frequency of one of the mutations, M694I. We also wanted to know if there was any relation with hsCRP and RF levels in people carrying MEFV mutations and polymorphisms. Our subgroup analysis has indicated a significant relationship between people carrying E148Q polymorphism with increased RF levels.

These two observations appear to contradict each other at first sight as the observed association of E148Q polymorphism with increased RF was not associated with chronic inflammation in the elderly. On the other hand, a review of MEFV associations with various inflammatory diseases in literature shows an increased frequency of the E148Q polymorphism in inflammatory arthritis and amyloidosis (20). A study by Rabinnovich *et al*. analysed MEFV variants along with RF levels in RA patients and reported that the median clinical severity score was significantly higher in the mutation carriers even after correction for the presence of RF. It was interesting to note that in the Rabinnovich *et al*. study 17/18 of their patients with MEFV mutations had RF positive while the same was true for 53% of the 80 patients who did not carry the MEFV mutations. On the other hand the association of more severe disease with being a carrier of MEFV mutations still persisted after correcting for the presence of RF. No analyses, however, were available for any specific mutations. A further and recent study showed that the frequency of MEFV mutations in rheumatic heart disease patients were not higher than in the general population (27). Similarly such mutations were not reported to be higher in gout (28). However, a significant association was found in patients with chronic obstructive pulmonary disease which typically runs a course of recurrent intervening infections (29).

Finally, it is interesting to note that while E148Q polymorphism has been associated with mild disease presentation in FMF (8).

These observations from the above cited studies, together with our preliminary finding of E148Q MEFV association with the presence of RF among the elderly, only suggest, at this stage, that different inflammatory pathways exist for different disease conditions.

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