Clinical and Experimental Rheumatology 2008; 26: 1091-1094.



# *MEFV* mutations in Japanese rheumatoid arthritis patients

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*Received on January 11, 2008; accepted in revised form on May 20, 2008.*



**Key words:** AA amyloidosis, familial Mediterranean fever, *MEFV*, rheumatoid arthritis.

*Competing interests: none declared.*

## ABSTRACT

**Objective.** *Familiar Mediterranean Fever (FMF) is common among Mediterranean populations, while other populations are rarely affected. The aim of this study was to assess the involvement of MEFV gene mutations among Japanese rheumatoid arthritis patients with or without amyloid A (AA) amyloidosis.*

**Methods.** *The frequency of the MEFV mutations, which were identified in Japanese FMF patients, was determined in 126 Japanese RA patients and 76 Japanese healthy subjects.*

**Results.** *The M694I mutation was not observed among RA patients and healthy subjects. Allele frequency of R408Q, P369S, E148Q, L110P mutations account respectively for 3.3%, 3.9%, 23.7%, 9.2% in healthy subjects and 5.6%, 6.7%, 24.2%, 9.5% in RA patients. The overall mutation rate was comparable between the RA patients and healthy subjects, as well as between the RA patients with and without amyloidosis.*

**Conclusions.** *This study shows the high prevalence of mutations of the MEFV genes in Japanese RA patients. However, our data suggest that the MEFV gene mutations may not be a genetic factor affecting the susceptibility of RA or the development of amyloidosis in a Japanese population.*

## Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive inherited inflammatory disease which is characterized by a recurrent episode of fever, peritonitis and arthritis (1). The gene responsible for the disease, *MEFV*, encodes a protein called pyrin or marenostrin (2). Pyrin is involved in inflammations through altered apoptosis, secretion of interleukin (IL)-1β and activation of the NF-κB pathway (3). Rheumatoid arthritis (RA) and FMF are both characterized by cytokine-mediated chronic inflammatory diseases, and AA amyloidosis may occur in both diseases, thus resulting in a fatal disorder (4). Recent reports have raised the possibility of the modifying effect of the *MEFV* gene on the expression of certain inflammatory diseases including RA (5). About 50 *MEFV* mutations have been associated with FMF, with the M694V, V726, M694I, M680I (in exon 10) and E148Q (in exon 2) mutations being the most frequently encountered types (1). The prevalence of FMF mutations among certain Arab populations has already been reported, but as far as we know, has rarely been reported in East Asia. In Japan, more than 20 cases with FMF have been reported to date, and *MEFV* gene mutations (L110P E148Q, R408Q, P369S, M69I) have been confirmed in these FMF patients (6-12). The E148Q mutation has been demonstrated in several inflammatory diseases (13, 14). Furthermore, E148Q was reported to be substantially over-represented in British and Indian patients with inflammatory arthritis complicated with AA amyloidosis (15). These findings suggest that this mutation is linked with the severity of inflammatory arthritis. In the present study, we investigated whether these *MEFV* mutations are associated with RA occurrence or RA-related AA amyloidosis in a Japanese population.

## Patients and methods

*Patients*

Seventy-six healthy subjects (41 men and 35 women) with no history of inflammatory joint diseases were recruited from blood donors living in Nagasaki province of Japan as controls. All control subjects were of Japanese ethnic origins. The mean age was 32.0±8.1 years. One hundred and twenty-six Japanese RA patients, who met the American College of Rheumatology (ACR) 1987 classification criteria for RA (16), were recruited consecutively from the outpatient clinic of Kumamoto Rheumatic Center and Nagasaki Medical Center. RA patients with the extra-articular manifestations such as recurrent attacks of fever and abdominal or thoracic pains were excluded from the study. All RA patients had been treated with at least one disease-modifying anti-rheumatic drugs (DMARDs; methotrexate, sulfasalazine, bucillamine, intramuscular and oral gold preparation). Some RA patients received glucocorticoids (prednisolone), immunosuppressive agents (cyclophosphamide, azathioprine) and

**Table I.** Characteristics of the RA patients.

| | Age | | RA duration | | CRP | |
|---|---|---|---|---|---|---|
| | No.of subjects | mean±SEM (years) | Gender (females/males) | mean±SEM (years) | RF (%) | mean±SEM (mg/dl) |
| RA patients with AA amyloidosis | 35 | 62.8 ± 9.9 | 27 / 8 | 13.9 ± 10.0 | 31 / 35 (88.6) | 3.6 ± 2.4 |
| RA patients without AA amyloidosis | 91 | 61.9 ± 14.1 | 76 / 15 | 12.5 ± 10.9 | 73 / 91(80.2) | 2.0 ± 1.9 |

anti-TNF-blockers (etanercept, infliximab). AA amyloidosis was diagnosed in 35 patients (8 men and 27 women, 62.8±9.9 yr) among these 126 RA patients. The diagnosis of AA amyloidosis was confirmed by histology based on the presence of Congo red staining, greenish birefringence on polarizing microscopy and anti-human amyloid A reactivity of tissue biopsy specimens. RA patients with AA amyloidosis exhibited clinical symptoms and signs associated with amyloidosis, including refractory diarrhea (21/35), renal manifestations, such as proteinuria and renal impairment (15/35), and cardiomegaly (7/35). All patients were diagnosed within the past five years. The remaining 91 RA patients (15 men and 76 women, 61.9±14.1 yr) showed no symptoms supposed to be AA amyloidosis, such as proteinuria, gastrointestinal symptoms, and cardiac involvement. The study protocol was approved by the Ethics Committees of the two institutes.

*DNA extraction*

Genomic DNA was isolated from whole blood using the QIAamp DNA blood protocol according to the manufacturer's instruction (Qiagen Ltd., UK).

*Genotyping*

As a first step, the PCR sequencing methods were used sequentially on only 40 patients. The coding region of *MEFV* gene was amplified by the PCR using Taq polymerase (Amplitaq: Perkin Elmer Cetus) and sequenced using Big Dye Terminator sequence chemistry and ABI310 sequencer. Four different mutations were detected by these methods (R408Q, P369S, E148Q, L110P). Next, the PCR restriction-digestion method was used for all patients and healthy subjects to detect these mutations. The M694I mutation was detected by the ARMS method. The polymerase chain reaction (PCR) amplification was conducted using the primers listed in Table II.

*Statistical analysis*

Results are expressed as mean±SD. Comparisons were made by Fisher's extract probability test and the $\chi^2$ test. All *p*-values were two-tailed, and *p*-values <0.05 were considered to indicate statistical significance.

## Results

Table I represents the clinical manifestations of RA patients with and without AA amyloidosis. We analyzed the genotype in these Japanese RA patients and the 76 healthy subjects. The *MEFV* allele encoding M694I was not identified among the healthy control population and RA patients (Table III). The overall allele frequencies of *MEFV* gene mutations (L110P, E148Q, P369S, R408Q) were listed in Table III. The mutation frequencies did not differ significantly among healthy subjects, RA patients without AA amyloidosis and RA patients with AA amyloidosis.

The distributions *MEFV* genotypes in RA patients with or without AA amyloidosis were presented in Table IV. Among RA patients without amyloidosis, 20.9% were heterozygotes, 2.2% were homozygotes and 33.0% were compound heterozygotes. Whereas 11.4% were heterozygotes, 8.6% were homozygotes and 20.0% were compound heterozygotes in RA patients with amyloidosis.

**Table II.** Primers and restriction enzymes used for mutational screening.

| Mutation | Primers | R.E | Profile (bands base pairs) Normal | Mutant |
|---|---|---|---|---|
| L110P | Forward:5'-AAACGTGGGACAGCTTCATC-3'<br>Reverse:5'-CGGCCAGCCATTCTTTCTCT-3' | SmaI | 430-170-133 | 333-170-133-97 |
| E148Q | Forward:5'-GCCTGAAGACTCCAGACCACCCCG-3'<br>Reverse:5'-AGGCCCTCCGAGGCCTTCTCTCTG-3' | BstNI | 157 | 93-64 |
| P369S | CGGGAAGCCTGAGC-3'<br>Reverse:5'-TTGGGAAAATGAAGTAAGGCCC-3' | SacI | 252 | 228-24 |
| R408Q | Forward:5'-CCCTGCCACAGTGTAAGC-3'<br>Reverse:5'-AGGCCCAGTGTGTCCAAGTG-3' | MspI | 207 | 111-96 |
| M694I | Common:5'-TATCATTGTTCTGGGCTC-3'<br>Normal:5'-CTGGTACTCATTTTCCTTC-3' | - | 184 | - |
| | Common:5'-TATCATTGTTCTGGGCTC-3'<br>Mutant:5'-CTGGTACTCATTTTCCTTT-3' | - | - | 184 |

RE; restriction enzyme.

**Table III.** Allele frequency of MEFV mutations in Japanese populations.

| Mutation | Control n=76(%) | RA patients without amyloidosis n=91(%) | RApatients with amyloidosis n=35(%) |
|---|---|---|---|
| M694I | 0 (0) | 0 (0) | 0 (0) |
| R408Q | 5 (3.3) | 12 (6.6) | 2 (2.9) |
| P369S | 6 (3.9) | 15 (8.2) | 2 (2.9) |
| E148Q | 36 (23.7) | 45 (24.7) | 16 (22.9) |
| L110P | 14 (9.2) | 18 (9.9) | 6 (8.6) |

**Table IV.** *MEFV* genotype frequencies in RA patients with or without amyloidosis.

| *MEFV* genotype | RA patients without amyloidosis n=91(%) | RA patients with amyloidosis n=35(%) |
|---|---|---|
| Heterozygotes | | |
| L110P/- | 0 (0) | 0 (0) |
| E148Q/- | 18 (19.8) | 4 (11.4) |
| P369S/- | 1 (1.1) | 0 (0) |
| R408Q/- | 0 (0) | 0 (0) |
| Homozygotes | | |
| E148Q/E148Q | 2 (2.2) | 3 (8.6) |
| Compound Heterozygotes | | |
| E148Q/L110P | 16 (17.6) | 5 (14.3) |
| E148Q/P369S | 1 (1.1) | 0 (0) |
| R408Q/P369S | 5 (5.5) | 0 (0) |
| E148Q/R408Q/P369S | 6 (6.6) | 1 (2.9) |
| E148Q/P369S/L110P | 1 (1.1) | 0 (0) |
| E148Q/R408Q/P369S/L110P | 1 (1.1) | 1 (2.9) |

There was no statistical significant difference in the frequency of *MEFV* genotypes between RA patients with and without AA amyloidosis.

## Discussion

Since the cloning of *MEFV* gene, 5 mutations have been discovered in Japanese FMF patients. These mutations observed in Japanese FMF patients are in exon 10 (M694I), in exon 2 (L110P, E148Q) and in exon 3 (P369S, R408Q) (6-12). Complex alleles were already seen in Japanese FMF patients fearing (E148Q/L110P), (E148Q/M694I), (E148Q/P369S/R408Q) (8-10, 12). Up-to-date genetic studies on *MEFV* have not made on the Japanese population, a population of interest. Since the population of Japanese is almost exclusively a single race and the frequency of AA amyloidosis, in which the connection of *MEFV* gene polymorphisms have been suggested (15), is relatively high. In Japan, AA amyloidosis has been reported to be found in 13.3% of RA patients by endoscopic biopsy (17). The difference in the frequency of AA amyloidosis among different races and the fact that AA amyloidosis is not consistently related to the severity of RA suggesting the contribution of genetic factors. SAA1 gene polymorphisms may participate in the development of AA amyloidosis in Japanese RA patients, in which SAA1.3 allele was increased and associated with the risk of AA amyloidosis (18).

In the present study, we investigated the genetic variations *MEFV* gene in Japanese healthy subjects and RA patients without the episodic manifestations consistent with FMF. Mutation analysis of the *MEFV* gene in Japanese RA patients showed an unexpectedly high frequency of mutations. However, the results of this study did not reveal a significant association between the presence of *MEFV* mutations confirmed in Japanese FMF patients, and the RA susceptibility as well as the development of AA amyloidosis. Previous findings have suggested that *MEFV* mutations affect the occurrence or presentation of chronic inflammatory diseases such as Behçet's disease and inflammatory bowel disease (13, 14). Booth *et al*. reported that E148Q variant may occur widely in Indian, Chinese and Caucasian British controls (15). They also reported that E148Q was substantially overrepresented in British and Indian patients with inflammatory arthritis complicated by AA amyloidosis suggesting that this variant might upregulate the inflammatory response (15). Rabinovich *et al*. demonstrated that RA patients carrying *MEFV* mutations, particularly the E148Q mutation had a higher RA severity score than the non-carrier group suggesting *MFFV* mutation is an modifier of the clinical manifestation of RA (5).

In this study, high frequencies of E148Q mutation were found among the Japanese healthy subjects; however, there was no significant difference in the frequencies of E148Q between the healthy subjects and RA patients as well as RA patients with amyloidosis in our study. Our findings suggest that the *MEFV* mutations may not be linked with the AA amyloidosis in Japanese RA patients. The discrepancy between previous findings and our results could be attributed to the different ethnic genetic background of the studied populations. One of the possible explanations is the heterogeneity of SAA1 genotype among different ethnic groups, which could result in a different frequency of AA amyloidosis. In Japanese RA patients, the frequency of the SAA1.3 allele was markedly increases in patients with AA amyloidosis (18). It is possible that these genetic factors other than *MEFV* mutations play a role in the association with AA amyloidosis in Japanese RA patients.

In conclusion, this study shows a high prevalence of *MEFV* gene mutations in Japanese population. However, our findings demonstrated no association between the presence of *MEFV* mutations and RA occurrence as well as RA-related AA amyloidosis.

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