MEFV gene mutations in familial Mediterranean fever phenotype II patients with renal amyloidosis in childhood: a retrospective clinicopathological and molecular study

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Abstract

Background. Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurring attacks of fever and serositis. The definition of the mutated gene has allowed molecular diagnosis of the disease. The most important complication of FMF is the development of AA type secondary amyloidosis. In a group of patients clinically designated as phenotype II amyloidosis patients, renal amyloidosis develops without being preceded by typical attacks of the disease. In this study, the mutations of the MEFV gene were analysed in a group of patients clinically recognized as phenotype II.

Methods. DNA samples were obtained from tissue samples of the subjects. PCR–RFLP methods were used to analyse the M694V, M680I, V726A and E148Q mutations that have been previously defined by us to be the most common mutations in our Turkish cohort.

Results. The distribution of the four most common mutations among phenotype II patients was 38% for M694V, 8% for M680I, 4% for V726A and 4% for E148Q.

Conclusions. In phenotype II amyloidosis patients, the distribution of the four common MEFV mutations was not significantly different from that found in all FMF patients with typical symptoms who do or do not develop amyloidosis. We therefore suggest that secondary genetic or environmental factors are operative in the development of secondary amyloidosis in patients with FMF.

Keywords: amyloidosis; childhood; familial Mediterranean fever; MEFV gene; phenotype II

Introduction

Familial Mediterranean fever (FMF) is an inherited inflammatory disease that is principally seen in Jewish, Armenian, Turkish and Middle-Eastern Arab populations. The disease is characterized by recurrent febrile episodes and inflammation in the form of sterile polyserositis [1]. Amyloid protein involved in inflammatory amyloidosis was named AA (amyloid-associated) protein and its circulating precursor was named SAA (serum amyloid-associated) [2]. Amyloidosis of the AA type is the most severe complication of the disease.

The gene responsible for FMF, MEFV, encodes a protein called pyrin or marenostrin and is expressed mainly in neutrophils [3,4]. To date, 29 mutations in the MEFV gene have been associated with the FMF phenotype [5].

The definition of the MEFV gene has permitted genetic diagnosis of the disease [1,6]. Nevertheless, as studies have unwrapped molecular data, problems have arisen with the clinical definitions of the disease. Patients with typical phenotypes who have been genetically confirmed to have mutations are defined as phenotype I, and those who have no phenotype but the required genotype are referred to as phenotype III patients [7]. To make things more complicated, papers from Turkey and Israel [2,8,9] have suggested another phenotype, II, where patients develop amyloidosis without any previous attacks typical of FMF.

The aim of this study is to confirm the presence of MEFV mutations in phenotype II patients. Establishing this will also be important for counselling families with phenotype II. Tissue samples from patients clinically accepted to have phenotype II disease were the materials of this study. Those samples were analysed for MEFV gene mutations (M694V, M680I, V726A and E148Q).
Subjects and methods

Materials

The clinical data of 150 consecutive case of tissue-diagnosed AA type amyloidosis were re-evaluated and analysed. AA type amyloidosis was confirmed by using the KMnO₄ test and immunoperoxidase staining with monoclonal anti-AA antibody [10]. Tissue samples from these patients had been collected over a 20-year period in the archives of the Nephropathology Unit, Hacettepe University. Among those patients, 25 were classified as phenotype II. Paraffin blocks from that group of patients—15 male, 10 female, between the ages of 6 and 18 years—were analysed for mutations in the MEFV gene. Our data is retrospective and dates from the 1970s and 1980s; thus, follow-up data was not available for some patients.

Mutation analysis

The DNA samples were isolated from paraffin-embedded tissue crossects [11]. Mutation analysis was performed by genomic DNA amplification. We searched for three mutations in exon 10 (M694V, M680I and V726A) and one mutation in exon 2 (E148Q).

The PCR was performed under the following conditions: 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and final extension at 72°C for 3 min. The amplified products were digested with restriction endonuclease enzymes to detect M694V, M680I, V726A and E148Q mutations (HphI, HinfI, AluI and BstNI, respectively).

Results

Clinical data on these patients were obtained from the records of Hacettepe University hospital. According to these files, all patients were Turkish and ranged between 6 and 18 years in age, their BUN levels varied between 8 and 150 mg/dl, none of them had an associated disease, all patients had presented with frank nephrotic syndrome and had proteinuria and oedema at presentation. All the studied material came from renal biopsies, and two were post-mortem specimens. Extra-renal amyloid deposits were not specifically sought.

At presentation, the renal function of six of the patients had deteriorated. Unfortunately, most were lost to follow-up. Those who were followed either developed end-stage renal failure or died of sepsis early in the follow-up period.

Mutations in both alleles were identified in nine of the 25 patients, three were homozygous for the M694V mutation and six were compound heterozygous for different combinations of M694V mutations (M694V/M680I, E148Q, V726A). In seven patients, no mutation could be detected. Mutation analysis showed that four missense mutations accounted for 54% of the 50 independent affected alleles. Six different genotypes were characterized in phenotype II patients (Table 1). The most common mutation was M694V (38%). Other mutations accounted for 16% of the alleles as shown in Table 2.

Discussion

According to previous studies, amyloidosis due to FMF occurs in 37% of Sephardic Jews, 27% of non-Ashkenazi Jews, 12% of Turks, 24% of Armenians, and 1–2% of Armenians living in the United States [12].

There is no close link between the severity of the inflammatory episodes and the occurrence of amyloidosis. Recent studies have compared the mutations found in patients with FMF-associated amyloidosis with those found in the general population of FMF patients. Amyloidosis was more frequent in the patients with homozygous M694V mutations than in the patients with other mutations in the MEFV gene [2,13]. Recently, Yağcınkaya et al. [14] described Turkish children with FMF and renal disease, all heterozygous for the V726A mutation, and Pras [15] reported a patient with amyloidosis who was homozygous for the V726A mutation. These results show that amyloidosis can and does occur in patients carrying other allels as well, and they suggest that the M694V mutation is an important factor in predicting the development of amyloidosis, or that it is associated with other factors or processes leading to amyloid development. The reasons underlying the regional or racial differences in the prevalence of amyloidosis are still unclear, but genetic factors may well play an important role. For example, the genetic disposition to convert SAA to amyloid fibrils (AA) is an important determinant of the rate of amyloid deposition [13]. We have recently found an association between the SAA1 gene polymorphism and the

| Table 1. Genotype distribution of the 25 cases with renal amyloidosis |
|----------------------|------------------|
| Genotype             | Patients (n)     |
| M694V/M694V          | 3                |
| M694V/M680I          | 2                |
| M680I/V726A          | 2                |
| M694V/E148Q          | 2                |
| M694V/u              | 9                |
| E148Q/u              | 1                |
| u/u                  | 9                |

u, Unidentified.

| Table 2. MEFV gene mutation frequency of the cases with renal amyloidosis |
|----------------------|------------------|
| Mutation             | Alleles (n)     |
| M694V                | 19/50            |
| M680I                | 4/50             |
| V726A                | 2/50             |
| E148Q                | 2/50             |
| Unidentified         | 23/50            |

n = 50 allele.
MEFV gene mutations in FMF phenotype II patients

susceptibility to AA amyloidosis in Turkish population with an increased relative risk of developing AA amyloidosis in FMF patients homozygous for the SAA1 gene polymorphism (unpublished data). Unfortunately, we could not screen the specimens used in this study for SAA1 gene polymorphism because the amounts of tissue samples were not sufficient.

The confirmation of phenotype II in patients with renal amyloidosis can be done by analysing the common MEFV mutations. One drawback of our work is that it was a retrospective study. The files were sufficient, however, and were thoroughly analysed for the necessary data.

In our previous study we analysed the most common MEFV mutations in Turkish FMF patients. These missense mutations accounted for 67.64% of the mutant alleles, and M694V (51.55%) was the most common mutation [16]. In our later study presented here, these missense mutations accounted for 54% of the mutant alleles and M694V (38%) was again the most common mutation. The allele frequencies of M680I, V726A and E148Q were 8, 4 and 4%, respectively. The distribution of the four common mutations among FMF phenotype II patients was not significantly different from that found in phenotype I patients (M694V 51.55%, M680I 9%, V726A 2.88% and E148Q 3.55%). The number of patients who developed amyloidosis was 16 in our phenotype I group and the majority of them were homozygous for the M694V mutation. In this study 47.7% of the alleles remain unidentified. Those alleles may be carrying rare mutations in the unscreened exons. Furthermore, in some patients the disease may not be linked to the known chromosome locus (16p13.3), as indicated by some previous studies [17,18].

This study is important, as it is the first one presenting MEFV gene mutation analysis results in renal amyloidosis patients who had carefully screened clinical files and biopsy reports. In this study we showed that the M694V mutation is the most common one in phenotype II patients. The majority of the other phenotype II patients reported to date, were also homozygous for the M694V mutation [2,19].

Six patients in our group were compound heterozygotes, all of whom had the M694V mutation in one allele. These results show that the M694V mutation is likely to be a risk factor for amyloidosis, since it can manifest itself not only in the homozygote but also in genetically compound states.

This study once again shows M694V to be the most common mutation in those developing secondary amyloidosis. Whether phenotype II patients are a distinct group with different modifying genetic factors, or simply have higher pain thresholds, are subjects for further study. This work also shows that in patients who have clinical phenotype II disease, the mutation analysis is similar to that of patients presenting with the typical phenotype of the disease. This fact further supports the concept that the phenotype or genotype in FMF do not necessarily predict the development of amyloidosis. Consequently, we once again suggest that the development of amyloidosis is associated with additional genetic or environmental factors.

References


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