The analysis of interleukin-1 receptor antagonist and interleukin-1β gene polymorphisms in Turkish FMF patients: do they predispose to secondary amyloidosis?

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ABSTRACT

Objective. *Amyloid development in familial Mediterranean fever (FMF) patients is associated with acute phase response and the acute phase reactant serum amyloid A which is induced by IL-1β. Its concentration can increase to more than 1000 fold during inflammation. In view of the inflammatory nature of FMF disease we have investigated whether IL-1β and IL-1 receptor antagonist gene polymorphisms may be involved in amyloid development in FMF patients.*

Methods. *Ninety-nine FMF patients without amyloidosis; 54 FMF patients with amyloidosis and 60 healthy controls samples were genotyped for IL-1β-511 (C/T) and IL-1β+3953 (C/T) polymorphisms using PCR-RFLP and for IL-1Ra VNTR polymorphism using PCR.*

Results. *The allele and genotype frequencies of IL-1β-511 (C/T), IL-1β+3953 (C/T) and IL-1Ra VNTR polymorphisms in FMF patients with and without amyloidosis were all compared with those in controls. There were no significant differences between FMF patients with and without amyloidosis and healthy control samples for these polymorphisms (all P-values are >0.05). These polymorphisms were not associated with M694V mutation in FMF patients with and without amyloidosis.*

Conclusion. *IL-1β-511 (C/T), IL-1β+3953 (C/T) and IL-1Ra VNTR polymorphisms are not associated with the development of amyloid in FMF patients.*

Introduction

Secondary amyloidosis is a serious and life threatening complication for patients with familial Mediterranean fever (FMF) (1). FMF is an autosomal recessive genetic disorder characterised by recurrent febrile episodes of fever and serosal inflammation manifested by sterile peritonitis, pleuritis and synovitis (2). The disease affects populations of eastern Mediterranean origin: Ashkenazi Jews, North African Jews, Armenians, Arabs, and Turks (3). Amyloidosis of the AA type, mainly renal, is the major complication of FMF (4, 5).

Amyloidosis is a protein misfolding disorder leading to the accumulation in tissues of protein aggregates in a fibrillar form having a cross-B sheet conformation (6, 7). The mechanisms of amyloid formation in systemic AA-amyloidosis are not fully understood but it is believed that continuously elevated serum amyloid A (SAA) levels in response to inflammatory diseases coupled with impaired catabolism of SAA leads to assembly of the partially degraded N-terminal fragment (AA) into protease resistant fibrils which are critical in the pathogenesis of amyloid development (8).

The gene causing FMF was cloned in 1997 and named MEFV (9, 10). The predicted protein was called pyrin (11). It is expressed in neutrophils and monocytes, and its expression is tightly regulated by cytokines (12). The role of pyrin has been speculated that wild type pyrin acts as a negative regulator of the inflammatory response (13).

IL-1β plays a crucial role in the pathogenesis of inflammation in autoinflammatory disorders such as FMF (14) IL-1β is a major mediator of fever and systemic inflammation (15). It also regulates inflammatory reaction and immune response through promoting expressions of other cytokines, such as IL-6 and IL-12 (16). These inflammatory cytokines are potent inducer of Serum Amiloid A

Table I. Sequences of the amplification primers, PCR conditions, PCR products and restriction endonuclease enzymes.

Polymorphisms	Primers	Tm	PCR Product (bp)	RE
IL-1B -511	F:5'-TGGCATTGATCTGGTTCATC-3' R:5'-GTTTAGGAATCTTCCCACTT-3'	55°C	190+114 or 304	Ava I
IL-1B +3953	F:5'-GTTGTCATCAGACTTTGACC-3' R:5'-TTCAGTTCATATGGACCAGA-3'	55°C	135+114 or 249	Taq I
IL-1Ra	F:5'-CTCAGCAACACTCCTAT-3' R:5'-TCCTGGTCTGCAGGTAA-3'	56°C	I:410, II:210 III:325, IV:500	VNTR

RE: Restriction endonuclease; VNTR: Variable Number Tandem Repeats.

Table II. IL-1Ra VNTR polymorphism genotype and allele frequencies in FMF patients with and without amyloidosis and healthy controls.

	FMF with amyloidosis (n=54)	FMF without amyloidosis (n=99)	Control group (n=60)
IL-1 Ra Genotype (p=0.598)			
1/1	30 (55.5)*	47 (47.4)	36 (60.0)
1/2	14 (25.9)	36 (36.3)	17 (28.3)
1/3	4 (7.4)	4 (4.1)	1 (1.7)
1/4	2 (3.7)	2 (2.1)	2 (3.3)
2/2	2 (3.7)	8 (8.1)	2 (3.3)
2/4	2 (3.7)	1 (1.0)	2 (3.3)
4/4	0	1 (1.0)	0
IL-1Ra alleles (P=0.395)			
1	80 (74)	136 (68.7)	92 (76.7)
2	20 (18.5)	53 (26.8)	23 (19.2)
3	4 (3.7)	4 (2.0)	1 (0.8)
4	4 (3.7)	5 (2.5)	4 (3.3)

*Parenthesis indicate the percentage of frequencies (%).

Table III. IL-1β -511 and IL-1β+3953 polymorphism genotype and allele frequencies in FMF patients with and without amyloidosis and healthy controls.

	FMF with amyloidosis (n=54)	FMF without amyloidosis (n=99)	Control group (n=60)
IL-1β-511 Genotype (p=0.763)			
C/C	18 (33.3)*	36 (36.4)	17 (28.3)
C/T	22 (40.7)	43 (43.4)	30 (50.0)
T/T	14 (26.0)	20 (20.2)	13 (21.7)
IL-1β-511 alleles (p=0.637)			
C	58 (53.7)	115 (58.1)	64 (53.3)
T	50 (46.3)	83 (41.9)	56 (46.7)
IL-1β+3953 Genotype (p=0.400)			
C/C	31 (57.4)	59 (59.6)	31 (51.7)
C/T	23 (42.6)	35 (35.4)	26 (43.3)
T/T	0	5 (5.0)	3 (5.0)
IL-1β+3953 alleles (P=0.598)			
C	85 (78.7)	153 (77.3)	88 (73.3)
T	23 (21.3)	45 (22.7)	32 (26.7)

*Parenthesis indicate the percentage of frequencies (%).

(SAA) expression by hepatocytes. Its concentration can increase to more than 1000-fold during inflammation (17).

Pyrin has been reported to be involved in the IL-1β pathway through interactions with apoptosis associated speck-like protein (ASC) (18). Pyrin, regulates caspase-1 activation and consequently IL-1β production through cognate interaction of its N-terminal PYRIN motif with the ASC adaptor protein (18).

We hypothesized that IL-1β, -511 C/T, +3953 C/T and IL-1Ra VNTR gene polymorphisms may have an association with the development of amyloidosis in FMF patients.

Materials and methods

Patients

Our study group was consisted of 60 (M/F:33/27) healthy controls (mean age: 50.2±5.9) and 54 (M/F:33/21) FMF patients with amyloidosis (mean age 18.02±0.60) and 99 (M/F:59/40) FMF patients without amyloidosis (mean age 21±0.70). The mean age and the proportion of treated patients were similar in the patients groups with and without amyloidosis. All of the patients are followed up at Hacettepe University, Department of Pediatric Rheumatology and Nephrology in Ankara. All had typical clinical features of FMF and fulfilled the Tel Hashomer criteria for FMF. MEFV gene mutations were analysed by Vienna Lab Strip Assay. Amyloidosis was diagnosed with a renal biopsy in all, demonstrating apple-green birefringence under a polarized light microscope after Congo Red Staining. Informed consent for genetic analysis was obtained from each of the patients and control group prior to the study.

Methods

Genomic DNA was extracted from EDTA anticoagulated peripheral blood according to a standard method. The genotypes of the ILRA VNTR polymorphism was identified by PCR and IL-1B -511, IL-1B +3953 polymorphisms were identified by polymerase chain reaction (PCR) followed by AvaI and TaqI RFLP analysis respectively. PCR was carried out in a total volume of 50 ul, containing genomic DNA, 2 pmol of each primer, 1X Taq DNA polymerase buffer, 1.5 mM $MgCl_2$ and 0.3 units of Taq DNA polymerase (Promega USA). The primers for IL-1β-511, IL-1β+3953 and IL-1Ra VNTR polymorphisms and PCR conditions are listed in Table I.

Statistical analysis

Statistical analysis was performed with SPSS 15 for windows. The allelic frequency distributions of the polymorphisms in the control group and FMF patients with and without amyloidosis were compared by the chi-squared test. A p-value of <0.05 was considered significant.

Results

The mean age of the FMF patients was 21±0.7. All the patients who developed amyloidosis had high CRP levels, at least ten-fold. The acute phase reactant of the patients varied in a wide range. Within each study group, the genotype distributions were consistent with those predicted by the Hardy-Weinberg equilibrium (Table II and Table III). For all three polymorphisms, there was no significant difference in genotype distribution between the FMF patients with amyloidosis, without amyloidosis and healthy controls. Also, these polymorphisms were not associated with M694V mutation in FMF patients with and without amyloidosis (data not shown).

Discussion

Clinical features of FMF are variable from a patient to another (3). More than 100 mutations have been identified on MEFV (19) and these mutations correlate with the severity of disease, within these mutations, M694V mutation is widely believed to introduce a major risk of amyloidosis (20, 21). It is not exactly known why some patients develop amyloidosis, whereas others do not. Our group previously investigated the SAA1 α/α genotype as a genetic factor which confers a significant risk for amyloidosis in FMF patients (21). Neither SAA1 nor SAA2 genotypes had a significant effect on SAA level (22) which demonstrates that other disease modifying effects may be existing for FMF.

In the present study, we aimed to determine a possible effect of the interleukin-1 gene polymorphisms on the development of secondary amyloidosis in FMF patients. IL-1 is an important mediator of the inflammatory response and is considered to contribute to several inflammatory diseases (23, 24). In the case of IL-1 mediated immune response, the pro-inflammatory response is downregulated by IL-1Ra (25). The balance between IL-1 and its competitive antagonist IL-1Ra may contribute to various inflammatory diseases (26).

IL-1 gene polymorphisms affect serum IL-1β level and certain reports demonstrated that these polymorphisms are associated with several inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, primary Sjögren's syndrome, systemic lupus erythematosus and Behçet's diseases (26-30). In the Turkish population, IL-1β+3953 allele 2 was reported to be increased in rheumatoid arthritis and Behçet's diseases patients in two separate studies (26, 27). There are controversial reports for the possible function of alleles of IL-1β and IL-1Ra (27). Some studies found an association between these polymorphisms and serum IL-1 levels (31, 32) but others found no affect (33, 34). The results of several studies suggest that caspase-1 which has been reported to be another pyrin interacting protein, is essential in processing of pro-IL-1β (14, 18). Thus we hypothesized that an association might exist between these polymorphisms and amyloid development in our patients which might lead up to an inbalance in the serum level of inflammatory cytokines. This would predispose these patients may have a tendency to develop amyloidosis due to an increased baseline of inflammation. However, in our study, neither IL-β gene polymorphisms; IL-1β-511, IL-1β+3953, nor IL-1Ra genotypes, showed an association with secondary amyloidosis development in FMF patients. The allele frequencies of these polymorphisms in our Turkish control group were strongly correlated with the results of another study from Turkey which confirms our data (27). We next examined whether these polymorphisms had an additive synergistic effect on amyloid development in the presence of a severe MEFV gene mutation, M694V but they were not associated with amyloidosis seen in M694V homozygote FMF patients.

However, some limitations of this study should be considered. The polymorphisms studied do not extensively cover the IL-1beta gene; therefore, the possibility that other polymorphisms and functional variants might show association with amyloidosis in FMF patients cannot be excluded. Another limitation of this study is the low sample size of FMF patients with amyloidosis for this kind of a polymorphism study. Testing these polymorphisms on a larger sample and in other populations will allow more accurate results and will lead to more definite conclusions. However, larger number of patients with amyloidosis is rather difficult since, colchicine, the drug of choice in FMF affected patients, is effective in preventing both the acute attacks of FMF and the development of amyloidosis. Furthermore, these observations can not exclude a weak effect of IL-1 hidden by a stronger effect of MEFV or SAA1 genotypes or a possible varying effect of IL-1 on the course of amyloid development in different populations which could be expected in the case of genes that have minor modifying effects on a disease phenotype.

Familial Mediterranean fever is a clinically heterogeneous disease. The factors governing amyloidogenesis in FMF remain, as in many other genetic diseases, complex and multiple. Many factors other than MEFV and SAA1 genotypes related with the course of amyloid development remain to be elucidated.

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