Arg753Gln TLR-2 Polymorphism in Familial Mediterranean Fever: Linking the Environment to the Phenotype in a Monogenic Inflammatory Disease

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ABSTRACT. ***Objective.*** Familial Mediterranean fever (FMF) is an autoinflammatory disease common in eastern Mediterranean populations. The most severe complication is the development of secondary amyloidosis. Toll-like receptor (TLR-2) plays a critical role in linking the recognition of microbes to immune activation. We investigated whether the Arg753Gln TLR2 polymorphism affected the development of secondary amyloidosis in patients with FMF.

Methods. We studied 75 patients with FMF, 40 patients with FMF who developed secondary amyloidosis, and 116 healthy controls. TLR2 gene Arg753Gln mutations were analyzed with a polymerase chain reaction-restriction fragment length polymorphism method.

Results. The frequency of the Arg753Gln TLR2 polymorphism among the Turkish population was 6%, whereas it was 25.2% among patients with FMF ($p < 0.01$). The difference of the frequency of the polymorphism between FMF patients with and without amyloidosis was significant: 15/40 (37.5%) and 14/75 (18.6%), respectively ($p = 0.02$).

Conclusion. The Arg753Gln polymorphism may affect the severity of this monogenic disease by influencing the innate immune response to pathogens. The presence of the polymorphism may influence the phenotype of FMF in geographic areas where bacterial insult is more common. (First Release Oct 1 2006; J Rheumatol 2006;33:2498–500)

Key Indexing Terms:
TLR2 POLYMORPHISM FAMILIAL MEDITERRANEAN FEVER AMYLOIDOSIS

Familial Mediterranean fever (FMF) is the most common of the monogenic autoinflammatory diseases caused by mutations in the MEFV gene[1,2]. The protein product is pyrin. The mutated pyrin is thought to result in overproduction of interleukin 1 (IL-1) and activation of nuclear factor-κB (NF-κB), with ensuing inflammation[1,2]. FMF is mainly a disease of the innate immune system.

Toll-like receptors (TLR) are important agents of the innate immune recognition of pathogens[3,4]. Stimulation of TLR by microbes initiates a signaling cascade involving a number of proteins including IL-1 receptor-associated kinase[3]; this signaling cascade then leads to NF-κB activation that induces the secretion of proinflammatory cytokines[5]. Among the TLR, TLR-2 is a key innate-immunity receptor for sensing both endogenous inflammatory mediators and ligands of several microbes[5-7]. Diverse pathogens activate cells through TLR-2 and suggest that this molecule is a central pattern recognition receptor in host immune responses to microbial invasion. Further, even heat shock protein-60 has been reported to activate cells through TLR-2, suggesting that TLR-2 may be an inducer of specific defense processes, including oxidative stress, initially stimulated by microbial compounds[4].

A single nucleotide polymorphism (SNP) of Arg753Gln in TLR-2 has been implicated in a number of infectious diseases, such as tuberculosis, as well as in atopic dermatitis[7]. Interestingly, this polymorphism has been associated with a risk for coronary restenosis in atherosclerotic patients[8].

The intriguing features of FMF include the enhanced inflammation in patients. It has been hypothesized that the enhanced inflammatory response was the reason for the selection of the MEFV mutations among the first settlers of the eastern Mediterranean, where the ancestors of populations with FMF come from[9,10]. Another feature is that only a portion of patients of FMF develop secondary amyloidosis, the severe complication of intense inflammation, whereas others do not, even without colchicine. Finally, the reason secondary amyloidosis is more frequent among the people living in the

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eastern Mediterranean, compared to the same ethnic groups living in the US, remains unresolved[11].

Our hypothesis was that patients with FMF, or clinical symptoms of FMF, were more prone to develop intense immune responses because of their SNP in the innate immune system. We investigated the Arg753Gln TLR2 polymorphism that was implicated in functional alterations and recently studied in atherosclerotic disease[8]. We report the frequency of this polymorphism of TLR2 in patients with FMF and amyloidosis.

MATERIALS AND METHODS

The study included 40 patients with secondary amyloidosis due to FMF and 75 patients with FMF who had not developed amyloidosis, a total of 115 patients. The latter group was taking colchicine treatment, although there were occasional patients with disease symptoms whose diagnosis had been delayed for more than 5 years. The 40 patients who had developed secondary amyloidosis had developed this complication either because they had not been diagnosed as having FMF and hence had not received colchicine, or because they had not been compliant with the colchicine treatment.

All FMF patients were diagnosed with the typical features of recurring fever and serositis accompanied by acute-phase inflammation. All the patients who were genotyped in this study had 2 identified MEFV mutations. M694V was the leading mutation among patients both with and without amyloidosis. The patients with FMF who had developed amyloidosis had the following mutations: M694V (66.6%), V726A (7.8%), M680I (25.4%), and E148Q (1.9%). FMF patients who had not developed this complication had M694V (44%), V726A (9.3%), M680I (18.6%), and E148Q (16%), and the remaining patients had the rare mutations associated with the disease.

All patients with amyloidosis were diagnosed by kidney biopsies because of proteinuria. All had confirmed amyloid deposits in the kidney tissue.

The control group consisted of 116 healthy controls.

TLR2 genotyping. TLR2 gene Arg753Gln mutations were analyzed from genomic DNA extracted from peripheral blood leukocytes using restriction fragment length polymorphism-polymerase chain reaction methods, and mutations were confirmed with a direct DNA sequencing method. The TLR2 gene Arg753Gln polymorphism was genotyped as reported[12].

Statistical methods. Data analysis was performed using SPSS 11.0 for Windows. Chi-square analysis or Fisher's exact test was used when appropriate. Results were corrected for multiple comparisons. A p value < 0.05 was considered statistically significant. The observed genotype distribution and allele frequencies were compared with the expected Hardy-Weinberg distributions by chi-square analyses.

RESULTS

The frequencies of TLR2 genotypes in 2 study groups were found in accord with those expected by the Hardy-Weinberg equilibrium.

Among 116 healthy controls, 7 (6.0%) had the Arg753Gln polymorphism, whereas 29 of 115 patients (25.2%) carried the polymorphism, a significant difference ($p < 0.001$). The polymorphism was present among 15/40 (37.5%) of the FMF patients with and 14/75 (18.6%) of those without amyloidosis (Table 1); again the difference was significant ($p = 0.02$). The odds ratio for developing amyloidosis with the Arg753Gln polymorphism was 2.02-fold.

There was a group of 15 adult patients who had not been diagnosed with FMF until they were adults, yet they did not develop amyloidosis despite lack of treatment. Among these 15 patients only one had the Arg753Gln polymorphism (6.6%), which is similar to the frequency of the general population.

Table 1. Arg753Gln polymorphism in the TLR2 gene.

	n (%)
Healthy controls, n = 116	7 (6.0)
FMF without amyloidosis, n = 75	14 (18.6)
FMF with amyloidosis, n = 46	15 (37.5)

The number of patients in each genotype group was not sufficient for analysis of correlations.

DISCUSSION

This is the first study suggesting that a genetic variation in the microbe recognition pathway may link an environmental factor(s) to the phenotype of a monogenic disease, affecting the severity of the disease expression.

Innate immunity is the first-line host defense of multicellular organisms that operates rapidly to limit infection upon exposure to infectious agents[13]. TLR induce expression of inflammatory cytokines, participating in the innate response and signaling the activation of adaptive immunity[3-5]. The frequency of the studied polymorphism is quite low among healthy controls in Western populations, ranging between 1% and 9%[6,14,15]. The frequency among our controls was 6%; this slight difference may be attributed to ethnic variations.

The Arg753Gln polymorphism has been associated with some infectious agents in preliminary studies; e.g., it has been suggested to predispose individuals to life-threatening bacterial infections, especially staphylococcal infections[6,7]. However, in one recent large study this association was not present[16]. Although TLR-2 may not play a central role in acute infections, it may be important in chronic inflammatory diseases. It has been hypothesized that these SNP may be more important in cases where commensal or low numbers of bacteria interact with the host to cause chronic inflammation, such as in Crohn's disease, associated with a variation in NOD2, and atherosclerosis[7]. A study from Germany assessed the effect of this SNP in restenosis in atherosclerotic patients[8]; the authors suggested that TLR were involved in the development of atherosclerosis by mediating inflammation, and indeed they found an increased frequency of the TLR2 Arg753Gln SNP among patients with restenosis compared to those who did not have restenosis ($p = 0.013$).

Berdeli, *et al*[12] have shown that this TLR2 polymorphism provokes the development of acute rheumatic fever: the Gln allele was 16 times more frequent among patients with acute rheumatic fever compared to healthy controls, whereas the Arg753Arg genotype was significantly decreased (OR 15.6, 95% CI 7.87–30.8, $p < 0.0001$)[12]. On the other hand, Sanchez, *et al*[15] have hypothesized that the Arg753Gln polymorphism of TLR2 may contribute to susceptibility to systemic lupus erythematosus and adult rheumatoid arthritis, since the TLR

response to bacteria could be involved in triggering lymphocytes. However, they failed to observe an association.

Colchicine treatment prevents the development of amyloidosis. Our unpublished observations and a report[11] showing the lack of amyloidosis among patients with FMF in the US suggest that the disease seems to have a more severe phenotype in the eastern Mediterranean. This suggests that an environmental factor in this area may be affecting the inflammatory pathway in this monogenic disease. Perhaps it was not just the pathogen but the response pattern to the pathogen(s) that made the difference. We believed the Arg753Gln TLR2 polymorphism was a good candidate to analyze since it is a key molecule for sensing both microbes and endogenous mediators that in turn initiate the inflammatory pathway through IL-1 and NF-κB, important molecules for the pyrin pathway as well[1,2]. Thus we would suggest that the SNP within TLR2 affects the course of FMF. Patients carrying this SNP seem to be more prone to develop amyloidosis, and this may be a contributing factor for the development of this severe complication. It is interesting that among the patients who had milder disease (late diagnosis), the frequency was lower than that of the remaining patient population.

There are no reports suggesting the association of infections and the occurrence of amyloidosis to directly support our hypothesis, although a well-planned study may do so. Nevertheless, TLR-2 has been involved in endogenous responses as well, such as its response to heat shock proteins[4]. Bornstein, *et al* have described an important role for TLR-2 in the adrenal stress response[17]. In TLR-2-deficient mice there was an impaired adrenal corticosterone release along with markedly reduced intraadrenal expressions of IL-1, IL-6, and tumor necrosis factor-α following lipopolysaccharide treatment[17]. This report also sheds light on the association of FMF attacks and elevated acute phase response with stress.

The mutations in the MEFV genes were selected in biblical times among the first settlers in the Eastern Mediterranean Basin. While conferring on them certain advantages with their fight against the microbes, these mutations also rendered these people more susceptible to inflammation[9,10]. Other genetic polymorphisms in the microbe-recognition pathways may have an influence on the final phenotype. The presented TLR2 polymorphism may prove to be useful in defining the risk of amyloidosis. It is tempting to speculate that this is an important link defining the severity of the disease in this area, where the common microbes are more frequently encountered than in Western countries.

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