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Arg753Gln polymorphism of the human Toll-like receptor 2 gene from infection to disease in pediatric tuberculosis

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ABSTRACT

The aim of this study is to examine the occurrence of the Arg753Gln polymorphism of the Toll-like receptor 2 (TLR2) gene in Turkish children with pulmonary and/or extrapulmonary tuberculosis (TB) disease compared with that in healthy children with latent TB infection (LTBI) and to assess the risk of progression from LTBI to active TB disease in children. The Arg753Gln polymorphism of the TLR2 gene was studied in 198 TB patients compared with 200 ethnically and age-matched children with LTBI. The culture confirmed TB patients were more frequently Arg753Gln heterozygous [odds ratio (OR) 5.05, 95% confidence interval (95% CI) 2.61–9.76, $p = 0.00$], and Gln allele frequency was significantly higher in the patient group (13.86% vs 3.5%, OR 4.40, 95% CI 2.34–8.30, $p = 0.00$). We also showed that the frequencies of the heterozygous Arg753Gln genotype and the Gln allele were significantly higher in patients with pulmonary TB alone and in patients with definitive pulmonary plus extrapulmonary TB than in children with LTBI. Our data suggest that the Arg753Gln polymorphism of the TLR-2 gene influences the speed of progression from infection to TB disease in children. Further investigations are needed to clarify whether this polymorphism has a strong impact on susceptibility to TB in children.

1. Introduction

Tuberculosis (TB), primarily caused by the pathogenic bacterium *Mycobacterium tuberculosis* (MTB), continues to be an enormous public health problem despite the widespread use of BCG vaccine, multidrug therapy, and national and international TB control programs. Almost a third of the world's population is infected with MTB [1]. The rate of progression from infection to disease is highly variable, and ≈90% of infected individuals never develop clinical disease. Of the 10% of MTB-infected persons who do develop clinically overt disease, approximately half will be diagnosed within <2 years of infection and are considered fast progressors of TB disease. This so-called primary TB disease is particularly common among children, and most pediatric cases present with primary TB disease. TB patients who progress more slowly from infection to TB disease and develop clinical disease >2 years after infection are referred to as "reactivation" cases. Little is known about the mechanisms that influence the rate of progression from infection to disease [2]. The evidence suggests that genetic factors may be important determinants of increased susceptibility to progressive disease development [3–5]. The majority of genetic studies have investigated adult pulmonary TB cases, and few have focused on pediatric TB disease. The exclusion from more intense study of primary TB disease is surprising, because approximately half of all TB patients are thought to represent primary disease [6]. Contrasting the results of primary and reactivational disease studies will provide a better understanding of the mechanisms that govern progression from infection to disease in 2 distinct stages of TB [2].

Mouse and human studies have demonstrated the importance of Toll-like receptor (TLR) signaling in host defense. TLR represents a primitive host defense mechanism against bacteria, fungi, and viruses and plays a major role in pathogen recognition and initiation of inflammatory and immune responses [7]. At least 10 mammalian TLRs are involved in the recognition of various bacterial lipoproteins belonging to *Borrelia, Mycoplasma*, MTB, and other bacteria. TLR2 is a principal mediator of macrophage activation in response to mycobacteria [8].

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide [adenine (A), thymine (T), cytosine (C), or guanine (G)] in the genome sequence is altered. Many SNPs have no effect on cell function, but some, especially if they affect the function of the gene, could predispose

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people to disease or influence their response to a drug [9]. SNPs in the TLR2 genes have been shown to be associated with suppressed macrophage response to mycobacteria in both animal and human studies. In a comparison of the resistance to airborne infection with MTB in TLR2, TLR4, CD14 knockout, and control mice groups, Reiling et al. found that TLR2 knockout mice showed decreased resistance to TB on high-dose exposure [10]. The Arg77Trp polymorphism of TLR2 is associated with pulmonary tuberculosis in Tunisian patients [11], whereas the Arg753Gln polymorphism of TLR2 is associated with tuberculosis in Turkish patients [1].

Pediatric and adult TB differ markedly in epidemiologic features (2 distinct peaks of incidence), clinical appearance (disseminated vs pulmonary disease), and pathogenesis (primary infection vs reactivation). These differences likely reflect differences in immunologic and genetic control [12]. Investigation of the effects of susceptible genes on pediatric TB will provide additional information about the molecular genetic mechanisms involved in pediatric TB development and also aid in the establishment of more efficient ways to prevent TB spread. This additional information will be particularly relevant for decreasing adult TB developed from latent childhood TB infection [13]. Although there is growing evidence of the role of candidate genes from the host immune response pathway to MTB in adults, data from children are scarce. Further studies of tuberculosis genetics in well-defined pediatric populations are needed.

The aim of this study is to examine the occurrence of the Arg753Gln polymorphism of the TLR2 gene in Turkish children with pulmonary and/or extrapulmonary TB disease compared with that in healthy children with latent TB infection (LTBI) and to assess the risk of progression from LTBI to active TB disease in children.

2. Subjects and methods

2.1. Study subjects

This study was conducted at Sisli Etfal Training and Research Hospital and Sureyyapasa Chest Diseases and Thoracic Surgery Teaching Hospital in Istanbul, Turkey, the country's 2 largest referral centers. The study consisted of 198 immune-competent Turkish children with pulmonary and/or extrapulmonary TB in the study group and 200 healthy children with LTBI in the control group. The control patients were matched with the TB patients by age, sex, and ethnicity. There were no significant differences between the groups for age and sex ($p > 0.05$).

The patients were categorized according to the certainty of their TB diagnosis. Those categorized as definite TB had a diagnosis confirmed by culture of MTB from sputum or gastric aspirate, bronchial lavage, and/or pleural fluid as well as samples from organ systems other than the lung (urine, cerebrospinal fluid, etc.) or biopsy material. Highly probable TB had acid-fast bacilli identified in sputum, gastric aspirate, cerebrospinal fluid, or other body fluids or on histologic examination of biopsy material resembling the appearance of TB-affected tissue (granulomatous tubercles or caseous necrosis) but with no culture confirmation having been obtained. Probable TB was diagnosed according to World Health Organization criteria for suspecting TB in children [14]. The clinical and microbiological results of all patients were reviewed by 2 pediatricians with experience in childhood TB, and only patients whose findings and response to treatment were consistent with TB were retained in the study. The nutrition status of patients was classified according to the *z* score and the World Health Organization tables for developing countries [15]. Patients with a *z* score of −3 or lower were considered to have malnutrition and were excluded from the study. Patients over 15 years of age, HIV seropositive, or with other underlying immunodeficiency or those who had been on prolonged treatment with steroids or other immunosuppressive drugs were also excluded from the study, as well as those from different ethnic backgrounds.

The control group consisted of 200 children with LTBI. The control patients were healthy, asymptomatic, had no history of TB and specific antimycobacterial treatment, had normal radiographic examination findings, had positive purified protein derivative skin test, and/or had a history of household contact with an index case. All controls were from the same ethnic and geographic origin and lived in the same city as the TB cases.

Demographic information was collected from individuals using a short questionnaire. Only Turkish subjects were included in the study; therefore, the study sample was composed totally of Caucasians (Turkish), without any other ethnicities (African or Asian).

The study design was approved by the institutional ethics committee. After written informed consent was obtained from each child's parent, blood samples were collected from the patients and controls in EDTA vials, and their clinical details were recorded. All samples ($n = 398$) were stored at 4°C before the analyses, which were performed in accordance with the principles of the Declaration of Helsinki.

The Arg753Gln polymorphism of TLR2 was investigated in patients with TB and in controls at the Institute of Forensic Medicine, Ankara University.

2.2. Genomic DNA preparation

Genomic DNA was extracted from EDTA anticoagulant peripheral whole blood samples using the QIAmp blood DNA mini-kit (Qiagen, Hilden, Germany) according to the method recommended by the manufacturer. DNA concentration was determined using the PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR) according to the manufacturer's instructions.

2.3. Determination of Arg753Gln polymorphism of TLR2 by restriction fragment length polymorphism

TLR2 gene Arg753Gln polymorphism was genotyped by the method previously reported by Schroder et al. [16]. To screen for Arg753Gln polymorphism of TLR2, a 341-bp fragment was amplified by polymerase chain reaction (PCR) using the following primers: forward: 5′-GCCTACTGGGTGGAGAACCT-3′ and reverse 5′-GGCCACTCCAGGTAGGTCTT-3′.

Amplification was carried out on a Techne Tc 512 PCR System in a 50-μl reaction mixture containing 200 μM dNTPs, 20 pmol each of forward (F) and reverse (R) primers, 1 U Hot Star Taq DNA polymerase (Qiagen), 1× PCR buffer (Qiagen), and 100 ng genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. Then, the PCR product (341 bp) was digested with *Aci*I (New England Biolabs, Hertfordshire, UK) and incubated at 37°C for 2 hours. The PCR products were separated on a 2% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned, and photographed using the Syngene Monitoring System (Fig. 1). Digestion of PCR product by *Aci*I yields 228-, 75-, and 38-bp fragments for the presence of the G allele and 266- and 75-bp fragments for the presence of the A allele.

2.4. Statistical analysis

Statistical analyses were carried out using SPSS software, version 16.0 (SPSS, Chicago, IL). Demographic and clinical data are expressed as means ± SD or a percentage. The Student *t* test was used for the comparison of numeric variables within groups. The overall genotype distributions and allele frequency at the 753 codon of the patients and controls were compared. Genotype distributions and allele frequencies between groups were compared

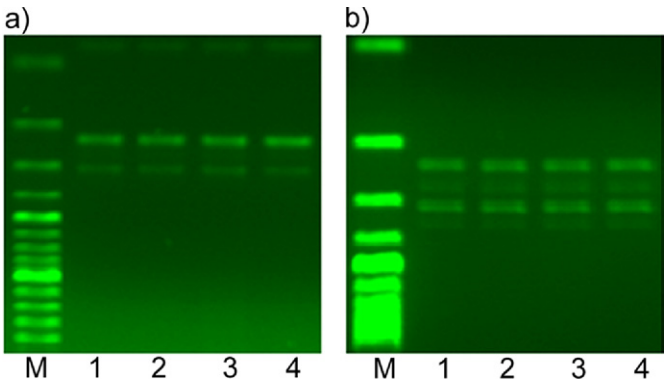

Fig. 1. Representative agarose gel image of digested PCR products with *AciI*: (M: 100-bp ladder). (a) Lanes 1 to 4: Arg753Arg, GG (normal) genotype. (b) Lanes 1 to 4: Arg753Gln, GA (heterozygous carrier of polymorphism) genotype. The upper bands represent the 470-bp internal control PCR fragment and the lower bands the G (lane 1) and A (lane 2) alleles.

using 2 × 2 contingency tables, Pearson χ^2, Yates χ^2, or Fisher's exact tests, as appropriate. Odds ratios (ORs) with 95% confidence intervals (95% CI) were also calculated for these comparisons; $p < 0.05$ was considered statistically significant. Allele and genotype frequencies among controls were compared with values predicted by Hardy–Weinberg equilibrium using the Haldane exact test.

3. Results

Of the 198 study patients, 104 (52.5%) were female and 94 (47.5%) were male. The mean age ± SD was 100.42 ± 57.91 months. The control group consisted of 108 (53.9%) female subjects and 92 (46.1%) male subjects with a mean age ± SD of 102.21 ± 54.65 months.

Pulmonary TB was diagnosed in 124 (62.6%) cases, central nervous system TB in 11 (5.5%), TB lymphadenitis in 10 (5.1%), abdominal TB in 9 (4.5%), miliary TB in 8 (4%), bone TB in 6 (3%), renal TB in 6 (3%), and TB pericarditis in 2 (1%). Twenty-two patients (11.1%) had both pulmonary TB and extrapulmonary TB (Table 1).

A total of 138 patients were confirmed to have definitive TB by the isolation of MTB from sputum ($n = 20$), gastric aspirates ($n = 50$), bronchial lavage ($n = 23$), or other sources (bone, $n = 3$; pleural effusion, $n = 15$; cerebrospinal fluid, $n = 8$; peritoneal fluid, $n = 5$; urine, $n = 9$; pericardial biopsy, $n = 3$; lymph node biopsy, $n = 2$). Fifteen of 60 patients with probable TB had a histological diagnosis (bone, $n = 3$; lymph nodes, $n = 12$; pleural biopsy, $n = 2$; pericardial biopsy, $n = 1$; renal biopsy, $n = 2$). Thirty-seven of 138 culture-confirmed patients exhibited positive acid-fast staining initially (sputum/bronchial lavage, $n = 25$; gastric aspirates, $n = 6$; cerebrospinal fluid, $n = 3$; urine, $n = 1$; pleural effusion, $n = 1$; peritoneal fluid, $n = 1$).

Using blood samples from children with TB and the infected control group, we tested whether an association exists between TB and Arg753Gln polymorphism in the TLR2 gene. The findings are summarized in Tables 2 and 3. The results have shown that the Arg753Arg genotype was significantly decreased in the entire group of definitive TB cases when compared with controls (OR 0.20, 95% CI 0.10–0.38, $p = 0.00$). The definitive TB patients were also found to be more frequently Arg753Gln heterozygous (OR 5.05, 95% CI 2.61–9.76, $p = 0.00$). No definitive TB patients and no children in the control group were homozygous carriers of the Gln753Gln polymorphism. In addition, Gln allele frequency was significantly higher (13.8% vs 3.5%, OR 4.40, 95% CI 2.34–8.30, $p = 0.00$), but Arg allele frequency was significantly lower (86.2% vs 96.5%, OR 0.23, 95% CI 0.12–0.43, $p = 0.00$) in patients with definitive TB compared with controls. There were no significant differences between the probable TB and control groups in genotype distribution and allele frequency ($p > 0.05$).

Because each form of TB may also be considered a different phenotype, the TB disease manifestation was classified as pulmonary in 124 (62.6%), extrapulmonary in 52 (26.3%), and mixed pulmonary and extrapulmonary in 22 (11.1%) cases; further analysis was performed between the TB patients who had different types of TB and the infected control group (Table 3).

The pulmonary TB group consisted of 61 (49.2%) males and 63 (50.8%) females with a mean age ± SD of 105.58 ± 56.54 months. Ninety-five of 124 pulmonary TB patients were confirmed to have definitive TB by the isolation of MTB from their samples. The heterozygous (Arg753Gln) genotype was detected in 25 (26.3%) definitive pulmonary TB patients and 14 (7%) controls, respectively, whereas the allelic frequency for Gln was 13.2% ($n = 25$) versus 2.8% ($n = 11$) in patients with definitive pulmonary TB and infected controls. When a χ^2 test was performed for evaluation of the differences in the ratio of the Arg753Gln genotype between the 2 groups, it was found to be significantly associated with TB. The odds ratios for the Arg753Gln genotypes for predisposition to TB was 4.74 (95% CI 2.33–9.65) in the definitive pulmonary TB group. The results also showed that the Arg753Arg genotype was significantly decreased in the entire group of definitive TB cases compared with the controls (OR 0.21, 95% CI 0.10–0.43, $p = 0.00$). In addition, Gln allele frequency was significantly higher (13.2% vs 2.8%, OR 5.36, 95% CI 2.58–11.14, $p = 0.00$), but Arg allele frequency was significantly lower (86.8% vs 97.2%, OR 0.19, 95% CI 0.90–0.39, $p = 0.00$) in patients with definitive pulmonary TB compared with controls.

Table 1
Characteristics of tuberculosis (TB) patients and control groups in the study

	Controls ($n = 200$)	Definitive tuberculosis (TB) ($n = 138$)	Probable TB ($n = 60$)
Age (mean months ± standard deviation [SD])	102.21 ± 54.65	97.35 ± 58.62	103.27 ± 55
Sex	92 (46.1%)	67 (48.6)	27 (45)
Male	108 (53.9%)	71 (51.4)	33 (55)
Female			
Weight (mean kg ± SD)	26.48 ± 13.94	24.40 ± 13.26	25.86 ± 12.77
Pulmonary TB	–	95 + 4[a] + 6[b] + 3[c] + 1d + 1e	29 + 4[a] + 2[b] + 1[c]
Central nervous system	–	6	5
Lymphadenitis	–	2	8
Abdominal	–	5	4
Miliary	–	7	1
Bone	–	3	3
Renal	–	4	2
Pericarditis	–	1	1
Positive smear	–	38 (27.53)	2 (3.33)

Data are number (%) of subjects, unless otherwise indicated.
Case also demonstrated coexisting [a]pleural TB, [b]endobronchial TB, [c]abdominal TB, [d]pericardial TB, or [e]bone TB.

Table 2
Distribution of TLR2 Arg753Gln genotype and allele frequencies and clinical types of TB disease

Type of TB	TB patients		Genotype distributions						Allele frequencies			
			Arg753Arg		Arg753Gln		Gln753Gln		Arg		Gln	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Pulmonary	124	62.6	93	75	31	25	—	—	217	87.5	31	12.5
Pulmonary plus extrapulmonary	22	11.1	15	68.2	7	31.8	—	—	37	84.1	7	15.9
Central nervous system	11	5.6	10	90.9	1	9.1	—	—	21	95.5	1	4.5
Lymphadenitis	10	5.1	8	80.0	2	20.0	—	—	18	90.0	2	10.0
Abdominal	9	4.5	8	88.9	1	11.1	—	—	17	94.4	1	5.6
Miliary	8	4.0	6	75.0	2	25.0	—	—	14	87.5	2	12.5
Bone	6	3.0	6	100	0	0	—	—	12	100	0	0
Renal	6	3.0	4	66.7	2	33.3	—	—	10	83.3	2	16.7
Pericarditis	2	1.0	2	100	0	0	—	—	4	100	0	0

n = number of individuals.

We also tested whether an association exists between probable pulmonary TB ($n = 29$) and Arg753Gln polymorphism in the TLR2 gene using blood samples of children with probable pulmonary TB and the infected control group. The results have shown that the Arg753Arg genotype frequency was significantly decreased in the entire group of probable pulmonary TB cases compared with controls (OR 0.29, 95% CI 0.10–0.82, $p = 0.03$). The probable pulmonary TB patients were also found to be more frequently Arg753Gln heterozygous (OR 3.47, 95% CI 1.21–9.90, $p = 0.03$). Also, Gln allele frequency was significantly higher (10.3% vs 2.8%, OR 4.08, 95% CI 1.45–11.50, $p = 0.01$), but Arg allele frequency was significantly lower (89.2% vs 97.2%, OR 0.24, 95% CI 0.09–0.69, $p = 0.01$) in patients with probably pulmonary TB compared with controls.

The pulmonary plus extrapulmonary TB (central nervous system, abdominal, bone, pleural, pericardial, bone, and renal) group consisted of 11 (50%) males and 11 (50%) females with a mean age ± SD of 94.82 ± 64.60 months. Fifteen of 22 patients with pulmonary plus extrapulmonary TB were accepted as having definitive TB according to their MTB culture results. The heterozygous (Arg753Gln) genotype was detected in 40% ($n = 6$), and the allelic frequency for Gln was 20% ($n = 6$) in patients with definitive pulmonary plus extrapulmonary TB. The results showed that the Arg753Arg genotype was significantly decreased in the group of definitive combined TB cases compared with controls (OR 0.11, 95% CI 0.03–0.36, $p = 0.00$). Definitive combined TB patients were also found to be more frequently Arg753Gln heterozygous (OR 8.85, 95% CI 2.76–28.45, $p = 0.00$). In addition, Gln allele frequency was significantly higher (20% vs 2.8%, OR 8.84, 95% CI 3.01–25.94, $p = 0.00$), but Arg allele frequency was significantly lower (80% vs 97.2%, OR 0.11, 95% CI 0.04–0.33, $p = 0.00$) in patients with definitive pulmonary plus extrapulmonary TB than in controls (Table 3). We did not find any significant differences between the probable pulmonary plus extrapulmonary TB and control groups in genotype distribution and allele frequency ($p > 0.05$).

The extrapulmonary TB group consisted of 22 (42.4%) males and 30 (57.6%) females with a mean age ± SD of 90.48 ± 57.84 months. Twenty-eight of 52 extrapulmonary TB patients were confirmed to have definitive TB by the isolation of MTB from their samples. There were no significant differences between the definitive extrapulmonary TB and control groups and between the probable extrapulmonary TB and control groups in genotype distribution and allele frequency ($p > 0.05$). We also did not find any significant differences between the pulmonary TB and extrapulmonary TB groups in genotype distribution and allele frequency ($p > 0.05$).

The control group was consistent with Hardy–Weinberg equilibrium. The Hardy–Weinberg exact test p value was 0.54 for the control group.

None of the infected control patients developed progression to active TB infection from LTBI during the 2-year follow-up period.

4. Discussion

The clinical spectrum of childhood TB reflects differences in the balance between the pathogen and the host immune response, with more severe disease resulting from either poor or overexuberant attempts by the immune system to contain the disease. Many cases of primary tuberculosis infection in children are asymptomatic, self-healing, and remain completely unnoticed or accidentally discovered at a later stage [17]. In previously healthy children it remains largely unknown what determines the differences in the host–pathogen interactions that lead to successful containment as opposed to progressive disease. Following infection, several factors influence the balance of risk between latent tuberculosis infection and progression to active disease, including host genetics, age, and nutritional, vaccination, and immune status [11].

TLRs play an important role in the innate immune recognition of invading microorganisms, initiating adaptive immune responses. Some individuals may not respond properly to TLR ligands because of SNPs within the TLR genes, causing defects in intracellular signal transductions and resulting in altered susceptibility to infectious diseases [18]. SNPs in the TLR2 gene have been implicated in increased risk of TB disease by a number of studies [1,10]. The focus of all of the studies was on susceptibility to smear-positive tuberculosis disease among adult populations. TB in children presents particularly difficult challenges, but research priorities and advances in pediatric TB research could provide wider insights and opportunities for TB control. We compared allele and genotype frequencies in primary TB patients with those in healthy control patients with LTBI, allowing us to distinguish susceptibility to progression of infection to disease from susceptibility to TB infection. The design of our study was different from that of previous investigations because we analyzed the effect of the Arg753Gln polymorphism of the TLR2 gene on risk of primary TB disease in pediatric population. This is the first study to evaluate whether the TLR polymorphisms are associated with progression active disease from LTBI in children.

A number of previous studies have explored the occurrence of Arg753Gln polymorphism of TLR2 in various diseases and healthy populations. Lorenz et al. [19] found that the Arg753Gln TLR2 polymorphism occurred in 2 of 91 septic shock patients, and most of the cases had staphylococcal infections. In 2004, however, a large study concluded that this SNP is not associated with severe diseases caused by *Staphylococcus aureus* [20]. In the same year, Ogus et al. [1] described a higher frequency of this SNP (Arg753Gln) among people diagnosed with TB in adults. Berdeli et al. [21] reported that polymorphism of the TLR2 gene leading to a replacement of arginine by glutamine at position 753 leads to increased susceptibility to group A β-hemolytic streptococcal infections and acute rheumatic fever. In this study, we identified 38 children among 138

Table 3
Distribution of the TLR2 polymorphism and ORs in patient and control groups

Genotype distributions		Tuberculosis (TB) patients											
		Definitive TB						Probable TB					
	Controls *n* (%)	*n* (%)		*p*	OR (95% CI)			*n* (%)		*p*	OR (95% CI)		
Arg753Arg	186 (93)	100 (72.5)		0.00	0.20 (0.10–0.38)			52 (86.7)		0.20	–		
Arg753Gln	14 (7)	38 (27.25)		0.00	5.05 (2.61–9.76)			8 (13.3)		0.20	–		
Gln753Gln	–	–		–	–			–		–	–		
Total	200 (100)	138 (100)		–	–			60 (100)		–	–		
Genotype distributions	Different types of TB patients												
		Pulmonary TB						Pulmonary plus extrapulmonary TB				Extrapulmonary TB			
		Definitive		Probable				Definitive		Probable		Definitive		Probable	
	Controls *n* (%)	*n*(%)	*p*	OR (95% CI)	*p*	OR (95% CI)		*n* (%)	*p*	OR (95% CI)		*n* (%)	*p*	*n* (%)	*p*
Arg753Arg	186 (93)	70 (73.7)	0.00	0.21 (0.10–0.43)	0.03	0.29 (0.10–0.82)		9 (60)	0.00	0.11 (0.03–0.36)		6 (85.7)	0.41	24 (85.7)	0.25	20 (83.3)	0.11
Arg753Gln	14 (7)	25 (26.3)	0.00	4.74 (2.33–9.65)	0.03	3.47 (1.21–9.90)		6 (40)	0.00	8.85 (2.76–28.45)		1 (14.3)	0.41	4 (14.3)	0.25	4 (16.7)	0.11
Gln753Gln	–	–	–	–	–	–		–	–	–		–	–	–	–	–	–
Total	200 (100)	95 (100)	–	–	–	–		15 (100)	–	–		7 (100)	–	28 (100)	–	24 (100)	–

n = number of individuals; OR = odds ratio; CI = confidence interval.

culture-confirmed patients who were heterozygous for Arg753Gln, whereas no homozygous patients were found. However, 14 child controls of 200 children with LTBI were heterozygous. These results clearly show that in the patient group, the frequency of the heterozygous Arg753Gln genotype was significantly higher than in the infected controls, and this difference remained significant even when the patients with definitive/probable pulmonary TB alone or the patients definitive pulmonary plus extrapulmonary TB were taken into account. Our result is the first in the literature to find a 5.05-fold tendency in an SNP's genotype and a 4.40-fold tendency in a specific allele of a gene for progression of TB infection to definitive TB disease in children.

Reiling et al. [10] studied resistance to airborne infection with MTB in TLR2, TLR4, and CD14 knockout and control mice groups and found that TLR2 knockout mice showed decreased resistance to TB on high-dose exposure, but no difference between the groups was observed under natural low-dose airborne infection conditions [1]. These data suggest that carrying a defective TLR2 gene could be a causative factor for increased susceptibility to progressive TB disease. However, there is no direct evidence that this polymorphism causes a decrease in immune response to MTB. Despite the significant difference between patients and healthy controls, the TLR2 polymorphism is present only in a small subset (23.2%) of patients with TB disease. Therefore, TLR2 gene polymorphism may be one of the factors influencing disease susceptibility, and other factors or defects in different steps of the immune response (even other polymorphisms of TLR2 or other TLRs) might also be responsible for an inability to prevent progression of TB infection to disease. It has been already shown that those individuals with interferon-γ receptor, interleukin 12, or interleukin 12 receptor deficiency or signal transducer and activator of transcription 1 mutation show increased susceptibility to mycobacterial infections [22–25].

In our study, all consecutive cases of TB disease were included; the number of pulmonary TB cases was 124 (62.6%), the number of extrapulmonary TB cases was 52 (26.2%), and the number of pulmonary TB plus extrapulmonary TB cases was 22 (11.1%). The statistical analysis demonstrated an association between the system involved and TLR2 polymorphism. Our data showed that the frequency of the heterozygous Arg753Gln genotype and Gln allele was significantly higher in patients with definitive/probable pulmonary TB alone and in patients with definitive pulmonary plus extrapulmonary TB than in healthy children with LTBI. The Arg753Gln genotype and Gln allele frequency were equally common in children with LTBI and children with definitive/probable extrapulmonary TB, indicating that this genotype and allele do not increase susceptibility to disease in children with extrapulmonary TB. The present authors are, therefore, of the opinion that larger series are needed to test for a possible association between TLR2 gene polymorphism and organ preference, as well as clinical severity of disease.

No statistically significant differences were observed between cases of pulmonary TB and cases of extrapulmonary TB (data not shown), demonstrating that these polymorphisms increase the likelihood of progression of TB infection to pulmonary TB disease rather than extrapulmonary TB disease in children. More extrapulmonary TB cases should be included in future studies.

In the Caucasian population, the Arg753Gln SNP was detected in 9.4% of the German whites; in independent studies, the Arg753Gln allele was observed among 10.34% [16] and 12.3% [26] of the healthy Turkish adult population. Lorenz et al. identified the Arg753Gln polymorphism among almost 3% of recruited subjects in another Caucasian population and speculated that the mutation could be a risk factor for developing septic shock after infection by Gram-positive bacteria [19]. Our data indicate that Arg753Gln polymorphism occurs in 7% in the Turkish child population, which

is lower than Berdeli et al. have previously reported (9.89% in the child population) [21].

In conclusion, on the background of the developing immune system in children, a combination of host, bacterial, and environmental factors contribute to the immunologic responses to MTB. Genetic and acquired defects in immune response pathways greatly increase the risk of progressive disease and infection frequency. By selecting children with clear phenotypes and by minimizing the effects of nongenetic risk factors, our results provide strong evidence that having the Arg753Gln genotype and Gln allele influence the speed of progression from TB infection to active pulmonary disease in children. No studies have been published regarding the association of Arg753Gln polymorphism with TB disease in children, and further investigations are needed to clarify whether this SNP has a strong impact on increased risk for progression of TB infection to active disease in children. Advances in our understanding of TB genetics in children would provide insights and opportunities to enhance efforts to control this disease.

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