# DRB1, DQA1, DQB1 genes in Turkish children with rheumatic fever

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## ABSTRACT

***Objectives.*** *Several studies have suggested that genetic susceptibility to rheumatic fever (RF) may be linked to HLA Class II alleles. The purpose of this study was to examine the association between HLA Class II genes and RF in Turkish children.*

***Methods.*** *DNA typing HLA Class II genes (DRB1, DQA1, DQB1) were performed in 55 children with RF and 50 healthy unrelated controls using sequence specific primers (SSP).*

***Results.*** *The frequency of the HLA DQA1*03 (OR: 0.462, $p < 0.05$) allele was significantly decreased in the patient group. Also, the frequency of the combination of DRB1*04 and DQA1*03 allele (OR: 0.42, $p < 0.01$) was more significantly decreased in the patient group. Differences in frequencies of the DRB1 and DQB1 alleles between groups were not significant.*

***Conclusions.*** *Our data indicate that the HLA DQA1*03 allele may be a protecting factor in Turkish children with RF. Our results also suggest that the combination of the DRB1*04 and DQA1*03 alleles may be a stronger protective factor than the DQA1*03 allele alone.*

## Introduction

The pathogenesis of rheumatic fever is thought to involve an aberrant immunological reaction triggered by an antecedent group A streptococcal infection. The association between rheumatic fever and HLA has been investigated but no definite relationship has been established (1, 6). It is known that the frequencies of HLA alleles and haplotypes vary considerably among races and ethnic groups (7). All of the previous HLA typing studies in children with rheumatic fever in Turkey were based on the serological method, which has technical limitations. Less accurate serological HLA typing methods can generate false results and fail to discriminate between allelic subgroups. These limitations can now be overcome by HLA typing at the DNAlevel, which is based on the polymerase chain reaction technique (11). In this study we used the DNA-typing technique to analyze the association of HLAclass II alleles with rheumatic fever in Turkish children.

## Materials and methods

The study was conducted on 55 Turkish children with an established diagnosis of rheumatic fever, who had been admitted to the Hacettepe University Faculty of Medicine, Section of Pediatric Cardiology, in Ankara. There were 32 male and 23 female patients between 7 and 21 years of age (mean age, $12.6 \pm 3.57$ years). Most of the patients came from the same geographic region, Ankara, which is in the middle of the Anatolia.

They presented initially with an illness which fulfilled the modified Jones criteria for the diagnosis of acute rheumatic fever. Polyarthritis alone was present in 24 cases, carditis alone in 9, combined carditis and polyarthritis in 17, combined carditis and chorea in 3 and chorea alone was diagnosed in 2. A control group was comprised of 50 healthy individuals with no history of RF living in the same geographic region. The institutional ethics committee of Hacettepe University approved this study. Informed consent was obtained from the parents of the subjects.

Genomic DNAwas extracted from peripheral mononuclear cells using the micro-spin colon method (Macherey-Nagel GmbH & Co, Düren, Germany) and subjected to 30 cycles of polymerase chain reaction (PCR) in a thermal cycler to amplify the second exon of the DRB1, DQA1, and DQB1 genes, using thermostable DNApolymerase (Olerup SSP, DQ-DR SSPCombi Tray, Saltsjöbaden, Sweden).

PCR analysis was performed for 24 alleles for DRB1, 8 alleles for DQB1, and 24 alleles for DQA1. PCR conditions for all alleles included a cycle at 94ºC for 2 min denaturation, followed by 10 cycles 94ºC for 10 sec denaturation, 65ºC for 60 sec annealing and extension, 20 cycles 94º C for 10 sec denaturation, 61ºC for 50 sec annealing, and 72ºC for 30 sec extension. PCR products were separated on 2% agarose and the amplified bands were visualized. W.M.C. Helmberg-SCORE Software can be helpful in the interpretation of the typing. The allele designations are those recommended by the official HLA Nomenclature Committee (2).

*Statistical analysis*

HLA-DRB1, DOA1, and DQB1 allele frequencies in the patients and healthy unrelated control subjects were compared. Tests for differences in the predisposing and protective effects of HLAclass II alleles were performed by use of the odds ratio (OR) method. Statistical significance was examined by Fisher's exact test.

## Results

*DRB1 alleles:* Frequencies of DRB1 alleles were not significantly different between the patients and controls (Table I).

*DQA1 alleles:* Frequency of the DOA1 *03 allele was detected in 12.4% of the patients, and in 24% of the controls. The frequency of the HLA DQA1*03 (OR: 0.462, $p < 0.05$) allele was significantly decreased in the patient group (Table II).

*DQB1 alleles:* Frequencies of DRB1 alleles were not significantly different between the patients and controls (Table III).

In this study, 14.5% of the patients and 22% of the controls carried the DRB1 *04 and DQA1*03 alleles together. The frequency of the combination of DRB1*04 and DQA1*03 alleles (OR: 0.42, $p < 0.01$) was significantly decreased in the patient group.

The allele frequencies of DRB1, DQA-1, and DQB1 were not different in patients with cardiac involvement; otherwise the DQA1*03 allele was significantly decreased in patients with noncardiac involvement, as in the general patients group (OR: 0.337, $p<0.05$).

**Table I.** Frequencies of HLA-DRB1 alleles in patients with rheumatic fever and controls.

| DRB1 allele | Patients (%) | Controls (%) | OR (% 95 CI interval) | | $\chi^2$ |
|---|---|---|---|---|---|
| 01 | 10.9 | 10 | 1.12 | (0.454 – 2.675) | n.s. |
| 03 | 17.3 | 13 | 1.397 | (0651 – 3.0) | n.s. |
| 04 | 12.7 | 20 | 0.583 | (0.277 – 1.228) | n.s. |
| 07 | 5.5 | 5.0 | 1.096 | (0.324 – 3.709) | n.s. |
| 08 | 9.1 | 3.0 | 3.233 | (0.864 – 12.10) | n.s. |
| 09 | 0.0 | 1.0 | – | – | |
| 10 | 3.6 | 0.0 | 4.72 | (0.542 – 41.09) | n.s. |
| 11 | 10 | 15 | 0.630 | (0.274 – 1.444) | n.s. |
| 12 | 2.7 | 1.0 | 2.776 | (0.284 – 27.12) | n.s. |
| 13 | 13.6 | 9.0 | 1.596 | (0.666 – 3.830) | n.s. |
| 14 | 6.4 | 5.0 | 1.291 | (0.396 – 4.207) | n.s. |
| 15 | 6.4 | 11 | 0.550 | (0.204 – 1.479) | n.s. |
| 16 | 1.8 | 7.0 | 0.246 | (0.050 – 1.213) | n.s. |

n.s.: not significant

**Table II.** Frequencies of HLA-DQA1 alleles in patients with rheumatic fever and controls.

| DQA1 allele | Patients (%) | Controls (%) | OR (95% CI interval) | | $\chi^2$ |
|---|---|---|---|---|---|
| 01 | 42.7 | 36 | 1.326 | (0.76 – 2.313) | n.s. |
| 02 | 5.5 | 6 | 0.904 | (0.282 – 2.899) | n.s. |
| 03 | 12.7 | 24 | 0.462 | (0.224 – 0.953) | P< 0.05 |
| 04 | 1.8 | 1.0 | 1.833 | (0.164 – 20.53) | n.s. |
| 05 | 37.3 | 32 | 1.263 | (0.713 – 2.235) | n.s. |
| 06 | 0.0 | 1.0 | - | - | |

n.s.: not significant

**Table III.** Frequencies of HLA-DQB1 alleles in patients with rheumatic fever and controls.

| DQB1 allele | Patients (%) | Controls (%) | OR (%95 CI interval) | | $\chi^2$ |
|---|---|---|---|---|---|
| 02 | 20.9 | 16 | 1.388 | (0.686 – 2.809) | n.s. |
| 03 | 37.3 | 46 | 0.698 | (0.402 – 1.211) | n.s. |
| 04 | 3.6 | 2.0 | 1.849 | (0.331 – 10.32) | n.s. |
| 05 | 24.5 | 29 | 0.796 | (0.432 – 1.469) | n.s. |
| 06 | 13.6 | 7.0 | 2.098 | (0.818 – 5.379) | n.s. |

n.s.: not significant

## Discussion

An abnormal humoral and cellular immune response to various streptococcal antigens suggests that it has role in the pathogenesis of RF. Epidemiological and familial studies indicate that genetic factors might determine the disease's susceptibility. Previous studies have described discrepant results regarding the disease-associated HLA alleles in RF, and no definite relationship has been established. However, it is well known that there are strong linkage disequilibria between the alleles in the HLA-DR and DQ loci. We therefore compared the frequencies of DRB1, DQA1 and DQB1 alleles in the patients with those in the controls.

In the present study, the frequencies of DRB1 and DQB1 alleles were not significantly different between the patients and controls. The association between HLA class II antigens and RF has been extensively investigated and some different results reported in the literature. Guedez *et al*. (4) found that the DRB1*0701 allele was significantly increased, whereas Koyanagi *et al*. (9) showed that the DQB1*0503 allele was significantly increased in patients with RF.

In our study, the DQA1*03 allele was significantly decreased in the patient group. In different populations increased frequencies of DQA1*0201 and DQA1*0104 alleles were shown in RF (4, 9). On the other hand, in one study no relationship between DQ alleles and RF was found (3). Our data indicate that the HLADQA1*03 allele may be a protective factor in Turkish children with RF, because the frequency of this allele was significantly decreased in

**Table IV.** Previously reported HLAclass II studies in patients with RF/rheumatic heart disease (RHD)

| Population | Year | HLAclass II associations Risk | Protection | Method | Patients | Controls | Disease |
|---|---|---|---|---|---|---|---|
| American (1) | 1986 DR4 | DR2 | | Serology | 72 | 349 | RHD |
| Black (10) | 1987 DRw6 | DR1 | | Serology | 103 | 120 | RHD |
| Indian (14) | 1989 DQw2 | DR3 | DR2 | Serology | 54 | 57 | RHD |
| Brazilian (5) | 1991 DR7 | DRw53 | | Serology | 40 | 617 | RF |
| Turkish (8) | 1992 | DR4 | | Serology | 93 | 80 | RF |
| Turkish (12) | 1993 | DRw11 | | Serology | 100 | 100 | RF |
| Turkish (13) | 1993 | DR3 DR7 | DR5 | Serology | 107 | 203 | RHD |
| Japanese (9) | 1996 | DQA1*0104 DQB1*0503 | | PCR-SSO[a] | 72 | 525 | RHD |
| Egyptian (4) | 1999 | DRB1 *0701 DQA1*0201 | DQA1*0103 DQB1*0603 | PCR-SSO[a] | 88 | 59 | RHD |
| Brazilian (15) | 2000 | DR7 | | Serology | 35 | 209 | RF |
| Turkish (*) | 2004 | | DQA1*03 DRB1*04/DQA1*03 | PCR-SSO | 55 | 50 | RF |

[a]PCR-SSO: PCR and sequence specific oligonucleotide; *present study data.

the patient group.

In this study, the frequency of DRB1 *04 allele was decreased although not significantly. We found that 14.5% of the patients and 22% of the controls were carrying the DRB1*04 and DQA 1*03 alleles together. The frequency of the combination of these two alleles was significantly decreased in the patient group. Our results suggest that the combination of the DRB1*04 and DQA1*03 alleles may be a stronger protective factor than the DQA1*03 allele alone.

Use of serological HLA typing methods showed different results in the various populations (1,5,10,14,15). Three investigations have reported the involvement of HLA class II antigens in rheumatic fever from Turkey and they found different results. Khosroshahi *et al.* (8) revealed an increased frequency of HLA-DR4 positivity in patients with RF; Olmez *et al.* (12) found a significant increase of DRB35 in RF and that HLA-DRw11 frequencies were increased in patients with carditis; Ozkan *et al.* (13) reported that HLA DR3 and DR7 were encountered in a significantly higher frequency in patients with rheumatic heart disease. There were some limitations to these studies, however: all of them investigated only HLA-DR loci and the less sensitive serological HLA typing methods were used. We know that PCR methods can be used in the routine HLA typing of patients with a greater precision than serology (16).

Nowadays, DNA typing HLA methods are preferred because of their high sensitivity. With these methods different associations between alleles and RF have been detected in various populations. For instance, a significant association between HLA-DQw2 and RF was reported in the Hindu population (14), and in the Japanese population the frequencies of the DQA1*0104 and DQB 1*0503 alleles was increased (9). Another DNA typing HLA study revealed that DRB1*0701 and DQA1*0201 alleles were increased in Egypt (4). On the other hand, Carlquist *et al.* (3) performed a DNAtyping of HLA-DQ alleles among American Caucasians and did not find any association. The reported alleles in these studies were not consistent, and the discrepancies could be explained by ethnic differences (Table IV).

Class II antigens and RF associations appear to be stronger in clinically homogenous patients (4). Our study group had both cardiac and non-cardiac involvement and so were not clinically homogenous. The allele frequencies of DRB1, DQA1, and DQB1 were not different in patients with cardiac involvement, otherwise the DQA1*03 allele was significantly decreased in patients with non-cardiac involvement as in the general patient group. We thought that the decrease in the DQA 1*03 allele that was detected in the general

patient group might be due to patients without cardiac involvement. In this study, the separation of the patients into clinical subgroups resulted in a decrease in the patient number in each group. These factors could have influenced our results and we could not find any further associations.

Recently, some studies have suggested that susceptibility to rheumatic heart disease is closely associated with DQ alleles (9). Our result partly support the idea that the HLA DQA1*03 allele may be a protective factor in Turkish children with RF. Moreover, our results also suggest that the combination of the DRB1*04 and DQA1*03 alleles may be a stronger protective factor than the DQA1*03 allele alone. However we did not find any allele related to susceptibility to RF. Further research must be done in this area in clinically homogenous patients, especially focusing on HLA-DQ loci.

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