

# The HLA Class II Associations with Rheumatic Heart Disease in South Indian Patients: A Preliminary Study

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## ABSTRACT

**Introduction:** Rheumatic heart disease (RHD) occurs in 30-45% of the patients with rheumatic fever (RF) and it leads to chronic valvular lesions. The human leukocyte antigen (HLA) might confer a susceptibility to RHD. The aim of the present study was to determine the prevalent HLA class II DR/DQ allelic types which were associated with rheumatic heart disease (RHD) in a small group of south Indian patients and to compare them with those in the control subjects.

**Methods:** A total of 23 patients who were diagnosed with RHD and 6 control samples were included in this study. A low resolution HLA Class II DR/DQ typing was performed on the blood samples by the PCR-SSP method.

**Results and Conclusion:** The DRB3\*01:01:02:01 allele showed a positive association with RHD, whereas the DQB1 loci alleles did not show any significant association.

**Key Words:** HLA Class II DR/DQ typing, Rheumatic Heart Disease, South India

## INTRODUCTION

Rheumatic fever (RF) and Rheumatic Heart Disease (RHD) are important public health problems which often lead to chronic valvular lesions [1]. The incidence per 1000 ranges from 0.6 to 11 in the reports from different parts of India [2]. It occurs as a sequel to the throat infections which are caused by *Streptococcus pyogenes*, it manifests as polyarthritis, carditis, chorea, erythema marginatum, and/or as subcutaneous nodules and it progresses to cause damage to the valve tissue of the heart, leading to congestive heart failure, stroke, endocarditis, and death [3].

In spite of the knowledge of the inciting agent, the pathogenesis of RHD is not completely understood and only 2-3% of the patients develop RF after occurrences of untreated streptococcal pharyngitis. Several studies have documented a high familial incidence of RF, thus suggesting the involvement of host genetic factors in the susceptibility to RF, with a consequential progression to RHD [4, 5].

As critical components in the antigen processing, the human leukocyte antigen (HLA) molecules are attractive candidate antigens that might confer a susceptibility to RHD. Bryant *et al.*, in his review, has provided an overall perspective of the several HLA alleles which are associated with RF and RHD across various regions of the world. A number of studies have suggested that the HLA class II molecules appear to have a closer association with an increased risk of RF or RHD than the class I molecules, although no single HLA haplotype or combination exists, that is consistently associated with a susceptibility to RHD [6].

The aim of the present study was to determine the prevalent HLA class II DR/DQ allelic types which were associated with Rheumatic Heart Disease (RHD) in a small group of south Indian patients and to compare them with those in the control subjects.

## MATERIALS AND METHODS

We determined the HLA class II DR/DQ allelic types in 23 south Indian patients who were diagnosed with rheumatic heart disease,

based on the clinical and the echocardiographic findings. The mean age of the patients was  $29.6 \pm 12.25$  years (mean age  $\pm$  S.D), and they included 16 males and 7 females. Six age and sex matched control samples from normal individuals in south India, with no history of RHD or any autoimmune disease, were also included in our study. The present study was conducted during the years 2010-2011. An ethical clearance was obtained from the institutional ethical committee. Of the 23 patients, 13 (56.52%) had mitral valve disease; 3(13.04%) had aortic valve disease; and 7(30.04%) had multi valvular disease.

A low resolution HLA Class II DR/DQ typing was performed by the PCR-SSP (sequence specific priming) method. The assay was performed by using the MICRO SSP™ HLA DNA TYPING TRAYS in accordance with the protocol which was provided by the manufacturers (One Lambda Diagnostics, USA). Two ml of blood was collected in EDTA from each individual, and the genomic DNA was isolated from whole blood by using the DNAeasy Blood and Tissue Kit (mini spin column-QIAGEN, Germany). Briefly, the procedure was as follows: The Micro SSP™ D mix, the primer set trays, and the DNA samples were thawed to room temperature. 2  $\mu$ l of Taq Polymerase (5units/ $\mu$ l) was added to the Micro SSP™ D-mix and the mixture was vortexed. 9  $\mu$ l of this was added to the negative control reaction tube on the primer set tray. The DNA sample (39  $\mu$ l) was added to the Micro SSP™ D-mix tube and it was vortexed for 5 seconds and centrifuged. 10  $\mu$ l of the sample-reaction mixture from the Micro SSP™ D-mix tube was pipetted into each reaction tube of the Micro SSP™ primer set tray, with the exception of the negative control reaction tube. The reaction tubes with the tray were sealed and the Micro SSP™ primer set tray was placed in the PCR thermocycler. After the PCR process, the amplified DNA fragments were separated by agarose gel electrophoresis and they were visualized by using a gel documentation system (Bio-Rad, USA). The interpretation of the PCR-SSP results was based on the presence or absence of a specific amplified DNA fragment. The pattern of the positive wells was matched with the information on

the Micro SSP™ worksheet to obtain the HLA typing of the sample DNA.

The statistical analysis was performed by using the Fisher's exact test to determine the degree of association.

## RESULTS

The frequency of DRB3\*01:01:02:01 was 39.13% in the RHD patients, whereas it was not present in the controls. The difference in the prevalence of the DRB3\*01:01:02:01 allele among the RHD patients and the controls was statistically significant ( $p=0.01$ ). DRB1\*09:01:02, on the other hand, occurred at a frequency of 16.6% in the controls but was not found in any of the RHD patients [Table/Fig-1]. The DQB1 alleles did not show a significant association in the RHD patients as compared to that in the controls [Table/Fig-2].

DRB allele	RHD patients		Controls		p value
		Allele frequency AF (%)		Allele frequency AF (%)	
DRB3*01:01:02:01	18/46	39.13	0/12	0	0.011
DRB4*01:01:01:01	16/46	34.78	6/12	50	0.505
DRB1*07:01:01:01	10/46	21.73	3/12	25	1
DRB1*15:01:01:01	6/46	13.04	4/12	33.33	0.191
DRB1*04:01:01	6/46	13.04	1/12	8.33	1
DRB1*03:01:01:01	5/46	10.86	0/12	-	0.354
DRB1*10:01:01	5/46	10.86	1/12	8.33	1
DRB5*01:01:01	6/46	13.04	4/12	33.33	0.190
DRB1*13:01:01	7/46	15.21	0/12	-	0.354
DRB1*14:01:01	2/46	4.3	0/12	-	1
DRB1*14:04	2/46	4.3	0/12	-	1
DRB1*14:02	1/46	2.17	0/12	-	1
DRB1*08:09	1/46	2.17	0/12	-	1
DRB1*08:01:01	0/46	-	1/12	8.33	0.207
DRB1*09:01:02	0/46	-	2/12	16.66	0.0399
DRB1*11:01:01	1/46	2.17	0/12	-	1

[Table/Fig-1]: Frequency of distribution of DRB locus alleles in RHD patients and controls

DQB allele	RHD patients		Controls		p value
		Allele frequency AF (%)		Allele frequency AF (%)	
DQB1*05:01:01	11/46	23.91	3/12	25	1
DQB1*06:01:01	12/46	26.08	2/12	16.66	0.710
DQB1*02:01:01	9/46	19.56	3/12	25	0.698
DQB1*03:03:02	5/46	10.86	2/12	16.66	0.626
DQB1*03:02:01	4/46	8.6	1/12	8.33	1
DQB1*03:01:01	3/46	6.5	0/12	-	0.597
DQB1*04:01:01	1/46	2.17	1/12	8.33	0.374
DQB1*05:03:01	1/46	2.17	0/12	-	1

[Table/Fig-2]: Frequency of distribution of DQB1 locus alleles in RHD patients and controls

## DISCUSSION

In a study which was done by EL-Hagrassy *et al.*, [7] on Egyptian children, DR\*04-02 and DR\*10-0101 were found to be common in the RHD patients. Stanevicha *et al.*, [8] reported that the HLA class

II DRB1\*07-DQB1\*0401-2 and DRB1\*07-DQB1\*0302 could be the risk alleles and that the HLA class II DRB1\*06 and DQB1\*0602-8 could be the protective alleles among the RHD patients in Latvia. The DRB1\*07 allele was also found to be associated with RHD in Pakistan [9]. Guedez *et al.*, [10] also found the DRB1\*0701-DQA1\*0201 haplotype to be significantly associated with RHD. On the contrary, in our study, we found that DRB1\*07:01:01:01 was present in both the RHD patients and the controls and therefore, it could not be linked with RHD in the south Indian patients.

Although several studies have been done in different parts of the world, studies on the HLA association with RHD is lacking in India, especially in the south Indian population. This was a pilot study on the HLA Class II types which were seen in the RHD patients in south India. Previous studies which had been done in north India were based on serological assays and the HLA alleles which were associated with RHD were reported to be DR3, DR4 and DQ3 [11-13].

In the present study, DRB3\*01:01:02:01 was seen with an allele frequency of 39.13% in the patients and it was seen in none of the controls. This difference was unlikely to be incidental and it was certainly worth investigating in future studies. We also found that the alleles of the DQB1 loci were not significantly associated with RHD.

The HLA types which were seen in the RHD patients in south India were different from the HLA types which had been reported in other countries, and hence there appeared to be ethnic differences in the distribution of the HLA alleles. In addition, the earlier studies which were done on HLA were based on serological assays and thus, a comparison of the results of our study with earlier reports was not possible. The molecular techniques for HLA typing are superior to the serological assays and they are also less cumbersome. The limitation of this study was the small number of patients in the control group.

## CONCLUSION

The present study was a pilot study; however, the preliminary results are interesting and there is a need to do a larger case-control study in India to confirm whether DRB3\*01:01:02:01 is a risk allele for RHD.

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