Original Article

# Human Leukocyte Antigen Class II Genetic Variants are Highly Associated with Rheumatic Heart Disease in Yemeni Patients

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## ABSTRACT

**Objectives:** To investigate the association of rheumatic heart disease (RHD) with human leukocyte antigen (HLA) class II alleles in Yemeni patients
**Design:** Case control study
**Setting:** Al-Thawra Modern General Hospital, Sana'a, Yemen
**Subjects:** One hundred RHD patients (case group) and 50 healthy subjects (control group) were recruited in this study.
**Interventions:** Echocardiography was used to include RHD patients (abnormal echocardiography) and healthy subjects (normal echocardiography).
**Main Outcome Measures:** HLA-DRB1 and HLA-DQB1 polymorphisms were genotyped by sequence-specific oligonucleotide-probe polymerase chain reaction (PCR-SSOP) reverse dot blot hybridization.
**Results:** The results showed that HLA-DRB1*07 and HLA-DQB1*0203 allele as risk factors for RHD (OR = 4.0; 8.7, p = 0.005; 0.02, respectively). In contrast, the HLA-DRB1*11, HLA-DQB1*0305 and HLA-DQB1*0602 alleles showed a protective association against RHD (OR = 0.32; 0.23; 0.24, p = 0.01; 0.03; 0.01, respectively).
**Conclusions:** HLA class II genetic variants were a predisposing factor for development of RHD in Yemeni people. This study also replicated the association of HLA-DRB1*07 with RHD and suggested that HLA-DQB1*0203 allele is a risk factor for RHD.

KEY WORDS: HLA-DRB1, HLA-DQB1, molecular mimicry, rheumatic heart disease

## INTRODUCTION

Rheumatic heart disease (RHD) is still a major public health burden in developing countries due to the morbidity and mortality resulting from the heart lesions that follow a rheumatic fever (RF) episode in 30 - 45% of patients. It was estimated worldwide that at least 15.6 million individuals have RHD, and this disease is leading to 233,000 deaths annually. The highest prevalence of RHD was reported from developing countries than developed countries[1-4]. Prevalence of RHD was reported to be 0.8 / 1000 in Oman[5], 2.4 / 1000 in Saudi Arabia[6], 3 / 1000 in Sudan[7], 6.2 / 1000 in Egypt[8], 5.7 / 1000 in sub-Saharan Africa and in Pakistan[2,9] and 5.8 / 1000 in India[10]. Prevalence of RHD in Northern parts of Yemen was reported to be 3.6 / 1000[11] while in southern parts of Yemen, it was estimated to be 36.5 / 1000[12], which is one of the highest prevalence in the world.

RHD is a consequence of autoimmune reaction triggered by group A streptococcal pharyngitis leading to severe heart valvular damage. The exact reason why only certain individuals exposed to group A streptococci develop RHD is unknown. Molecular mimicry between human cardiac myosin and the M proteins of the group A streptococcal membranes has been proposed as a triggering factor, leading to autoimmunity in individuals with a genetic predisposition factor[13-16]. M protein plays an important role in the bacterial adherence to throat epithelial cells. It shares structural homology with a-helical coiled-coil human proteins like cardiac myosin, tropomyosin, vimentin and several valvular

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proteins resulting in cross-reactivity by both T and B lymphocytes[17,18].

Genetic factors have been postulated to be important in determining susceptibility patterns to RHD. RHD is found to be more common in individuals with family history[19-22]. Human leukocyte antigen (HLA) class II polymorphisms were a predisposing factor for RHD. HLA class II molecules are normally expressed on particular immune cells known as professional antigen presenting cells (APCs) such as dendritic cells and macrophages. Professional APCs engulf extracellular protein antigens, degrade them into peptide fragments, couple peptide fragments (epitopes) to the HLA class II molecules for presentation and activation of resting T lymphocytes. The molecular mechanism by which certain MHC class II molecules confer susceptibility to RHD is not clear. However, risk HLA class II alleles may encode HLA molecules that facilitate the presentation of some streptococcal peptides that later will trigger autoimmune responses[23,24]. The predisposing allelic HLA class II variants were different in various populations with HLA-DR7 being predominantly observed in different ethnicities[25-33]. In Yemen, the predisposing HLA class II alleles remain practically unknown. The aim of this study was to investigate the association of HLA class II alleles with RHD in Yemeni patients.

## SUBJECTS AND METHODS

### Study Subjects

Yemeni RHD patients aged 14 to 45 years who attended the outpatient clinic at Al-Thawra Hospital in Sana'a for treatment were invited to participate in this study. One hundred RHD patients (57 females, 43 males) freely participated in the study (case group). The diagnosis of RHD was confirmed using echocardiogram in each patient. For control group, normal subjects aged 18 to 47 years in the same geographical area as the patients were invited to participate in this study. Fifty healthy individuals (28 females, 22 males) freely participated to act as control group. In order to exclude presence of subclinical RHD in this group, echocardiogram was used in each healthy study subject. Venous blood, 3 - 4 ml was collected from each participant. The study was approved by ethical committee at Sana'a University.

### HLA class II genotyping

Genomic DNA was extracted from peripheral blood leukocytes using PureLink Genomic DNA kit (Invitrogen, USA). HLA class II DRB1 and DQB1 genotyping were carried out using sequence-specific oligonucleotide-probe polymerase chain reaction with Dynal RELI SSO HLA-DRB1 and Dynal RELI SSO HLA-DQB1 kits, respectively (Invitrogen, USA).

### Statistical analysis

Statistical Package for Social Science (SPSS) 12 (SPSS Inc, Chicago, IL, USA) was used for comparing the frequency of HLA-DRB1 and HLA-DQB1 alleles between the patients and the controls using the chi-square test or Fisher's exact test, as appropriate. Odds ratio (OR) with their 95% confidence intervals were calculated and alleles showing an association with RHD at 5% or less level of significance were deemed to be significant.

## RESULTS

The echocardiographic data of the patients with RHD are depicted in Table 1. The results showed that

**Table 1:** Echocardiographic data of 100 patients with rheumatic heart disease

| Echocardiographic parameter | Patients with rheumatic heart disease number (%) |
|---|---|
| Site of lesion | |
| Mitral valve | 29 (29) |
| Mitral and aortic | 52 (52) |
| Mitral, aortic and tricuspid | 19 (19) |
| Main Echocardiographic abnormality | |
| Mitral regurgitation | 47 (47) |
| Mitral stenosis | 3 (3) |
| Mitral regurgitation and stenosis | 50 (50) |

**Table 2:** Association of HLA-DRBI alleles with rheumatic heart disease

| HLA-DRB1 alleles | N (frequency) | | OR | 95% CI | p-value |
|---|---|---|---|---|---|
| | Cases (n = 100) | Controls (n = 50) | | | |
| HLA-DRB1*01 | 24 (0.24) | 9 (0.18) | 1.43 | 0.61 - 3.38 | 0.4 |
| HLA-DRB1*03 | 19 (0.19) | 9 (0.18) | 1.1 | 0.44 - 2.57 | 0.88 |
| HLA-DRB1*04 | 40 (0.40) | 18 (0.36) | 1.2 | 0.59 - 2.39 | 0.64 |
| HLA-DRB1*07 | 31(0.31) | 5 (0.10) | 4.0 | 1.5 - 11.2 | 0.005 |
| HLA-DRB1*08 | 9 (0.09) | 5 (0.10) | 0.89 | 0.28 - 2.81 | 0.84 |
| HLA-DRB1*10 | 2 (0.02) | 3 (0.06) | 0.32 | 0.05 - 1.98 | 0.1[a] |
| HLA-DRB1*11 | 10 (0.10) | 13 (0.26) | 0.32 | 0.13 - 0.79 | 0.01 |
| HLA-DRB1*12 | 4 (0.04) | 5 (0.1) | 0.38 | 0.1 - 1.5 | 0.15 |
| HLA-DRB1*13 | 44 (0.44) | 23 (0.46) | 0.92 | 0.47 - 1.82 | 0.82 |
| HLA-DRB1*15 | 5 (0.05) | 4 (0.08) | 0.6 | 0.16 - 2.36 | 0.5 |
| HLA-DRB1*16 | 9 (0.09) | 4(0.08) | 1.14 | 0.33 - 3.89 | 0.84 |

OR = odds ratio, CI = confidence interval, [a] = The p-value was generated using Fisher's exact test.

**Table 3:** Association of HLA-DQBI alleles with rheumatic heart disease

| HLA-DQB1 alleles | N (frequency) | | OR | 95% CI | p-value |
|---|---|---|---|---|---|
| | RHD cases (n = 100) | Controls (n = 50) | | | |
| HLA-DQB1*0201 | 23 (0.23) | 8 (0.16) | 1.6 | 0.65 - 3.81 | 0.32 |
| HLA-DQB1*0202 | 17 (17) | 5 (10) | 1.8 | 0.64 - 5.33 | 0.25 |
| HLA-DQB1*0203 | 15 (0.15) | 1 (0.02) | 8.7 | 1.1 - 67.5 | 0.02 |
| HLA-DQB1*0301 | 21 (0.21) | 8 (0.16) | 1.4 | 0.6 - 3.4 | 0.5 |
| HLA-DQB1*0302 | 25 (0.25) | 9 (0.18) | 1.5 | 0.65 - 3.6 | 0.33 |
| HLA-DQB1*0303 | 8 (0.08) | 6 (0.12) | 0.64 | 0.21 - 2 | 0.43 |
| HLA-DQB1*0304 | 5 (0.05) | 3 (0.06) | 0.83 | 0.19 - 3.6 | 0.8 |
| HLA-DQB1*0305 | 3 (0.03) | 6 (0.12) | 0.23 | 0.1 - 0.95 | 0.03 |
| HLA-DQB1*0401 | 12 (0.12) | 6 (0.12) | 1 | 0.35 - 2.84 | 1 |
| HLA-DQB1*0402 | 10 (0.10) | 3 (0.06) | 1.7 | 0.5 - 6.6 | 0.41 |
| HLA-DQB1*0501 | 15 (0.15) | 6 (0.12) | 1.3 | 0.47 - 3.57 | 0.62 |
| HLA-DQB1*0502 | 11 (0.11) | 9 (0.18) | 0.56 | 0.22 - 1.5 | 0.23 |
| HLA-DQB1*0503 | 7 (0.07) | 6 (0.12) | 0.6 | 0.18 - 1.74 | 0.31 |
| HLA-DQB1*0504 | 4 (0.04) | 1 (0.02) | 2 | 0.22 - 18.7 | 0.5 |
| HLA-DQB1*0601 | 8 (0.08) | 6 (0.12) | 0.64 | 0.21 - 1.95 | 0.43 |
| HLA-DQB1*0602 | 5 (0.05) | 9 (0.18) | 0.24 | 0.1 - 0.8 | 0.01 |
| HLA-DQB1*0603 | 7 (0.07) | 7 (0.14) | 0.5 | 0.15 - 1.4 | 0.2 |

OR = odds ratio, CI = confidence interval, RHD = rheumatic heart disease.

HLA-DRB1*07 was strongly associated with RHD (OR = 4, p = 0.005) (Table 2). Moreover, HLA-DRB1*11 allele showed a protection against RHD (OR = 0.32, p = 0.01). HLA-DRB1*10 and HLA-DRB1*12 alleles showed a protective effect against RHD; however, they were not statistically significant (OR = 0.32; 0.38, p = 0.1; 0.15, respectively). The other alleles of HLA-DRB1*03, *04, *08, *13, *15, and *16 were not associated with RHD (Table 2).

The HLA-DQB1*0203 allele was a risk factor for RHD (OR = 8.7, p = 0.02). However, HLA-DQB1*0305 and –DQB1*0602 alleles were associated with a protection against RHD (OR = 0.23; 0.24, p = 0.03; 0.01, respectively) (Table 3). The HLA-DQB1 *0201, *0202 and *0402 alleles were not statistically significant risk factors for RHD (OR = 1.6; 1.8; 1.7, respectively). On the other hand, HLA-DQB1*0502 and HLA-DQB1*0603 alleles showed a protective effect against RHD, but statistical significance was not reached (OR = 0.56; 0.5, respectively). The other HLA-DQB1 alleles that were included in this study showed no association with RHD (Table 3).

## DISCUSSION

This study aimed to investigate the association of HLA-DRBI and HLA-DQBI with RHD among Yemeni patients. The present study found that the HLA-DRB1*07 were strongly associated with RHD in Yemen, which is in agreement with previous reports from Egypt[34], Pakistan[32], Turkey[29,35], Latvia[30] and Brasilia[33]. In contrast, the association of HLA-DRB1*07 allele with RHD could not be replicated in Taiwan[36] and Mexican Mestizo[37]. Furthermore, a study from north India showed an association between HLA-DR*14 and RHD[31] while another study performed in south India reported an association with HLA-DRB3*01 and HLA-DRB3*02[38]. Gene-gene and gene-environmental interactions may have contributed to many of the reported differences in gene-disease associations between different racial or ethnic groups[39].

Our study revealed that HLA-DQB1*0203 allele was a risk factor for RHD while HLA-DQB1*0201 and HLA-DQB1*0202 alleles were not statistically significant risk factors, which may be due to small sample size, particularly the control group. HLA-DQB1*0201 and HLA-DQB1*0202 alleles have been reported to be risk factors for RHD among Egyptian[34], Latvian[30] and Mexican[40] populations. On the other hand, the HLA-DRB1*11 allele showed a protective effect against RHD in this study which is in concordance with, other studies in Egypt[34], Turkey[29] and Mexico[37]. Moreover, our data suggest that HlA-DQB1*0602 allele may confer a protective effect against RHD, which is consistent with other studies[30, 34]. HLA class II molecules play a central role in T cells activation and mounting an immune response against extracellular microorganisms such as group A β-hemolytic streptococci. Exogenous antigens are engulfed and processed into small peptide fragments by antigen presenting cells. Antigenic peptides are then associated with HLA class II molecules and transported to the cell surface to be recognized by the T cells receptor (TCR), thus, triggering the activation of the adaptive immune response[41].

The molecular mechanism by which HLA class II variants confer susceptibility to autoimmune diseases is not clear[24]. However, the HLA-DRB1 and HLA-DQB1 risk alleles were the most associated alleles with the RHD patients and seems to be associated with the development of valvular lesions in the patients with RHD. Those risk alleles may facilitate the presentation

of some streptococcal peptides that later will trigger autoimmune reactions. Streptococcal epitopes that have similarity to cardiac proteins might activate autoreactive T lymphocytes. These autoreactive T cells may activate B cells to produce antibodies which cross react with the endothelial surface protein of heart valvular and cardiac myosin peptides. The cross-reactive antibodies might up-regulate the expression of vascular cell adhesion molecule 1 (VCAM-1) which facilitates migration of immune cells leading to inflammation of heart valve resulting in damaging the heart valve and formation of valve scar[42,43]. The limitation of this study was the poor response of subjects to participate, which resulted in small sample size, particularly the control group. This limitation will seriously affect the results and conclusions of the present study and a larger population study will be needed to confirm the results suggested by the study.

## CONCLUSION

This study suggests that certain HLA class II genetic variants were a predisposing factor for developing RHD in Yemeni people, in spite of the small sample size. This study replicated the association of HLA-DRB1*07 with RHD seen in other published reports. In addition, this study suggested that HLA-DQB1*0203 is a risk factor for RHD in Yemen which needs to be confirmed with a larger population study.

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