

# The Frequency of Human Leukocyte Class II Antigens in Patients with Rheumatic Heart Disease in an Iranian Population

Shohreh Rezaie, MD; Ali Mostafaie, PhD; Masoum Ali Masumi, MD; Hamid Rahi, PhD and Mohammad Jafar Rezaie, PhD

## Abstract

**Background-** With respect to the high incidence of rheumatic heart disease (RHD) and the almost identical racial background of the western population of Iran and the importance of investigation on HLA typing as a new research tool, this study was conducted with the aim of better understanding the mechanisms involved.

**Methods-** The frequencies of class II HLA antigens (DQ, DR) in 35 patients with a diagnosis of RHD were studied and compared with a control group of 36 healthy individuals.

**Results-** An increase was found in the frequency of HLA-DR53 (57.1% in the patient group versus 16.7% in the control group:  $P=0.00099$ ,  $RR=6.66$ ), HLA-DR2 (31.4% in the patient group versus 2.8% in the control group:  $P=0.0037$ ,  $RR=16.04$ ) and HLA-DR15 (28.6% versus 2.8%:  $P=0.007$ ,  $RR=14$ ). The increased frequency of HLA-DQ2 and HLA-DQ5 in the patients compared with the control group was also significant ( $P<0.05$ ); however, frequency differences for other antigens was not significant.

**Conclusion-** HLA-DR53, HLA-DR2 and HLA-DR15 may be markers for susceptibility to RHD in our patients. These results could be explained by genetic differences resulting from racial or geographical diversity (*Iranian Heart Journal 2005; 6 (1,2): 83-88*).

**Key words:** RHD ■ HLA Class II antigens ■ Iran

Acute rheumatic fever (ARF) develops in only a relatively small percentage of patients (3%) following even the most virulent bouts of streptococcal pharyngitis, and not all patients with ARF develop rheumatic heart disease (RHD). Therefore, the question of host predisposition is often raised by investigators.<sup>1,2,3</sup>

For more than a century, researchers have tried to determine a genetic pattern of susceptibility to RHD and RF. Cheadle in 1889 assigned an increased susceptibility to RHD or RF.<sup>4</sup> Some researchers have assumed an autosomal recessive model.<sup>5</sup> Occurrence of RF or RHD in identical twins suggests that if a Mendelian pattern is present, penetrance must be incomplete.<sup>6</sup> Recent studies have tried to uncover specific markers for RHD or RF susceptibility.

Some studies have analyzed human leukocyte class I antigens, but no consistent association of these antigens with RF has been found.

Subsequent studies of class II antigens have disclosed an association with different HLA-DR alleles according to the population analyzed.<sup>7</sup>

Therefore, considering the high incidence of RHD and the almost identical racial background of the population in western Iran and the importance of research in this field, this study was conducted with the aim of better understanding the mechanisms involved.

## Methods

### Patients

To determine the frequencies of class II antigens, HLA typing was performed in 35 patients with rheumatic heart disease. In all the cases, the diagnosis was made by a cardiologist and was supported by echocardiography, cardiac catheterization and histological findings (in patients who underwent surgery for heart valve replacement). The patients were from the western regions of Iran and were aged 20-

63 years old (mean age, 40.17). There were 32 females and 3 males.

The control group comprised 36 healthy individuals, racially and geographically similar to the patient group, who were 20-57 years old. HLA class II typing was performed in all the patients and controls.

### HLA typing

The HLA class II antigens were determined by the microlymphocytotoxicity test using specific DQ and DR anti-sera (Pel-Freez). This test is achieved by the isolation of mononuclear cells from the peripheral blood and purification of the peripheral blood B cells by adherence to nylon wool columns [Teresi method<sup>8</sup>]. Tests were performed on fresh blood samples in all the cases.

### Statistical analysis

The frequencies of HLA class II antigens were compared using Yates's correction  $\chi^2$  test and Fischer Exact test when the number of the individuals with a specified antigen was less than five. Probability values and relative risk (RR) were calculated.

## Results

Detailed results are presented in Table I.

**Table I. Frequency of HLA-DQ and HLA-DR antigens in patients and controls**

YC=Yates' correction

HLA	Patients (n=35) (%)	Controls (n=36) (%)	P value (YC)	P value (FE)	RR
DQ1	11.4	55.5	NS	-	...
DQ2	57.1	25.0	0.0119	-	4
DQ3	42.58	22.2	NS	-	...
DQ4	2.85	2.77	-	NS	...
DQ5	22.8	5.55	-	0.038	5.03
DQ7	11.4	13.8	-	NS	...
DR1	0.0	5.55	-	NS	...
DR2	31.4	2.77	0.0037	-	16.04
DR3	2.85	13.88	-	NS	...
DR4	14.28	8.3	-	NS	...
DR5	11.4	8.3	-	NS	...
DR6	14.2	5.5	-	NS	...
DR7	37.1	19.4	NS	-	...
DR8	8.57	13.88	-	NS	...
DR9	0.0	8.2	-	NS	...
DR10	5.7	2.8	-	NS	...
DR11	8.5	8.35	-	NS	...
DR12	8.6	8.3	-	NS	...
DR13	5.8	8.2	-	NS	...
DR15	28.57	2.77	0.007	-	14
DR52	57.1	47.2	NS	-	...
DR53	57.1	16.66	0.00099	-	6.66

FE= Fisher Exact test

RR=Relative risk

NS= Not significant

The class II HLA antigens were typed in 35 patients with RHD and compared with a control group of 36 healthy individuals. HLA-DR53 was positive in 20 patients (57.1%) compared with 16.7% in the control group ( $P=0.00099$ ,  $RR= 6.66$ ). The frequencies of HLA-DR2 (31.4% in patients versus 2.8% in the control group:  $P=0.0037$ ,  $RR=16.04$ ) and HLA-DR15 (28.6% in patients versus 2.8% in the control group:  $P=0.007$ ,  $RR= 14$ ) were found to have increased. Also, HLA-DQ2 and HLA-DQ5 frequencies were found to be higher ( $P<0.05$ ) in the patients compared with the control group, but as for the other antigens, the frequency difference was not significant.

## Discussion

Although an abnormal response following streptococcal infection has been mentioned to be a potential cause of RF and RHD, the exact mechanism has not yet been clarified. HLA antigens interfere in the presentation of the rheumatogenic determinants of beta-hemolytic streptococci to T lymphocytes, and certain HLA alleles increase the responsiveness to beta-hemolytic streptococci and the immune response against streptococci causes tissue damage, especially in the heart, due to cross-reaction of streptococcal antigens with heart valve antigens. Therefore, investigators have attempted to establish an association between HLA antigens and RF/RHD. Other immunological factors of RF and RHD are under investigation.<sup>3,9</sup>

HLA class II typing, first performed in the 1980s, has led to conflicting results. Jhinghan studied an Indian population and described a positive association between RF with HLA-DR3 and HLA-AW33 and a negative association with HLA-DR2 (in contrast to our results).<sup>7</sup> Anastasiou Nana described a higher frequency of HLA-DR4 and a lower frequency of HLA-DRW6 in Caucasian patients with RHD. Monplaisir showed a significant decreased in HLA-BW14 and BW42 frequencies and an increase in HLA-B35 and HLA-DR1 frequencies.<sup>3</sup> Ayoub et al. disclosed an association of RF with HLA-DR2 in black patients, and with HLA-DR4 and HLA-DRW9 in Caucasian patients.<sup>10</sup> Rajapakse defined HLA-DR4 as a genetic marker of RHD in the Saudi Arabian population.<sup>11</sup> In Ozkan's study, an increased frequency of DR3, DR7 and B16 phenotypes and a decreased frequency in DR5 was reported.<sup>12</sup>

Afana suggested an increased frequency of HLA-B17, HLA-B21 and HLA-CW4 phenotypes in patients with rheumatic myocardium.<sup>13</sup> Reddy found an increased frequency of DR3 phenotype and decreased frequency of DR2 in RHD patients.<sup>14</sup> Guilherme et al. found that

HLA-DR7 and HLA-DR53 were markers for susceptibility to RF and RHD in Brazil.<sup>9</sup>

Gu et al., having studied the genetic susceptibility of HLA-DQA1 alleles to RF or RHD in Chinese Hans by PCR-PAGE and then silver dyeing, suggested that DQA1\*0101 contributes to genetic susceptibility for RF and RHD in Guangdong Hans.<sup>15</sup> Guedez et al. studied class II allele/haplotype distribution in patients with RHD and found significant increases in DRB1\*0701 and DQA1\*0201 alleles and DRB1\*0701-DQA1\*0201 haplotypes.<sup>16</sup> Maharaj et al. performed HLA-A, HLA-B, HLA-DR and HLA-DQ surveys in Indian patients with severe chronic rheumatic heart disease requiring cardiac surgery and found that there was no significant difference in HLA-A, HLA-B, HLA-DR and HLA-DQ frequencies between patients and controls.<sup>17</sup>

Koyanagi et al. performed DNA typing of HLA class II genes (DRB1, DQA1, DQB1 and DPB1) in patients with RHD and suggested that the susceptibility to mitral stenosis is in part controlled by a gene (or genes) in close linkage disequilibrium with HLA-DQA1\*0104 and DQB1\*05031.<sup>18</sup>

Carlquist et al. examined HLA-DR frequencies in rheumatic heart disease by a meta-analysis study that showed a significant negative association with DR4 in all studies, increased DR1 and DR6 in black patients, increased DR3 in Eastern Indian patients, decreased DR2 (in contrast to our results) and DR5 and increased DR4 in American whites.<sup>19</sup> Bhat et al. studied the distribution of HLA class I (A,B,C) and class II (DR and DQ) antigens in patients with rheumatic heart disease in Kashmir. They found that susceptibility to RHD in the studied population was HLA-related, with HLA-DR4 influencing its occurrence and HLA-B5 conferring protection against disease.<sup>20</sup> Guedez et al. reported a significant increase in DRB1\*0701 and DQA1\*0201 alleles and DRB1\*0701-DQA1\*0201 haplotypes in patients with RHD and decreased

frequency of DQA1\*0103 allele and absence of DQB1\*0603 allele and concluded that certain class II alleles/haplotypes were associated with risk for or protection from RHD.<sup>21</sup>

Guilherme et al. in their study reported that T cells sensitized in the periphery by M5 protein during streptococcal infection, in particular the M5 (81-96) peptide in HLA DR7 and DR53-positive RHD patients, migrated to the heart and initiated heart tissue damage after activation due to cross-reactive recognition of the relevant heart antigen.<sup>22</sup>

The present work is the first study on the population of the west of Iran in order to determine HLA phenotype frequencies in patients with RHD. In this research, the frequencies of HLA-DR53, HLA-DR52 and HLA-DR15 were found to have significantly increased in the patients with RHD compared with the control group. The frequency of HLA-DR53 was especially higher, and this finding is similar to Guilherme's report.<sup>9</sup> HLA-DR9, which is included in the HLA-DR53 group, was absent in the patient group. Frequencies of HLA-DR4 and HLA-DR7 (other antigens in the HLA-DR53 group) were not statistically significantly different. The high frequency of HLA-DR2 and statistical significance of this is in concordance with Ayoub's results<sup>10</sup> and in contrast with Reddy's results<sup>14</sup>.

The frequencies of HLA-DQ2 and HLA-DQ5 in the present study were also high and notable ( $P < 0.05$ ), but the increased frequency of HLA-DR4 which has been found in other populations<sup>9,23</sup> was not high in the present study. HLA-DR1 and HLA-DR9 were absent in our patients.

Based on these results, HLA-DR53, HLA-DR2 and HLA-DR15 antigens or a gene out of the MHC complex, which has preferential motivation with this, may be involved in an abnormal immune response against streptococcal antigens, causing valvular damage and rheumatic heart disease. The difference between our results and those in other reports is probably

related to geographical or racial differences in populations and different patterns of reactivity to allo-antisera. Consequently, we can conclude that HLA-DR53, HLA-DR2 and HLA-DR15 may be markers for susceptibility to RHD, but other genetic factors might have roles in determining this susceptibility. Indeed multiple genetic factors may be interacting to define this susceptibility. On the other hand, we cannot underestimate the role of non-genetic factors, such as social and economic factors in the analysis of RHD and RF, as well as different streptococcal strains that may elicit different patterns of immune response.

### Conclusion

According to the present study and other reports, genetic difference resulting from racial and geographical variations must have a role in susceptibility to RHD, but major influence must be in the form of a "susceptibility gene" situated in or near the HLA-DR locus.

Further studies and evaluation of all the important limbs of the immune system, cellular and humoral immune parameters, as well as immunogenetic profiles may provide convincing evidence in the pathogenesis of rheumatic heart disease and rheumatic fever.

### References

1. Goldman L, Bennett JC, Alan L. Infectious Disease. In: Cecil Textbook of Medicine. 21<sup>st</sup> Edition, Vol. 3, Philadelphia, W. B. Saunders Co., 2000, pp. 1624-1631.
2. Schlant RC, Alexander RW, Charles E. Acute rheumatic fever. In: Hurst's The Heart. 8<sup>th</sup> Edition, Vol. 2, New York, McGraw-Hill Inc, 1994, pp. 1451-1457.
3. Olmez, U, Turgay M. Association of HLA class I and class II antigens with rheumatic fever in a Turkish population. Scand J Rheumatol. 1993; 22 (2): 49-52.
4. Cheadle WB. Harveian lectures on the various manifestations of the rheumatic state as exemplified in childhood and early life. Lancet 1889, 1:821-827.
5. Wilson MG, Schweitzer M. Pattern of hereditary susceptibility in rheumatic fever. Circulation 1954; 10: 699-704.
6. Taranata A, Torosdags-Metrakos JD, Jegier W, Uchida I. Rheumatic fever in monozygotic and dizygotic twins. Circulation 1959; 20: 788.
7. Jhinghan B, Mehra NK, Reddy KS. HLA, blood groups and secretor status in patients with established rheumatic fever and rheumatic heart disease. Tissue Antigens 1986, 27: 172-178.
8. Zachary A, Teresi G, Ashi Laboratory Manual. 2<sup>nd</sup> Ed, American Society for Histocompatibility and Immunogenetics, P. O. Box 15804, Lenexa, KS, USA, 66215.
9. Guilherme L, Weidebach W, Kiss MH, Sintcowsky R, Kalil J. Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a population. Circulation 1991; 83 (6): 1995-8.
10. Ayoub EM, Barret DJ. Association of class II histocompatibility leukocyte antigens with rheumatic fever. J Clin Invest 1986; 77: 2019-2026.
11. Rajapakse CN, Halim K. A genetic marker for rheumatic heart disease. Br Heart J 1987; 58: 659-662.
12. Ozkan M, Sonmez G, Senocak M, Ozdemir M, Yakut C. HLA antigens in Turkish race with rheumatic heart disease. Circulation 1993; 87 (6): 1974-8.
13. Afanas E, Kadyrova R, Iazdovskii W. The HLA associated immunological mechanisms in rheumatic involvement of the myocardium. Ter Arkh 1995; 67 (8): 56-9.

14. Reddy KS, Narula J, Bhatia R. Immunologic and immunogenetic studies in rheumatic fever and rheumatic heart disease. *Indian J Pediatr* 1990; 57 (5): 693-700.
15. Gu J, Yu B, Zhou J. HLA-DQA1 genes involved in genetic susceptibility to rheumatic fever and rheumatic heart disease in southern Hans. *Zhonghua Nei Ke Za Zhi* 1997; 36 (5): 308-11.
16. Guedez Y, Kotby A, El-Demellawy M. HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients. *Circulation* 1999; 99 (21): 2784-90.
17. Maharaj B, Khedun SM, Hammond MG. HLA-A, B, DR and DQ antigens in Indian patients with severe chronic rheumatic heart disease. *Jpn Heart J* 1997; 38 (5): 663-8.
18. Koyanagi T, Koga Y, Nishi H, et al. DNA typing of HLA class II antigens in Japan's patients with rheumatic heart disease. *Molecular Cell Cardiol* 1996; 28 (6): 1349-53.
19. Carlquist JF, Ward RH, Meyer KJ, et al. Immune response factors in rheumatic heart disease: meta analysis of HLA-DR associations and evaluation of additional class II alleles. *J Am Coll Cardiol* 1995; 26 (2): 452-7.
20. Bhat MS, Wani BA, Koul PA, et al. HLA antigen pattern of Kashmiri patients with rheumatic heart disease. *Indian J Med Res* 1997; 105: 271-4.
21. Guilherme L, Ohiro SE, Fae KC, et al. T- cell reactivity against streptococcal antigens in the periphery mirrors reactivity of heart infiltrating T lymphocytes in the rheumatic heart disease patients. *Infect Immune* 2001; 69 (9): 5345-51.
22. Kelley WN, Harris ED, Ruddy S. Immune and inflammatory response. In: *Textbook of Rheumatology*. Vol. 1, Fifth edition, Philadelphia, W. B. Saunders Co, 1993: pp. 95-122.
23. Maharaj B, Hammond MG. HLA-A, B, C and DR antigens in black patients with severe chronic rheumatic heart disease. *Circulation* 1987; 76: 259-261.
24. Patarroyo ME, Winchester RJ, Vejerano A. Association of a B-cell alloantigen with susceptibility to rheumatic fever. *Nature* 1979; 278: 173-174.
25. Zabriskie JB, Lavenchy D. Rheumatic fever associated B-cell alloantigens as identified by monoclonal antibodies. *Arthritis Rheum* 1985; 28: 1047-1051.
26. Taneja V, Mehra NK, Reddy KS. HLA-DR/DQ antigens and reactivity to cell alloantigen D8/17 in Indian patients with rheumatic heart disease. *Circulation* 1989; 80: 335-340.
27. Khana AK, Buskirk DR, Williams R. Presence of a non-HLA B cell antigen in rheumatic fever patients and their families as defined by monoclonal antibody. *J Clin Invest* 1989; 83: 1710-171.
28. Hafez M, Abdalla A, Shennawy F, Tonbary Y. Immunogenetic study of the response to streptococcal carbohydrate antigen of the cell wall in rheumatic fever. *Ann Rheum Dis* 1990; 49 (9): 708-14.
29. Khosroshahi HE, Kahramany. HLA and Rheumatic fever in Turkish children. *Pediatr Cardiol* 1992; 13 (4): 204-7.
30. Benjamin D, Schwartz A, McCarthy DJ. Structure, function and genetics of the HLA complex in rheumatic disease. *Arthritis and Allied Conditions*. In: *A Textbook of Rheumatology*. 20<sup>th</sup> ed., 1993; pp. 509-523.
31. Wolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1955; 19: 251-3.
32. Kemeny E, Husby G, Williams RC, Zabriskie JB. Tissue distribution of antigens defined by monoclonal antibody D8/17 reacting with B lymphocytes of patients with rheumatic heart disease. *Clin Immunol Immunopathol* 1994 72:35-43.