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RESEARCH ARTICLE

Association of HLA-DRB1*14 with rheumatic heart disease patients from Chandigarh, North India

Devinder Toor[1,2], Komal Leal[1], Rajesh Kumar[2], Yash Paul Sharma[3], and Anuradha Chakraborti[1]

[1]*Department of Experimental Medicine and Biotechnology,* [2]*School of Public Health, and*
[3]*Department of Cardiology; Postgraduate Institute of Medical Education and Research, Chandigarh, India*

Abstract

Context: Acute rheumatic fever (ARF)/rheumatic heart disease (RHD) is a major cause of morbidity and mortality in India, yet, few studies are available on susceptibility markers.

Objective: To associate human leukocyte antigen (HLA) class II alleles with north Indian RHD patients as genetic susceptibility markers.

Materials and methods: HLA alleles were analysed using sequence specific primer-polymerase chain reaction and nucleotide sequencing, while HLA-B27 expression by flowcytometry.

Results: Few HLA-DQB1/DRB1 alleles were associated with RHD and HLA-DRB1*14 gene polymorphism revealed two single nucleotide polymorphisms (SNPs) in patients. Bioinformatic predictions showed influence of SNPs on protein function. HLA-B27 was positive in 42.85% ARF patients.

Conclusion: The study showed association of different HLA class II alleles with RHD in North Indian population.

Keywords: Group A streptococcus (GAS), HLA-B27, HLA gene polymorphism, single nucleotide polymorphism

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Introduction

Streptococcus pyogenes or group A streptococci (GAS) causes a spectrum of diseases including its most serious sequelae, i.e. acute rheumatic fever (ARF) and rheumatic heart disease (RHD). It is a major cause of morbidity and mortality throughout the world (Carapetis et al. 2005) including India (Shet & Kaplan 2004). Siegel et al. (1961) suggested a genetic basis of this disease as ARF develops only in 0.3%–3% cases of GAS pharyngitis while Read et al. (1983) and Schwentker (1952) revealed that ARF occurred 4–8 times more frequently in relatives of patients.

Recent data on the molecular genetics of the human leukocyte antigen (HLA) provides direct support to a genetic basis for susceptibility to several diseases. The association of specific HLA antigen with many autoimmune diseases has been well established (Bowness 2002). It has led to an extensive search for a specific HLA associated with rheumatic fever/rheumatic heart disease (RF/RHD). Although HLA-B27 has been associated with several autoimmune and infectious diseases, however, its association with RF/RHD has not been studied. The inconsistency of HLA class I association with RHD prompted further studies to focus on class II antigens. Several studies suggest that genetic susceptibility to RF/RHD is linked to different HLA class II alleles in different ethnic population of the world (Bryant et al. 2009).

The inconsistency among susceptibility and/or protective alleles in earlier studies might be because of technical inefficiency, besides the ethnic differences in the distribution of HLA alleles. However, the present study has overcome this drawback using sequence specific primer-polymerase chain reaction to identify specific HLA type as a genetic susceptibility marker for RHD patients in Chandigarh, North India which could provide valuable clues about susceptible individuals.

Address for Correspondence: Dr. Anuradha Chakraborti, Professor, Department of Experimental Medicine & Biotechnology, PGIMER, Chandigarh - 160012, India. Tel: +91 172 2746277. Fax: +91 172 2744401, 2745078. E-mail:superoxide@sify.com

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Methods

Patients of RF/RHD from the North Indian states of India, who visited the Advanced Cardiac Center, Department of Cardiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh were enrolled in the study. The subjects were categorized into following groups: (1) 20 ARF patients diagnosed using Jones criteria, (2) 60 RHD patients diagnosed clinically by a cardiologist having a valvular lesion [severe mitral stenosis (MS) or regurgitation (MR)] confirmed by auscultation or electrocardiography and (3) equal number of age and sex matched apparently healthy controls. Ten milliliter of peripheral venous blood was withdrawn after informed consent of the subjects and their care takers. The study was carried out after approval by the ethics committee of PGIMER, Chandigarh.

Preparation of genomic DNA

The genomic DNA was isolated using DNA isolation kit (Real Biotech Corporation, Taiwan) as per the manufacturer's instruction.

HLA typing using sequence specific primer polymerase chain reaction

HLA typing was carried out using HISTO TYPE/DNA-DR/DQ kits (Biologische Analysensystem GmbH, Germany). Positive control provided in the kit was also run along with samples. The protocol followed was as per the manufacturer's instruction. For interpretation of HLA type, the specificity table and evaluation diagrams provided with the kit were used and bands that corresponded in size with that mentioned in the specificity table were considered positive.

HLA-DRB1*14 polymerase chain reaction

Amplification of HLA-DRB1*14 gene was carried out at the described conditions using specific published primers (Lee et al. 1996). The amplified products were visualized on ChemiImager™ 4400 (Alphainnotech, USA) after electrophoresis on 0.8% agarose gel. To detect size of the amplified product, 100 bp DNA ladder (Fermentas, Canada) was used as a marker. Negative control was also run along the samples and each experiment was repeated thrice.

Polymerase chain reaction product purification

Purification of the polymerase chain reaction (PCR) product was done using RBC HiYield™ PCR DNA purification kit (RBC, Taiwan), as per the manufacturers instruction and the DNA was eluted in 15 μL of double distilled water.

DNA sequencing reaction

Sequencing reaction was carried out using ABI PRISM® dGTP BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA). For sequencing reaction, PCR product was used as template DNA (1.0–2.0 μL as per the concentration of the purified PCR product) along with forward primer (1.6 μL of 1 pmol concentration), PCR buffer (1.5 μL of 1x), water (variable) and the ABI PRISM BigDye. Ten micro liter of the reaction was set up for sequencing and the samples were subjected to thermocycler GeneAmp® PCR system 9700 (Applied Biosystems, USA). The cycling parameters for 25 cycles were 95°C for 10 s, 55°C for 5 s and 60°C for 4 min.

Big dye terminator cleanup

Unincorporated Big Dye Terminators were removed by clean up of the sequencing reaction product. Briefly, 12 μL of Master Mix I (10 μL milliQ water and 2 μL of 125 mM EDTA per reaction) was added to 10μL of the reaction product along with 52 μL of Master Mix II (2 μL of 3 M sodium acetate and 50 μL of ethanol per reaction). Then the mixture was incubated for 15 min and centrifuged at 12,000×*g* for 20 min. Seventy percent ethanol was added to the pellet and centrifuged at 12000×*g* for 10 min at room temperature and the DNA pellet was air dried. Twelve micro liter of Hi-Di formamide was added and denatured at 95°C for 5 min. The samples were snap chilled and proceeded for automated sequencing in 3130xl Genetic Analyzer (Applied Biosystems, USA).

HLA-B27 expression

The HLA-B27 antigen detection was done using BD HLA-B27 kit (BD, USA) which is a qualitative, two color direct immunofluorescence method for rapid detection of HLA-B27 antigen expression in erythrocyte-lysed whole blood using BD flowcytometer. The protocol followed was as per the manufacturer's instruction. Acquisition of the stained samples was done with BD HLA-B27 software for BD FACSCalibur. Approximately, 15,000 total events or 2,000 T lymphocytes were acquired. The acquisition software automatically gated the T lymphocytes in dot plots of CD3 PE versus scatter, calculated the log median fluorescence and generated the result with respect to the decision marker as positive or negative.

Bioinformatics analysis

Basic Local Alignment Search Tool (BLAST) was carried out using the obtained nucleotide sequences of HLA-DRB1*14 at http://blast.ncbi.nlm.nih.gov/. The sequences were used further to detect single nucleotide polymorphism (SNP).

To find out SNPs, the nucleotide sequences of controls and patients were aligned with the known sequence from the GenBank database. Multiple alignments were carried out using online Multalin (http://multalin.toulouse.inra.fr/multalin/) program.

Each SNP has the potential to affect protein function. Sorting Intolerant from Tolerant (SIFT) makes prediction on the fact that a highly conserved position should be intolerant to most substitutions, whereas a poorly conserved position can tolerate more substitutions and on the nature of amino acid that is substituted. SIFT analysis for HLA-DRB1*14 was carried out online at http://sift.jcvi.org/.

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Statistical analysis

Statistical analysis was carried out using GraphPad PRISM (version 5) software. p value, odds ratio (OR) and 95% confidence interval (CI) was calculated using Fisher's exact test.

Results

Patient characteristics

Twenty ARF patients (mean age 10 years) clinically diagnosed as ARF without carditis (75%, $n=15$), ARF with carditis (20%, $n=4$) and chorea (5%, $n=1$) were enrolled. Sixty RHD patients (mean age 33 years) were enrolled out of which, 70% ($n=42$) were diagnosed with severe MS, 25% ($n=15$) with severe MR, while 5% ($n=3$) had severe aortic stenosis.

HLA typing

Genomic DNA prepared from RHD patients ($n=60$) and controls were used to carry out PCR using HLA typing kit.

HLA-DQ typing

Out of the six HLA-DQ alleles, we observed a significantly high frequency of DQB1*08 (82%; $p<0.0001$) followed by DQB1*16 (79%; $p<0.0002$) and DQB1*03 (39%; $p<0.0001$). The frequency of DQB1*06 allele was found to be higher in patients as compared to controls ($p>0.05$). In contrast, control samples showed more of DQB1*02 and DQB1*05 allele than patients (Table 1).

HLA-DR typing

Similarly, HLA-DR typing was carried out using HLA-DRB typing kit (BAG, Germany). Out of the 14 DR alleles, significantly high frequency ($p<0.0001$) of DRB1*14 was observed followed by DRB1*04 ($p=0.01$). All other alleles, i.e. DRB1*01, *07, *08, *11, *12, *13, DRB3*01 and DRB4*01 also showed high frequency but were not statistically significant when compared to controls. A strong negative association was observed with DRB1*15 ($p<0.01$). DRB1*03, *10 and DRB5*01 also showed low frequency in patients, but the association was not statistically significant (Table 2).

Gene polymorphism in HLA-DRB1*14

Since HLA-DRB1*14 allele was the predominant HLA type in RHD patients of north India, further studies were carried out on all RHD samples to rule out any polymorphism in this gene. HLA-DRB1*14 specific PCR product of 117 bp was purified and subjected to nucleotide sequencing. BLAST analysis showed hit with HLA-DRB sequence on the short arm of human chromosome 6 at 6p21 region. The obtained patient sequence was aligned using online MultAlin program with HLA-DRB1*14

Table 1. Frequency of HLA-DQ alleles among RHD patients and controls of Chandigarh, North India.

Allele	Patients ($n=60$) %	Controls ($n=60$) %	Odds ratio (OR)	95% CI	p value	Association
DQB1*02	20.3	27.0	0.61	0.34–1.3	0.3	NS
DQB1*03	38.6	00.0	222.3	13–3779	<0.0001*	Positive
DQB1*05	9.0	55.0	0.01	0.005–0.051	<0.0001*	Negative
DQB1*06	61.9	35.5	1.69	0.96–2.98	0.09	NS
DQB1*08	81.8	37.5	7.75	4.04–14.8	<0.0001*	Positive
DQB1*16	78.8	37.5	3.36	1.8–6.2	<0.0002*	Positive

*Statistically significant.
NS, non significant.

Table 2. Frequency of HLA-DR alleles among RHD patients and controls of Chandigarh, North India.

Allele	Patients ($n=60$) %	Controls ($n=60$) %	Odds ratio (OR)	95% CI	p value	Association
DRB1*01	34.3	22.5	1.82	0.97–3.42	0.09	NS
DRB1*03	18.1	25.0	0.67	0.28–1.61	0.5	NS
DRB1*04	63.6	37.5	2.77	1.32–5.82	0.01*	Positive
DRB1*07	09.0	00.0	11.99	0.64–222	0.06	NS
DRB1*08	36.3	25.0	1.73	0.79–3.81	0.2	NS
DRB1*10	01.6	12.5	0.12	0.02–1.67	0.06	NS
DRB1*11	54.5	37.5	1.97	0.95–4.09	0.09	NS
DRB1*12	25.2	14.0	2.04	0.99–4.22	0.07	NS
DRB1*13	54.5	25.0	1.97	0.95–4.09	0.09	NS
DRB1*14	100	25.0	595.1	35.6–9939	<0.0001*	Positive
DRB1*15	18.1	50.0	0.21	0.11–0.41	<0.01*	Negative
DRB3*01	72.7	62.5	1.46	0.67–3.15	0.4	NS
DRB4*01	27.2	12.5	2.36	0.92–6.04	0.1	NS
DRB5*01	22.1	35.5	0.52	0.27–0.98	0.059	NS

*Statistically significant.
NS, non significant.

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Table 3. HLA-B27 expression by flowcytometry in different subgroups.

Categories	No. of cases	–ve	+ve
RF cases	20	14	6
RF parents	20	16	4
RHD cases	50	50	0
Controls	50	50	0

RF, rheumatic fever; RHD, rheumatic heart disease.

sequence available on Genbank (GenBank:GQ302516.1). On sequence comparison, SNP was observed at two positions. The first point mutation was transversion (A→T) at position 43 and the other was transition (T→C) at position 118.

To understand the effects of these point mutations, the nucleotide sequence was translated to peptide sequence (http://expasy.org/tools/dna.html) and the protein BLAST matched with human HLA-DRB1 fragment and HLA-DRB1*14. To know the position of substituted amino acid in RHD patients, the peptide sequences were aligned using MultAlin tool. First SNP caused substitution of asparagine (hydrophilic amino acid) with isoleucine (hydrophobic amino acid) at position 14 (N14I) of the peptide sequence, while second SNP caused substitution of valine with alanine (both belong to hydrophobic aliphatic amino acid group) at position 39 (V39A) of the peptide sequence. The amino acid substitutions were further analysed using the online SIFT (Sorting Intolerant from Tolerant) program which predicted that substitution N14I would affect protein function, while V39A was tolerated and did not reveal affect on protein function.

Expression of HLA-B27

Flowcytometric HLA-B27 expression study was carried out on 20 ARF patients, 50 RHD patients and controls using 50 μL of whole blood sample. Parent (either mother or father) of ARF patients were also included in the study. Out of 20 RF patients screened for HLA-B27, 42.85% (14/20) were positive for this allele. Interestingly, four parents out of six (66.66%) HLA-B27 positive cases were also positive for HLA-B27. However, all RHD patients ($n=50$) tested negative for HLA-B27 antigen. The result in normal healthy controls was similar to that in RHD patients, i.e. all controls were also negative for this antigen (Table 3).

Discussion

The pathogenesis of RF/RHD is believed to involve the triad of a genetic susceptible individual infected with rheumatogenic strain of GAS with an anomalous host immune response. Host genetic factors have been associated with susceptibility to ARF and subsequent progression to RHD as earlier studies have shown low attack rate (up to 3%) of ARF after untreated streptococcal pharyingitis (Bowness 2002), relatively high concordance rate for RF in monozygotic twins (19%) in comparison to dizygotic twins (2.5%) (DiSciascio & Taranta 1980) and high familial incidence of ARF (Pickles 1943). Interest in the genetic aspects of RF/RHD occurred with the recognition that gene product of HLA is significantly associated with a number of autoimmune and rheumatic diseases. The fact that there are few Indian studies done using serological typing method way back during 1980's necessitated the screening for HLA types using more accurate molecular method in order to find the prevalent HLA type as the genetic susceptibility marker of RHD in north Indian population.

In our study, HLA-DQB typing showed highest positive association of RHD with DQB1*08 which has also been associated with RHD patients in Turkey (Gundogdu et al. 2007). In this study, high frequency of HLA-DQB1*16, DQB1*06 and DQB1*03 was found. HLA-DQB1*03 has been associated with RHD in Mexican (Hernandez-Pacheco et al. 2003), Latvian (Guedez et al. 1999) and Indian Kashmiri patients (Wani 1997). No study so far had shown association of RHD with DQB1*06 and DQB1*16. A statistically significant negative association of RHD was observed with DQB1*05 in our study and Latvian RHD patients (Stanevicha et al. 2003), while positive association was seen among Japanese patients (Koyanagi et al. 1996). DQB1*02 has been associated with Mexican RHD patients, but Indian study of Kashmiri RHD patients showed a negative association, as in this study.

HLA-DR typing results showed statistically significant positive association with HLA-DRB1*14 and DRB1*04. Although earlier studies did not report any association of DRB1*14 with RHD, Nepam et al. (1995) showed association of rheumatoid arthritis with DRB1*01, DRB1*04 and DRB1*14. All these three alleles showed high frequency in RHD patients, suggesting their association with rheumatism. DRB1*04 has been associated with RHD patients from various ethnic populations of the world like Caucasion–Americans (Anastasious-Nana et al. 1986; Ayoub et al. 1986), Turkey (Khosroshahi et al. 1992) and Saudi Arabians (Rajapakse et al. 1987). Indian studies reported so far on RHD patients have shown high frequency of DR4 (Wani 1997; Jhinghan et al. 1986; Reddy et al. 1990) which is consistent with this study. HLA-DR4 is significantly common in patients with post streptococcal glomerulonephritis (Layrisse et al. 1983). In ARF and glomerulonephritis, the abnormal immunological response directed against connective tissue is initiated by *S. pyogenes* which suggests that an enhanced response to streptococci was another factor predisposing HLA-DR4 positive individuals to RF and its complications.

Apart from DRB1*14 and DRB1*04, high frequency of HLA-DRB1*01, *07, *08, *11, *12, *13, DRB3*01 and DRB4*01 was also observed. Out of these, DRB1*01 was associated with RHD patients of Martinique (Monplaisir et al. 1986), Black South Africans (Maharaj et al. 1987) and showed a negative association in Turkish RHD

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patients (Gundogdu et al. 2007). Earlier Indian studies showed low frequency of DR1 which was not the case in this study. DR*07 has been associated with Brazilian (Guilherme et al. 1991; Visentainer et al. 2000), Turkish (Gundogdu et al. 2007), Latvian (Stanevicha et al. 2003), Pakistani (Rehman et al. 2007) and Egyptian (Guedez 1999) patients and is associated with the development of valvular lesion. HLA-DR11 contradicts the results as it showed negative association in other populations (Hernandez-Pacheco et al. 2003). DRB1*13 was only found associated in Egyptian RHD patients (Stanevicha et al. 2003). No reports of association with other alleles were found. Earlier studies on patients of North India showed high frequency of DRB1*07 and DRB1*08 (Jhinghan et al. 1986; Reddy et al. 1990) which is consistent with results of present study. However, it contradicts the study on Kashmiri patients, where both these alleles showed low frequency.

Present study showed statistically significant negative association of HLA-DRB1*15 with RHD. However, there is no earlier study which had shown any association with this allele along with DRB1*10 and DRB5*01. DR3 had shown positive association in patients of India (Jhinghan et al. 1986; Reddy et al. 1990), Turkey (Ozkan et al. 1993), Latvia (Guedez 1999) and Mexico (Hernandez-Pacheco et al. 2003), but it showed low frequency in this study.

Reasons for the observed discrepancies could be the use of serological HLA typing method in the previous studies which were reported to be prone to discrepancies, that may reach 40% (Ayoub & Majeed 2000; Carlquist & Anderson 1993; Erlich et al. 2001; Mytilineos et al. 1994) and secondly the heterogeneity among the study population (Khosroshahi et al. 1992; Olmez et al. 1993). Also, since HLA class II antigens play an important role in antigen presentation to T cells, the inconsistent association with HLA antigens in different populations is in harmony with the possibility that different GAS *emm* types are associated with ARF/RHD in different countries.

For the first time, HLA-DRB1*14 was found to be associated with RHD and was present in all the RHD patients of North India. Therefore, further study on this allele was done to detect gene polymorphism, if any. Although only a small proportion of polymorphisms may be of functional relevance, all are of potential value as genetic markers for mapping regions of DNA that determine disease susceptibility. HLA-DRB1*14 gene sequence from RHD patients revealed two point mutations and SIFT analysis predicted that the substitution at position 14 can affect protein function, while the other substitution at position 39 can be tolerated.

According to the structural model of the class II molecule, polymorphism can change the charge on the class II α and β chain, thereby disrupting antigen presentation (Deschamps & Khalil 1993). Promoter polymorphism may induce some cells to express class II molecules that would not ordinarily transcribe the HLA II genes. These abnormalities may cause an autoimmune response (Leech et al. 1995; Christiansen et al. 1999). The function of HLA is to present the antigen to the T cells, so it could be assumed that these SNPs may be associated with over expression of HLA-DRB1*14 on the surface of B cells which may cause high antigen presentation to T cells, thereby leading to abnormal immune response and further causing damage to the heart valves.

Our next focus was on HLA-B27, as it has been strongly associated with various autoimmune diseases (Bowness 2002). RHD is considered an autoimmune disorder, however, none of the earlier report showed association of HLA-B27 with RHD. Our study showed for the first time that HLA-B27 was positive in ~43% ARF patients, as there is no report of HLA-B27 association with RF. Our study showed all RHD patients to be negative for HLA-B27 which was consistent with earlier Indian study carried out serologically on North Indian population (Maharaj et al. 1997). All controls were also negative for HLA-B27 comparable to earlier Indian study which showed presence of HLA-B27 in 2.1% controls of north India (Maharaj et al. 1997). Thus, the study suggests that HLA-B27 could be one of the susceptibility marker expressed during the acute phase of ARF to initiate an immune response and subsides during the progression towards chronicity.

Conclusion

The data from the present study has focused on screening the susceptible individuals for RHD and to fill in the void of studies on susceptibility markers in RHD patients of north India. Besides HLA, several non-HLA markers have also been associated in ARF/RHD disease progression. Therefore, further studies are needed to understand the overall mechanism of genetic susceptibility.

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Declaration of interest

The authors report no conflicts of interest.

References

Anastasiou-Nana MI, Anderson JL, Carlquist JF, Nanas JN. (1986). HLA-DR typing and lymphocyte subset evaluation in rheumatic heart disease: A search for immune response factors. *Am Heart J* 112:992–997.

Ayoub EM, Majeed HA. (2000). Poststreptococcal reactive arthritis. *Curr Opin Rheumatol* 12:306–310.

Ayoub EM, Barrett DJ, Maclaren NK, Krischer JP. (1986). Association of class II human histocompatibility leukocyte antigens with rheumatic fever. *J Clin Invest* 77:2019–2026.

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Bowness P. (2002). HLA B27 in health and disease: A double-edged sword? *Rheumatology (Oxford)* 41:857–868.

Bryant PA, Robins-Browne R, Carapetis JR, Curtis N. (2009). Some of the people, some of the time: Susceptibility to acute rheumatic fever. *Circulation* 119:742–753.

Carapetis JR, Steer AC, Mulholland EK, Weber M. (2005). The global burden of group A streptococcal diseases. *Lancet Infect Dis* 5:685–694.

Carlquist JF, Anderson JL. (1993). HLA, autoimmunity, and rheumatic heart disease. Apparent or real associations? *Circulation* 87:2060–2062.

Christiansen OB, Ring M, Rosgaard A, Grunnet N, Gluud C. (1999). Association between HLA-DR1 and -DR3 antigens and unexplained repeated miscarriage. *Hum Reprod Update* 5:249–255.

Deschamps I, Khalil I. (1993). The role of DQ α-β heterodimers in genetic susceptibility to insulin-dependent diabetes. *Diabetes Metab Rev* 9:71–92.

DiSciascio G, Taranta A. (1980). Rheumatic fever in children. *Am Heart J* 99:635–658.

Erlich HA, Opelz G, Hansen J. (2001). HLA DNA typing and transplantation. *Immunity* 14:347–356.

Guédez Y, Kotby A, El-Demellawy M, Galal A, Thomson G, Zaher S, Kassem S, Kotb M. (1999). HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients. *Circulation* 99:2784–2790.

Guilherme L, Weidebach W, Kiss MH, Snitcowsky R, Kalil J. (1991). Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a Brazilian population. *Circulation* 83:1995–1998.

Gündogdu F, Islamoglu Y, Pirim I, Gurlertop Y, Dogan H, Arslan S, Sevimli S, Aksakal E, Senocak H. (2007). Human leukocyte antigen (HLA) class I and II alleles in Turkish patients with rheumatic heart disease. *J Heart Valve Dis* 16:293–299.

Hernández-Pacheco G, Aguilar-García J, Flores-Domínguez C, Rodríguez-Pérez JM, Pérez-Hernández N, Alvarez-Leon E, Reyes PA, Vargas-Alarcón G. (2003). MHC class II alleles in Mexican patients with rheumatic heart disease. *Int J Cardiol* 92:49–54.

Jhinghan B, Mehra NK, Reddy KS, Taneja V, Vaidya MC, Bhatia ML. (1986). HLA, blood groups and secretor status in patients with established rheumatic fever and rheumatic heart disease. *Tissue Antigens* 27:172–178.

Khosroshahi HE, Kahramanyol O, Doganci L. (1992). HLA and rheumatic fever in Turkish Children. *Pediatr Cardiol* 13:204–207.

Koyanagi T, Koga Y, Nishi H, Toshima H, Sasazuki T, Imaizumi T, Kimura A. (1996). DNA typing of HLA class II genes in Japanese patients with rheumatic heart disease. *J Mol Cell Cardiol* 28:1349–1353.

Layrisse Z, Rodríguez-Iturbe B, García-Ramírez R, Rodríguez A, Tiwari J. (1983). Family studies of the HLA system in acute post-streptococcal glomerulonephritis. *Hum Immunol* 7:177–185.

Lee K, Park T, Park Y, Oh M, Kim Y. (1996). DNA polymorphism analysis of the HLA-DRB1 gene using polymerase chain reaction-sequence specific primer (PCR-SSP) among Korean subjects. *J Biochem Mol Bio* 29:45–51.

Leech N, Sorrentino R, McCulloch DK, Nepom GT. (1995). Ultrastructural allelic variation in HLA-DQB1 promoter elements. *Hum Immunol* 43:251–258.

Maharaj B, Hammond MG, Appadoo B, Leary WP, Pudifin DJ. (1987). HLA-A, B, DR, and DQ antigens in black patients with severe chronic rheumatic heart disease. *Circulation* 76:259–261.

Maharaj B, Khedun SM, Hammond MG, van der Byl K. (1997). HLA-A, B, DR, and DQ antigens in Indian patients with severe chronic rheumatic heart disease. *Jpn Heart J* 38:663–668.

Monplaisir N, Valette I, Bach JF. (1986). HLA antigens in 88 cases of rheumatic fever observed in Martinique. *Tissue Antigens* 28:209–213.

Mytilineos J, Scherer S, Dunckley H, Chapman J, Middleton D, Opelz G. (1994). Comparison of serological and DNA HLA-DR typing results for transplantation in Western Europe, Eastern Europe, North America and South America. *Transpl Int* 7 Suppl 1:S519–S521.

Nepom GT. (1995). Class II antigens and disease susceptibility. *Annu Rev Med* 46:17–25.

Olmez U, Turgay M, Ozenirler S, Tutkak H, Düzgün N, Duman M, Tokgöz G. (1993). Association of HLA class I and class II antigens with rheumatic fever in a Turkish population. *Scand J Rheumatol* 22:49–52.

Ozkan M, Carin M, Sönmez G, Senocak M, Ozdemir M, Yakut C. (1993). HLA antigens in Turkish race with rheumatic heart disease [see comment]. *Circulation* 87:1974–1978.

Pickles WN. (1943). Rheumatic fever. *Lancet* 2:241–244.

Rajapakse CN, Halim K, Al-Orainey I, Al-Nozha M, Al-Aska AK. (1987). A genetic marker for rheumatic heart disease. *Br Heart J* 58:659–662.

Read FEM, Ciocco A, Taussing HB. (1983). Frequency of rheumatic manifestations among siblings, parents, uncles, aunts and grandparents of rheumatic and control patients. *Am J Hyg* 27:719–737.

Reddy KS, Narula J, Bhatia R, Shailendri K, Koicha M, Taneja V, Jhingan B, Pothineni RB, Malaviya AN, Mehra NK. (1990). Immunologic and immunogenetic studies in rheumatic fever and rheumatic heart disease. *Indian J Pediatr* 57:693–700.

Rehman S, Akhtar N, Ahmad W, Ayub Q, Mehdi SQ, Mohyuddin A. (2007). Human leukocyte antigen (HLA) class II association with rheumatic heart disease in Pakistan. *J Heart Valve Dis* 16:300–304.

Schwentker FF. (1952). The epidemiology of rheumatic fever. In: Thomas L, ed. *Rheumatic fever*. Minneapolis university of Minneapolis Press, 17–27.

Shet A, Kaplan E. (2004). Addressing the burden of group A streptococcal disease in India. *Indian J Pediatr* 71:41–48.

Siegel AC, Johnson EE, Stollerman GH. (1961). Controlled studies of streptococcal pharyngitis in a pediatric population. Factors related to the attack rate for rheumatic fever. *New Eng J Med* 265: 559–565.

Stanevicha V, Eglite J, Sochnevs A, Gardovska D, Zavadska D, Shantere R. (2003). HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. *Arthritis Res Ther* 5:R340–R346.

Visentainer JE, Pereira FC, Dalalio MM, Tsuneto LT, Donadio PR, Moliterno RA. (2000). Association of HLA-DR7 with rheumatic fever in the Brazilian population. *J Rheumatol* 27:1518–1520.

Wani BA. (1997). Study of HLA-A, B, C, DR, DQ profile of patients with established rheumatic heart disease in Kashmir. *Indian Heart J* 49:152–154.

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