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HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia

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Abstract

Genetic control of immune reactions has a major role in the development of rheumatic heart disease (RHD) and differs between patients with rheumatic fever (RF). Some authors think the risk of acquiring RHD is associated with the HLA class II DR and DQ loci, but other views exist, due to the various HLA-typing methods and ways of grouping cases. Our goal was to determine the relations between HLA class II alleles and risk of or protection from RF in patients with relatively homogeneous clinical manifestations. A total of 70 RF patients under the age of 18 years were surveyed in Latvia. HLA genotyping of DRB1*01 to DRB1*18 and DQB1*0201-202, *0301-305, *0401-402, *0501-504, and *0601-608 was performed using polymerase chain reaction sequence-specific primers. Data for a control group of 100 healthy individuals typed for HLA by the same method were available from the databank of the Immunology Institute of Latvia. Of the RF patients, 47 had RHD and 8 had Sydenham's chorea. We concluded that HLA class II DRB1*07-DQB1*0401-2 and DRB1*07-DQB1*0302 could be the risk alleles and HLA class II DRB1*06 and DQB1*0602-8, the protective ones. Patients with mitral valve regurgitation more often had DRB1*07 and DQB1*0401-2, and patients with multivalvular lesions more often had DRB1*07 and DQB1*0302. In Sydenham's chorea patients, the DQB1*0401-2 allele was more frequent. Genotyping control showed a high risk of RF and RHD in patients with DRB1*01-DQB1*0301-DRB1*07-DQB1*0302 and DRB1*15-DQB1*0302-DRB1*07-DQB1*0303.

Keywords: alleles, genetic, HLA class II, rheumatic fever, rheumatic heart disease

Introduction

According to data from the Latvian Rheumatic Disease Patient Registry, 1298 children (born between 1984 and 2002) suffer from rheumatic diseases, of which rheumatic fever (RF) is the third most frequent. RF is an autoimmune connective tissue disease that develops after group A beta haemolytic streptococcal infection. The typical feature of the disease is the formation of autoantibodies against the connective tissue structures in the heart, synovial tissue, and neurons in the central nervous system. Rheumatic heart disease (RHD) is one of the most severe consequences of RF and is the main cause of acquired valvular RHD in the world [1–9]. In Latvia, since 1991 the number of RF cases in children under the age of 18 has increased, reaching an incidence of 7.5/100,000 in 1998. From 1999 to 2002, the incidence was stable, at 2.1/100,000 children.

Several authors have documented the familial occurrence of the disease [10–12], and it has been concluded that susceptibility to RF is inherited as a single recessive gene [13]. More substantial evidence for a genetic association was provided by Khanna and associates, who reported that B-cell alloantigens, designated D8-17, were present in 99% of patients with RF [13]. Further support for the role of genetic factors in susceptibility was provided by studies on the associations of this disease with inheritance of the HLA major histocompatibility antigens [14–23]. Several studies have suggested that genetic susceptibility to RF and RHD is linked to HLA class II alleles [20,24–34].

AVR = aortal valve regurgitation; MVL = multivalvular lesion; MVR = mitral valve regurgitation; OR = odds ratio; PCR–SSP = polymerase chain reaction with amplification with sequence-specific primers; PCR = polymerase chain reaction; RF = rheumatic fever; RHD = rheumatic heart disease.

However, there has been an apparent discrepancy concerning the nature of susceptibility or protective alleles [27]. One explanation is that most investigations used serological HLA genotyping methods that were less than accurate, leading to false results and failure to discriminate between allele subgroups.

Genetic associations are more likely to be detected in clinically homogeneous groups of patients, and therefore it is important to separate carditis patients from patients without carditis [27]. In studies in which RF patients with carditis were analysed separately from those without carditis, or in which only RHD patients mostly with mitral valve disease were studied, the reported HLA associations were rather similar [20,25–28,34,35].

We analysed the case histories of all white children under 18 years of age living in Latvia who had been affected by RF, with the aim to determine risk/protective alleles for RF and RHD. The HLA class II alleles were determined using polymerase chain reaction (PCR) in clinically homogeneous patient groups: RF patients with or without carditis and in patients with a diagnosis of an RHD – mitral valve regurgitation (MVR), mitral and aortal valve regurgitation or multivalvular lesion (MVL), and aortal valve regurgitation (AVR), and also cases of Sydenham's chorea.

Materials and methods

Subjects

The study included 70 white children (48 boys [68.5%] and 22 girls [31.4%]) in Latvia under the age of 18 (born between 1984 and 2002) who had RF. Of these, 23 (32.8%) were less than 7 years old and 47 (67.1%) were 7 or older. The RF diagnosis was confirmed according to Jones criteria. Eight RF patients had Sydenham's chorea. As a result of RF, 47 patients (67.1%) had developed RHD. Cardiac valve damage was diagnosed by echocardiography and/or heart catheterisation. RHD patients were further split into groups with MVR ($n=24$; 34.3%), AVR ($n=3$; 4.3%), and MVR+AVR or MVL ($n=20$; 28.6%). Only 23 of the patients (32.8%) had fully recovered by the age of 18. Recurrence of RF was recorded for 15% of the patients because they had not received prolonged penicillin treatment. Data for healthy individuals ($n=100$) were obtained from the Databank of the Immunology Institute of Latvia. The control individuals were free of autoimmune disease and had no family history of RF. In both groups (RF patients and healthy individuals), HLA class II alleles were determined by PCR.

DNA isolation

Genomic DNA was extracted from proteinase-K-treated peripheral blood leukocytes using the routine salt-off method. The DNA was stored in TE buffer (10 ml Tris–HCl, pH 7.5, and 2 ml 0.5 M Na_2 EDTA per litre of distilled water). The DNA obtained was used immediately for genotyping or was stored at –20°C. The DNA concentration, around 100–200 μg/ml, was determined by fluorescence with a DNA fluorimeter [36].

HLA-DR and -DQ genotyping by PCR

Low-resolution HLA-DR typing for DRB1* 01 to 18 and for DQB1*0201-202, *0301-305, *0401-402, *0501-504, and *0601-608 was performed by PCR with amplification with sequence-specific primers (PCR–SSP) [37]. The reaction mixture (15 μl) included 1 μl DNA, 1.5 μl PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, (pH 8.3)], 0.6 μl dNTPs (25 mmol/l), 1.0 μl specific primers (0.2 mmol/l), and 0.5 U of the *Taq* DNA polymerase (Promega). In addition, the internal positive control primer pair C3 and C5 was included in all reaction mixtures at a concentration one-fifth that of the allele- and group-specific primers.

The reaction mixture was subjected to 35 amplification cycles, each consisting of denaturation at 94°C (60 s), followed by one cycle, annealing at 94°C (20 s), 67°C (2 s) followed by seven cycles and extension at 93°C (5 s), 65°C (4 s), with a final extension in step with 28 cycles. PCR products were visualized by agarose-gel electrophoresis. After addition of 2 M loading buffer, the PCR reaction mixtures were loaded in agarose gels prestained with ethidium bromide (0.5 μk/ml gel). Gels were run for 15 min at 10 V/cm gel in 0.5 mM TBE (0.89 M Tris, 0.89 M Boric acid and 0.02 M EDTA in aqueous solution) buffer and then examined under UV illumination and recorded [15,38–40].

Statistical

The HLA-DRB1 and DQB1 frequency of each allele and genotype was compared between the patients and the controls using the chi-square test. The *P* value and odds ratio (OR) were calculated using EPI INFO software, version 06, with 95% confidence intervals and Fisher exact correction for small numbers [41].

Results

Frequencies of DRB1* and DQB1* alleles in RF patients and control subjects

In RF patients, HLA class II DRB1*07 (OR=4.18, $P<0.01$), DQB1*0302 (OR=3.13, $P<0.0002$), and DQB1*0401-2 (OR=4.33, $P<0.0001$) alleles were found more frequently than in the control group, while the DRB1*06 (OR=0.18, $P<0.0023$), DQB1*0602-8 (OR=0.4, $P<0.0127$), and DQB1*0501 (OR=0.26, $P<0.0027$) alleles were less frequent (Tables 1 and 2).

Frequencies of DRB1 and DQB1 alleles in RF, RHD, and Sydenham's chorea patients compared with control subjects

In the homogeneous patient groups, DRB1*07 had the highest OR in all RF groups (Table 3): patients with no acquired valvular heart disease developing after RF carditis (OR=7.35, $P<0.001$), MVR patients (OR=4.45,

Table 1

Frequencies of DRB1* alleles in RF patients and healthy controls

DRB1* allele	RF ($n = 140$)	%	Controls ($n = 200$)	%	Odds ratio	*P*
*01	24	17	26	13	0.17	<0.643
*15	27	19	44	22	0.88	<0.3603
*03(*17;*18)	18	13	22	11	1.17	<0.184
*04	18	13	24	12	0.017	<0.305
*05(*11;*12)	30	21	33	16.5	1.3	<0.1288
*06(*13;*14)	4	3	28	14	0.18	<0.0023
*07	11	8	4	2	4.18	<0.01
*08	3	2	12	6	0.6**	<0.403
*09	4	3	2	1	1.81**	<0.495
*10	1	0.7	5	2.5	0.28**	<0.252

**Confield not accurate. Extract limits preferred. RF, rheumatic fever.

Table 2

Frequencies of DQB1* alleles in RF patients and healthy controls

DQB1* allele	RF ($n = 140$)	%	Controls ($n = 200$)	%	Odds ratio	*P*
*0201-2	17	12	32	16	0.76	<0.306
*0301	31	22	39	19.5	1.14	<0.29
*0302	25	18	13	6.5	3.13	<0.0002
*0303	7	5	13	6.5	0.77	<0.624
*0304	1	0.7	2	1	Undefined	Undefined
*0305	1	0.7	–	–	Undefined	Undefined
*0401-2	19	14	7	3.5	4.33	<0.0001
*0501	4	3	22	11	0.26**	<0.0027
*0502-4	2	1	12	6	0.23**	< 0.03
*0601	11	8	11	5.5	1.43	<0.053
*0602-8	22	16	49	24.5	0.4	<0.0127

**Confield not accurate. Extract limits preferred. RF, rheumatic fever.

$P<0.03$), MVL patients (OR=5.44, $P<0.01$), and Sydenham's chorea patients (OR=11.31, $P<0.002$). The least frequent allele was DRB1*06 (OR=0.18, $P<0.0023$). The DQB1 allele frequencies differed for RHD patients with MVR and MVL: DQB1*0401-2 (OR=8.20, $P<0.001$) was more common in MVR patients and DQB1*0302 (OR=4.18, $P<0.001$) in MVL patients (Table 3). In Sydenham's chorea patients, a high frequency of DQB1*0401-2 (OR=6.36, $P<0.005$) was found.

DRB1* and DQB1* genotypes associated with RF patients

The strongest associations between DRB1* alleles and RF patients were for DRB1*01-07 (OR=8.57, $P<0.05$), DRB1*15-07 (OR=2.86, $P<0.02$), DRB1*04-05 (OR=3.57, $P<0.04$), and DRB1*05-07 (OR=2.86, $P<0.02$) (Table 4). The DRB1*03-06 allele (OR=0.48, $P<0.01$) had the lowest OR.

Common alleles associated with RF are: DQB1*0301-0302 (OR=5.44, $P<0.05$), DQB1*0302-0303 (OR=4.43, $P<0.02$), DQB1*0302-0601 (OR=2.91, $P<0.01$), and DQB1*0302-0602-8 (OR=2.19, $P<0.01$) (Table 5). The lowest allele frequencies in RF patients were observed for DQB1*0201-2-0201-2 (OR=0.28, $P<0.001$) and DQB1*0303-0602-8 (OR=0.22, $P<0.01$).

Discussion

HLA associations with RF and RHD have been frequently investigated, but there is no unanimity of opinion about the

Table 3

Frequencies of DRB1 and DQB1 alleles in RF and RHD patients compared with control subjects

Group ($n = 140$)		DRB1*06	DRB1*07	DQB1*0302	DQB1*0401-2
All RF ($n = 140$)	gf(P) + OR/χ^2	**(0.0023) 0.18/11.96**	**(0.01) 4.18/6.68**	**(0.001) 3.13/10.67**	**(0.015) 4.33/11.79**
MVR ($n = 48$)	gf(P) + OR/χ^2	0.15	**(0.03) 4.45/4.95**	1.09	**(0.001) 8.20/21.59**
MVL ($n = 40$)	gf(P) + OR/χ^2	0.18	**(0.01) 5.44/6.59**	**(0.001) 4.18/10.21**	**1.25**
Sydenham's chorea ($n = 16$)	gf(P) + OR/χ^2	0	**(0.002)11.31/13.19**	1.79	**(0.005) 6.36/7.17**
without RHD ($n = 46$)	gf(P) + OR/χ^2	0.31	**(0.001) 7.35/11.65**	**(0.005) 3.50/7.81**	**(0.01) 4.14/6.78**
Control subjects ($n = 200$)	gf	0.14	0.02	0.07	0.04

Boldface type highlights statistically significant associations for patients vs controls. gf (gene frequency) P (probability), OR (odds ratio), and Mantel–Hanszel values (χ^2) are reported only for significant associations ($P < 0.05$). n = number of haplotypes (e.g. 140 haplotypes from 70 individuals). The nature of valve lesions was not reported for two patients. MVL, multivalvular lesions; MVR, mitral valve regurgitation; RF, rheumatic fever; RHD, rheumatic heart disease.

Table 4

Significant association of HLA-DRB1 genotypes with predisposition/protection in RF patients

DRB1	RF ($n = 70$)	%	Controls ($n = 100$)	%	Odds ratio	P (Fisher)
***01/*07**	**6**	**8.6**	**1**	**1**	**8.57**	**<0.05**
*15/*03	6	8.6	2	2	4.29	<0.05
***15/*07**	**2**	**2.9**	**1**	**1**	**2.86**	**<0.02**
*03/*06	1	1.4	3	3	0.48	<0.01
***04/*05**	**5**	**7.1**	**2**	**2**	**3.57**	**<0.04**
***05/*07**	**2**	**2.9**	**1**	**1**	**2.86**	**<0.02**

Boldface type highlights statistically significant associations for patients vs controls. RF, rheumatic fever.

Table 5

Significant association of HLA-DQB1 genotypes with predisposition/protection in RF patients

DQB1	RF ($n = 70$)	%	Controls ($n = 100$)	%	Odds ratio	P (Fisher)
*0201-2/*0201-2	1	1.4	3	3	0.28	<0.001
*0201-2/*0602-8	4	5.7	8	8	0.71	<0.34
***0301/*0302**	**7**	**10**	**2**	**2**	**5.44**	**<0.05**
***0302/*0303**	**3**	**4.3**	**1**	**1**	**4.43**	**<0.02**
***0302/*0601**	**2**	**2.9**	**1**	**1**	**2.91**	**<0.01**
***0302/*0602-8**	**3**	**4.3**	**2**	**2**	**2.19**	**<0.01**
*0303/*0602-8	1	1.4	6	6	0.22	<0.01

Boldface type highlights statistically significant associations for patients vs controls. RF, rheumatic fever.

association of specific alleles in connection with the disease [20,32,33,35]. One reason for this discrepancy might be the use of old HLA genotyping methods [27]. Besides the technical issues, it has also been suggested that it is necessary to distinguish clinically homogeneous patient groups, for example, patient groups with and without rheumatic carditis, proved RHD, or Sydenham's chorea [27,33]. When patients have been divided into clinically homogeneous patient groups, no differences between ethnic groups have been found, with the DRB1*0701, DRB1*0301, and DQB1*0201 alleles being found at similar frequencies [18,21,32,42,43]. In medical

Table 6

Summary of HLA alleles or genotypes associated with rheumatic heart disease

Allele associated with risk	Allele associated with protection
DRB1*07	DRB1*06
DQB1*0401-2	DQB1*0602-8
DQB1*0302	
DRB1*07-DQB1*0401-2	DRB1*06-DQB1*0602-8
DRB1*07-DQB1*0302	
DRB1*01-DQB1*0301	
DRB1*07-DQB1*0302	
DRB1*15-DQB1*0302	
DRB1*07-DQB1*0303	

An asterisk preceding the allele number indicates the allele molecular designation (typing at DNA level). Boldface type highlights association cross-validated in this study.

publications, there is no unanimity of opinion about whether HLA class II alleles differ with the ethnic origin of the patients, but this may be connected to the use of different methods of typing HLA alleles.

The data in our investigations were obtained by investigating RF and RHD patients classified into clinically homogeneous patient groups. We found that the HLA class II DRB1*07, DQB1*0401-2, and DQB1*0302 alleles were risk factors for acquiring RF and RHD. The DRB1*07 allele was common and had a comparatively high frequency of incidence in patients with RF (Tables 1 and 3). This suggests that DRB1*07 could be a risk allele for RF and RHD. Our data on the high frequency of DRB1*07 in white patients differs from data on Brazilian patients, where HLA-DR7 was detected in the mulatto group of patients [20,44]. In our study, the HLA II allele DRB1*07 was rather frequent in white RF patients, both with and without permanent acquired valvular heart disease.

The HLA class II allele DQB1*0401-2 was found more often in MVR patients, and DQB1*0302 in MVL patients. Thus, it can be assumed that HLA-DQ genes control the risk of development of RHD, both MVL and MVR. This observation confirms previous findings for RHD patients in Japan [32]. Our data obtained using the PCR–SSP method suggests that a risk of developing RHD exists for the carrier DQ allele exists in Brazil and Japan, as well as in Latvia.

However, both the DQB1*0401-2 and *0302 allele frequencies were significantly elevated in the RF patient group and in patients who did not develop permanent valvular heart disease.

The genotyping showed that a high risk of acquiring RF and developing RHD was associated with DRB1*01-DQB1*0301-DRB1*07-DQB1*0302 and DRB1*15-DQB1*0302-DRB1*07-DQB1*0303. This, however, is only a supposition, and further experiments with a larger group will be necessary to establish whether it is correct.

Protective HLA class II alleles are as important as the risk alleles. Alleles that can be designated protective include DRB1*03-*06, DQB1*0201-2-*0201-2, and DQB1*0303-*0602-8. Higher protection against RF and RHD is provided by the allele genotype DRB1*06-DQB1*0602-8 (Table 6).

Among the alleles found to be protective in our study, DQB1*0602-8 is most often mentioned in the literature [20,26,27,29]. Several risk/protective HLA class II suballeles can be misperceived as other suballeles not connected with RF and RHD. Therefore it is important to determine genotypes [45–47].

HLA alleles regulate the immune response to infections [48,49], binding and presenting autoantigens with different affinities and regulating selection of T cells, and at the same time they themselves can serve as target autoantigens [50–52]. Different autoimmune peptide presentations with protective or risk alleles are very important in the development and research of the pathogenesis of autoimmunity.

The basis of autoimmune processes that contribute to the development of RHD is T-cell molecular mimicry between streptococcal and heart proteins. RHD is initiated by certain serotypes connected with group A streptococcus M protein. Guilherme and colleagues suggest that M5 peptide causes severe RHD in patients who have HLA-DRB1*07 combined with severe RHD [53]. In our study, patients with severe RF who developed RHD in cases of MVR as well as MVL all had the DRB1*07 allele.

Conclusion

Our findings indicate that the HLA class II alleles DQB1* and DRB1* are associated with a trend to risk/protection relating to RF and the development of RHD. HLA class II DRB1*07-DQB1*0401-2 and DRB1*07-DQB1*0302 can be considered to be risk genotypes, while HLA class II DRB1*06 and DQB1*0602-8 are probably protective. Considering the association of the HLA-DRB1 and DQB1 genotype with RHD risk/protection, patients with DRB1*07-DQB1*0401-2 are at risk of acquiring MVR, and DRB1*07-DQB1*0302 patients are at risk of acquiring MVL. By genotype analysis, we found that the RHD risk is increased in individuals with DRB1*01-DQB1*0301-DRB1*07-DQB1*0302 and DRB1*15-DQB1*0302-DRB1*07-DQB1*0303 genotypes. As we mentioned before, this conclusion is only a supposition, and study of a larger group of patients would be required to confirm it.

The genotype DRB1*06-DQB1*0602-8 may be associated with protection against RF and the development of RHD.

It is significant that the patients were grouped into clinically homogeneous groups as opposed to the total RF patient group, as the various subgroups differed in DQ allele frequencies.

The severity of RF is probably associated with the DRB1*07 allele and development of certain RHD may be dependent on specific DQ alleles.

Our study provides further information about the hypothesis that there is a genetic predisposition to RF and about the protective immune responses in RHD. Further insight into the molecular mechanisms of the disease will be a useful tool for predicting the clinical outcome in RF patients and may offer new means of and approaches to treatment and prophylaxis design in the future.

Competing interests

None declared.

References

1. Markowitz M, Gerber MA: **The Jones criteria for guidance in the diagnosis of rheumatic fever: another perspective.** *Arch Pediatr Adolesc Med* 1995, **149:**725-726.
2. Stollerman GH: **Rheumatic fever.** *Lancet* 1997, **349:**935-942.
3. Shulman ST: **Complications of streptococcal pharyngitis.** *Pediatr Infect Dis J* 1994, **Suppl 1:**S70-S74.
4. Kaplan E: **Global assessment of rheumatic fever and rheumatic heart disease at the close of the century: influences and dynamics of populations and pathogens: a failure to realize prevention?** *Circulation* 1993, **88:**1964-1972.
5. Bisno AL: **Group A streptococcal infections and acute rheumatic fever.** *N Engl J Med* 1991, **325:**783-793.
6. Zabriskie JB, Gibofsky A: **Genetic control of the suspectibility to infection with pathogenic bacteria.** *Curr Top Microbiol Immunol* 1986, **124:**1-20.
7. Ayoub EM: **Acute rheumatic fever.** In *Heart Disease in Infants, Children, and Adolescents.* 5th edition. Edited by Emmanouilides GC, Riemenschneider TA, Allen HD, Gutgesell HP. Baltimore, MD: Williams & Wilkins; 1995:1400-1416.
8. Veasy LG, Hill HR: **Immunologic and clinical correlations in rheumatic fever and rheumatic heart disease.** *Pediatr Infect Dis J* 1997, **16:**400-407.
9. Sanyal SK: **Long-term sequelae of the first attack of accute rheumatic fever during childhood.** In *Heart Disease in Infants, Children, and Adolescents.* 5th edition. Edited by Emmanouilides GC, Riemenschneider TA, Allen HD, Gutgesell HP. Baltimore, MD: Williams & Wilkins; 1995:1416-1440.
10. Read SE, Reid H, Poon-King T: **HLAs and predisposition to the nonsuppurative sequelae of group A streptococcal infections.** *Transplant Proc* 1977, **9:**543-546.
11. Wilson MG, Schweitzer MD, Lubschez R: **The familial epidemiology of rheumatic fever: genetic and epidemiologic studies.** *J Pediatr* 1943, **22:**468-492.
12. Wilson MG, Schweitzer M: **Pattern of hereditary susceptibility in rheumatic fever.** *Circulation* 1954, **10:**699-704.
13. Khanna AK, Buskirk DR, Williams RC Jr, Gibofsky A, Crow MK, Menon A, Fotino M, Reid HM, Poon-King T, Rubinstein P, *et al.*: **Presence of a non-HLA B cell antigen in rheumatic fever patients and their families as defined by a monoclonal antibody.** *J Clin Invest* 1989, **83:**1710-1716.
14. Ayoub EM, Barret DJ, Maclaren NK, Krischer JP: **Association of class II human histocompatibility leukocyte antigens with rheumatic fever.** *J Clin Invest* 1986, **77:**2019-2026.
15. Anastasiou-Nana MI, Anderson JL, Carquist JF, Nanas JN: **HLA–DR typing and lymphocyte subset evaluation in rheumatic heart disease: a search for immune response factors.** *Am Heart J* 1986, **112:**992-997.
16. Jhinghan B, Mehra NK, Reddy KS, Taneja V, Vaidya MC, Bhatia ML: **HLA blood groups and secretor status in patients with established rheumatic heart disease.** *Tissue Antigens* 1986, **27:**172-178.
17. Rajapakse CNA, Halim K, Al-Orainey I, Al-Nozha M, Al-Aska AK: **A genetic marker for rheumatic heart disease.** *Br Heart J* 1987, **58:**659-662.
18. Maharaj B, Hammond MG, Appadoo B. Leary WP, Phil D, Pudifin DJ: **HLA-A, B, DR and DQ antigens in black patients with severe chronic rheumatic heart disease.** *Circulation* 1987, **76:** 259-261.
19. Taneja V, Mehra NK, Reddy S, Narula J, Tandon R, Vaidya MC, Bhatia ML: **HLA-DR/DQ antigens and reactivity to B cell alloantigen D8/17 in Indian patients with rheumatic heart disease.** *Circulation* 1989, **80:**335-340.
20. Guilherme L, Weidebach W, Kiss MH, Snitcowsky R, Khalil J: **Association of human leukocyte class II antigens with rheumatic heart disease in Brazilian population.** *Circulation* 1991, **83:**1995-1998.
21. Ozkan M, Carin M, Sonmez G, Senocak M, Ozdemir M, Yakut C: **HLA antigens in Turkish race with rheumatic heart disease.** *Circulation* 1993, **87:**1974-1978.
22. Weidebach W, Goldberg AC, Chiarella JM, Guilherme L, Snitcowsky R, Pileggi F, Khalil J: **HLA class II antigens in rheumatic fever: analysis of the DR locus by restriction fragment-length polymorphism and oligotyping.** *Hum Immunol* 1994, **40:**253-258.
23. Ahmed S, Ayoub EM, Scornik JC, Wang CY, She JX: **Post streptococcal reactive arthritis: clinical characteristics and association with HLA-DR alleles.** *Arthritis Rheum* 1998, **41:**1096-1102.
24. Cassidy JT, Petty RE: *Textbook of Pediatric Rheumatology.* 2nd edition. Philadelphia: Churchill Livingstone; 1999.
25. DiSciascio G, Taranta A: **Rheumatic fever in children.** *Am Heart J* 1980, **99:**635-658.
26. Visentainer JE, Pereira FC, Dalalio MM, Tsuneto LT, Donadio PR, Moliterno RA: **Association of HLA-DR7 with rheumatic fever in the Brazilian population.** *J Rheumatol* 2000, **27:**1518-1520.
27. Guedez Y, Kotby A, El-Demellawy M, Galal A, Thomson G, Zaher S, Kassem S, Kotb M: **HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients.** *Circulation* 1999, **99:**2784-2790.
28. Maharaj B, Khedun SM, Hammond MG, van der Byl K: **HLA-A, B, DR, and DQ antigens in Indian patients with severe chronic rheumatic heart disease.** *Jpn Heart J* 1997, **38:**663-668.
29. Bhat MS, Wani BA, Koul PA, Bisati SD, Khan MA, Shah SU: **HLA antigen pattern of Kashmiri patients with rheumatic heart disease.** *Indian J Med Res* 1997, **105:**271-274.
30. Gu J, Yu B, Zhou J: **HLA-DQA1 genes involved in genetic susceptibility to rheumatic fever and rheumatic heart disease in southern Hans [in Chinese].** *Zhonghua Nei Ke Za Zhi* 1997, **36:** 308-311.
31. Afanas'ev II, Kadyrova RT, Iazdovskii VV: **[The HLA-associated immunological mechanisms in rheumatic involvement of the myocardium. — in Russian].** *Terr Arkh* 1995, **67:**56-59.
32. Koyanagi T, Koga Y, Nishi H, Toshima H, Sasazuki T, Imaizumi T, Kimura A: **DNA typing of HLA class II genes in Japanese patients with rheumatic heart disease.** *J Mol Cell Cardiol* 1996, **28 :**1349-1353.
33. Donadi EA, Smith AG, Louzada-Junior P, Voltarelli JC, Nepom GT: **HLA class I and class II profiles of patients presenting with Sydenham's chorea.** *J Neurol* 2000, **247:**122-128.
34. Olmez U, Turgay M, Ozenirler S, Tutkak H, Duzgun N, Duman M, Tokgoz G: **Association of HLA class I and class II antigens with rheumatic fever in a Turkish population.** *Scand J Rheumatol* 1993, **22:**49-52.
35. Carlquist JF, Ward RH, Meyer KJ, Husebye D, Feolo M, Anderson JL: **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:**452-457.
36. Walsh PS, Metzger D, Higuchi R: **Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material.** *Biotechniques* 1991, **10:**506-513.
37. Alexseyev LP, Boldyreva MN, Trofimov D: **Use of new variant HLA-DNA typing -mSSP at perspective selection of the donor**

of a nephros. *Proceedings of 2nd All-Russia scientific practical conference. Polymerase chain reaction (PCR) at diagnostics and the control of treatment contagious disease.* Moscow. 1998:133-134.
38. Olerup O, Zetterquist H: **HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation.** *Tissue Antigens* 1992, **39:**225-235.
39. Erlich H, Bugawan T, Begovich A, Scharf S, Griffith R, Saiki R, Higuchi R, Walsh PS: **HLA-DR, DQ and DT typing using PCR amplification and immobilized probes.** *Eur J Immunogenet* 1991, **18:**33-35.
40. Walsh PS, Erlich H, Higuchi R: **Preferential PCR amplification of alleles: mechanisms and solutions.** *PCR Methods Appl* 1992, **1:**241-250.
41. Mehta CR, Patel NR, Gay RJ: *Pascal program by ELF Franco & N Compos-Filho Ludwig Cancer Institute: Mathematics Software StatCalc.* São Paulo, Brazil: Am. Stat. Assoc; 1985, **78:**969-973.
42. Sirithunyanont Ch, Sura Th, Thamneum S, Busabarat M, Yamwong S, Sritara P, Vejbaesya S, Tanomsup S, Boonbaichaipruk S, Likitthanasombat K, Chandnanamatha P: **Association of human leukocyte antigens in Thais with rheumatic heart disease.** *Intern Med J Thai* 2001, **17:**326-334.
43. Debaz H, Olivo A, Perez-Luque E, Vasquez-Garcia MN, Burguete A, Chavez-Negrete A, Velasco C, Arguero R, Gorodeszky C: **DNA analysis of class II alleles in rheumatic disease in Mexicans [abstract]. 22nd Annual ASHI Meeting Abstracts.** *Human Immunology* 1996, **49(suppl):**63.
44. **HLA Antigens in Brazilian Patients with Rheumatic Heart Disease.** [www.brazilpednews.org.br/].
45. Thorsby E: **HLA associated diseases. A summary of the 12th International Histocompatibility workshop component.** In *HLA: Genetic Diversity of HLA: Functional and Medical Implications.* Edited by Charron D. Paris, France: EDK; 1996:91-96.
46. Kotb M: **Infection and autoimmunity: a story of the host, the pathogen, and the copathogen.** *Clin Immunol Immunopathol* 1995, **74:**10-22.
47. Schmidt D, Verdager J, Nuzhat A, Santamaria P: **A mechanism for the major histocompatibility complex-linked resistance to autoimmunity.** *J Exp Med* 1997, **186:**1059-1075.
48. Hill AVS: **HLA and infection [review].** *J R Coll Physicians Lond* 1992, **26:**11-16.
49. Ayoub EM, Kotb M, Cunningham MW: **Rheumatic fever pathogenesis.** In *Streptococcal Infections.* Edited by Stevens DL, Kaplan EL. New York, NY: Oxford University Press: in press.
50. Nepom GT: **Class II antigens and disease suspectibility.** *Annu Rev Med* 1995, **46:**17-25.
51. Sinha AA, Lopez MT, McDevit HO: **Autoimune diseases: the failure of self tolerance.** *Science* 1990, **248:**1380-1388.
52. McDevitt HO, Chinitz A: **Genetic control of the antibody response: relationship between immune responses and histocompatibility (H-2) type.** *Science* 1969; **163:**1207-1208.
53. Guilherme L, Oshiro SE, Fae KC, Cunha-Neto E, Renesto G, Goldberg AC, Tanaka AC, Pomerantzeff PM, Kiss MH, Silva C, Guzman F, Patarroyo ME, Southwood S, Sette A, Kalil J: **T-cell reactivity against streptococcal antigens in the periphery mirrors reactivity of heart-infiltrating T lymphocytes in rheumatic heart disease patients.** *Infect Immun* 2001, **69:** 5345-5351.

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