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Association between HLA-DQ alleles and leprosy in Indonesian Javanese population

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ABSTRAK

Hardyanto Soebono – *Hubungan antara HLA-DQ dan lepra pada populasi Jawa Indonesia.*

Penelitian sebelumnya menunjukkan bahwa kerentanan terhadap penyakit lepra dan respon antibodi anti *M. leprae* pada populasi Jawa berhubungan dengan faktor genetik HLA-DR. Untuk mengetahui apakah kerentanan tersebut juga berhubungan dengan HLA-DQ, telah dilakukan penelitian lanjutan dengan memeriksa fenotip-fenotip HLA-DQ dan pemeriksaan antibodi terhadap 79 penderita lepra (terdiri atas 41 lepra TT/BT dan 38 lepra LL/BL) dan 50 kontrol sehat dari populasi yang sama. Penggolongan HLA-DQ dilakukan dengan metode SSO (*sequence specific oligonucleotide typing*), sedangkan pemeriksaan antibodi dilakukan dengan tes ELISA dan ELISA-INHIBISI.

Hasil penelitian menunjukkan adanya hubungan antara HLA-DQB501 dengan lepra, baik TT maupun LL (OR: 3,27;95% CI 1,42-7,60). Jika HLA-DQ1 secara keseluruhan dianalisis, hubungan bermakna hanya dijumpai terhadap lepra lepromatosa (OR:9,18; 95% CI 1,89-86,30). Antibodi IgG anti protein 36 kD ternyata berhubungan dengan HLA-DQA102. Titer IgG anti 36 kD dijumpai lebih tinggi pada HLA-DQA102 positif dibandingkan HLA-DQA102 negatif. Tidak ada hubungan antara HLA-DQ dengan seropositivitas baik IgM maupun IgG anti *M. leprae* ($p>0,05$).

Disimpulkan bahwa selain HLA-DR, ternyata kerentanan terhadap lepra pada populasi ini diatur juga oleh gen pada lokus HLA-DQ. Penelitian ini juga menyokong penelitian-penelitian lain sebelumnya bahwa HLA-DQ1 merupakan penanda universal kerentanan terhadap lepra lepromatosa; sedangkan infeksi subklinis *M. leprae* tidak diatur oleh faktor genetik HLA.

Key words : leprosy – *M. leprae* – genetic factors – HLA-DQ – HLA-DQ1 – HLA-DQB501

ABSTRACT

Previous studies showed that susceptibility to leprosy and antibody response toward M. leprae in Javanese population was under control of HLA-DR alleles. To investigate whether this susceptibility was also associated with HLA-DQ, the study had been continued with phenotyping of HLA-DQ alleles and antibody assay to the same population which consisted of 79 leprosy patients, 41 tuberculoid (TT/BT) and 38 lepromatous (LL/BL) type, and 50 healthy controls. The HLA-DQ typing had been performed by using a sequence specific oligonucleotyping (SSO) method, while the anti M. leprae antibody had been tested by ELISA and INHIBITION-ELISA.

The results show that HLA-DQB501 is associated with leprosy, either tuberculoid or lepromatous type (OR 3.27; 95% CI 1.42-7.60). When all HLA-DQ1 alleles are analyzed, a significant association is found only with lepromatous leprosy (OR 9.18; 95% CI 1.89-86.30). IgG antibody anti 36 kD M. leprae is found to be associated with HLA-DQ102. The level of this antibody is higher in HLA-DQ102 positive individuals compared to those negative one (P). No correlation is found between HLA-DQ alleles and the seropositivity of either IgM or IgG.

In conclusion, the susceptibility to leprosy in this population is also controlled by genes in HLA-DQ locus. This study also supports the previous findings that HLA-DQ1 is a universal marker for the susceptibility to lepromatous leprosy, while the infection with M. leprae per se is not controlled by HLA genetic factor.

Key words : leprosy – *M. leprae* – genetic factor – HLA-DQ – HLA-DQ1 – HLA-DQB501

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INTRODUCTION

Leprosy is a chronic infectious disease of man caused by an intracellular microorganism, *Mycobacterium leprae*. Clinically, the disease is manifested as a spectrum with two polar forms, tuberculoid (TT) and lepromatous (LL) types. Between these two polars, there are intermediate forms: borderline tuberculoid (BT), mid-borderline (BB), and borderline lepromatous (BL). However, before clinical manifestations of the disease develop, patients may demonstrate a transitional form called indeterminate (I) which frequently heals spontaneously. The development of the clinical spectrum of the disease is related to the degree of cell mediated immunity which is regulated by genetic factors[1].

Leprosy remains a public health problem in Indonesia. Although the number of registered cases has decreased significantly since the implementation of multidrug therapy (MDT), the incidence of new cases is apparently unchanged[2]. Therefore, the fundamental question of why some people contract leprosy (infection) and others do not is still unanswered. One possibility is genetic difference in susceptibility. In Indonesia, the distribution of leprosy is not uniform in either the prevalence or type of disease. These differences may be due to the effect of many variables, one of which is genetic.

Studies of genetics on leprosy have been reviewed by Fine.[3,4] Among the genetic factors, human leucocyte antigen (HLA) has extensively been studied by several investigators[5,6,7,8,9]. From these studies, HLA Class II played a major role in disease susceptibility. Due to the inconsistency in the frequency of HLA Class-I antigens on leprosy in these studies, it was concluded that these antigens were not directly responsible for the differential susceptibility to leprosy[9]. Todd *et al.*[10] from their cases in Louisiana and meta-analysis of pooled reports of cases it was found that only HLA-DR2 and HLA-DQw1 were associated with leprosy, in either tuberculoid or lepromatous forms. Based on his own and many other studies, de Vries[11] came to the revolutionary conclusion that HLA-DR3 was better associated with TT leprosy and HLA-DQ1 was more universally associated with LL leprosy, whereas the susceptibility to *M. leprae* infection itself was not controlled by HLA-linked genes. A study conducted among Javanese population in Yogyakarta demonstrated that HLA-DR2 is associated with the susceptibility to lepromatous leprosy, in contrast with HLA-DR12 which is related to the resistance to leprosy in general. Whereas, the susceptibility to *M. leprae* infection is not controlled by HLA-DR genetic factor[12].

To investigate further whether other HLA loci are associated with leprosy, leprosy types and *M. leprae* infection, the study has been continued on HLA-DQ typing of the subjects.

MATERIALS AND METHODS

Subjects

Seventy-nine leprosy patients (41 TT/BT and 38 LL/BL) and 50 healthy controls were recruited from Yogyakarta area, Indonesia. They were all Javanese ethnic group. The diagnosis of leprosy was based on clinical, bacteriological and histopathological examinations; and was classified according to Ridley and Jopling[1]. Blood samples were taken for HLA typing and serological assays and had been drawn before chemotherapy started for leprosy. The healthy controls consisted of non-relative household contacts (spouses) and healthy blood donors.

HLA-DQ typing

According to the protocol of the Department of Immunohematology and Blood Bank Leiden, the Netherlands, for typing of HLA-DQ alleles oligonucleotide type method was used as previously described[12,13]. Briefly: DNA was extracted from peripheral blood lymphocytes. Amplification of this DNA was performed by the polymerase chain reaction (PCR) using sets of primers specific for DQA1 and DQB1. Typing for the alleles was carried out by dot blotting the PCR fragments and hybridization with sequences specific oligonucleotide (SSO) probes. The SSOs and their sequences used in this typing were listed in TABLE 7. Using those sets of primers and SSO probes, a number of 8 DQA1 and 13 DQB1 specificities could be typed.

Serological assays

Sera collected from the subjects were tested for specific antibody against *M. leprae* antigens i.e. IgM anti PGL-I and IgG anti 36 kD protein antigens, by using ELISA and INHIBITION-ELISA. The procedure and interpretation of ELISA and INHIBITION-ELISA were described elsewhere[12,14].

Statistical analysis.

Frequencies of HLA-DQ alleles in leprosy patients and controls were compared using a X^2-test. Association of the HLA phenotypes and (subclinical) leprosy was expressed in Odds ratio (OR)[15,16]. This ratio was equal with Relative Risk as defined by Woolf & Haldane (reviewed by Svejgaard *et al.*[17]). Attributable risk (AR) or prevented fraction (PF) was calculated using the formula as suggested by Kleinbaum *et al.*[18] and Kramer[16].

RESULTS

Using the sets of primers and SSO probes available so far, a number of HLA-DQ alleles were found. As shown in TABLE 1, 17 DQ alleles were identified. High frequency was found in the distribution of HLA-DQA601,-DQB501, and -DQB301 alleles both in leprosy and healthy controls. Alleles of HLA-DQA101, -DQA102 were distributed in moderate frequency, whereas the remaining alleles were found in low frequency. Four alleles were not detected in this population : HLA-DQB604, -DQB303, -DQB4-1 and -DQB402.

HLA-DQ association with leprosy

Among the HLA-DQ, only HLA-DQB501 allele showed a highly significant association with leprosy (either TT or LL type). The OR of this association was almost equal among TT, LL and total leprosy i.e: 3.27, 3.26 and 3.27 respectively (see TABLE 1). The attributable risk was calculated for TT, LL and total leprosy as 49.4 %, 49.3 % and 49.4 % respectively.

Considering that DQ1 was previously reported to be associated with (lepromatous) leprosy in several population[9,10] and that DQB501 belong to this specificity in serological testing, an analysis were carried out to know whether HLA-DQ1 which comprised of DQB501, DQB502, DQB503, DQB601, DQB602, DQB603 and DQB604 showed an association with (lepromatous) leprosy. The data are shown in TABLE 2.

A significant association was found between HLA-DQ1 and lepromatous leprosy (p=0.003), but not with TT (p=0.08). The attributable risk of this association was 83.3 % for LL and 63.4 % for all leprosy.

DQ1 is composed of DQ5 (which contains DQB501, DQB502 and DQB503 alleles) and DQ6 (which contains DQB601, DQB602, DQB603 and DQB604[19]). These specificities for an association with leprosy were analyzed (TABLE 3). In fact, only HLA-DQw5 was associated with leprosy (OR 2.95, 95% CI 1.27-6.93 p=0.006). This association was greater for LL than TT (OR 3.57 vs 2.56, p=0.012 vs. 0.039), and the AR was 55.7 % for LL and 48.9% for total leprosy.

Association of HLA-DQ and serum anti *M. leprae* antibody levels in leprosy patients

The association of HLA-DQ alleles and serum antibody levels in leprosy is presented in TABLE 4. IgM anti PGL-I antibody level was associated with DQA201, and DQA401 (P), whereas IgG anti 36 kD antibody level was associated with DQA102, DQA301, DQA401 and DQB302 (P). However, these associations did not meet the statistical requirements because the number of individuals carrying these alleles was small (less than 10 %). If we looked at the DQB501 allele, both IgM anti PGL-I and IgG anti 36 kD antibody levels were higher in individuals carrying this allele, although this was not statistically significant. In other words, no association between HLA-DQ and serum anti *M. leprae* antibody levels was found in leprosy patients.

Association of HLA-DQ and subclinical *M. leprae* infection

TABLE 5 presents a correlation between HLA-DQ and subclinical *M. leprae* infection. Among -DQ alleles, DQB201 was the only one associated with IgM seropositivity (OR= 5.63; 95% CI 2.21-14.33 χ^2=5.65 p=0.017), but not with IgG seropositivity.

TABLE 1. n Frequence and the Odds ratio of HLA-DQ alleles in lerposy patients and healthy controls

Alleles	Controls (n=50)	Leprosy patients											
		TT (n=41)				LL (n=38)				Total (n=79)			
	freq.	freq.	OR	(95%CI)	*p*	freq	OR	(95%CI)	*p*	freq	OR	(95%CI)	*p*
DQA101	28.6	46.3	2.16	(0.83-5.68)	0.083	45.7	2.11	(0.77-5.79)	0.108	31.6	2.13	(0.93-4.95)	0.051
DQA102	32.7	29.3	0.85	(0.32-2.30)	0.731	34.2	1.08	(0.39-2.97)	0.876	30.4	0.95	(0.41-2.20)	0.900
DQA103	6.1	12.2	2.13	(0.40-12.20)	0.524	11.4	1.98	(0.34-12.16)	0.640	11.3	2.06	(0.47-10.2)	0.453
DQA201	14.3	12.2	0.83	(0.21-3.28)	0.983	2.9	0.18	(0.01-1.56)	0.166	8.8	0.51	(0.14-1.85)	0.255
DQA301	8.2	9.8	1.22	(0.23-6.32)	0.914	11.4	1.45	(0.28-7.63)	0.900	10.5	1.32	(0.33-5.60)	0.899
DQA401	8.2	9.8	1.22	(0.23-6.32)	0.914	2.9	0.33	(0.01-3.40)	0.585	6.6	0.79	(0.17-3.75)	0.984
DQA501	0	2.4	NT			0	NT			1.3	NT		
DQA601	73.5	61.0	0.56	(0.21-1.51)	0.209	60.0	0.54	(0.19-1.51)	0.195	58.2	0.54	(0.23-1.23)	0.125
DQB501*	42.9	71.1	3.27	(1.22-8.95)	0.009	71.0	3.26	(1.13-9.58)	0.015	71.0	3.27	(1.42-7.60)	0.002
DQB502	8.2	2.6	0.30	(0.01-3.11)	0.525	12.5	1.61	(0.30-8.50)	0.795	6.3	0.87	(0.19-4.11)	0.884
DQB503	2.0	2.6	1.30	(0-49.41)	0.590	9.7	1.71	(0-65.82)	0.718	3.8	2.95	(0.30-71.67)	0.593
DQB601	16.3	10.5	0.60	(0-49.41)	0.642	16.1	0.99	(0.25-3.83)	0.774	11.4	0.77	(0.25-2.42)	0.618
DQB602	0	5.3	NT			3.2	NT			3.8	NT		
DQB603	2.0	2.6	1.30	(0-49.41)	0.590	0	NT			1.3	0.71	(0.02-26.58)	0.632
DQB201	8.2	5.3	0.63	(0.07-4.31)	0.918	0	NT			2.9	0.34	(0.24-2.26)	0.391
DQB301	71.4	60.5	0.61	(0.23-1.65)	0.287	51.6	0.43	(0.15-1.20)	0.074	49.4	0.52	(0.22-1.22)	0.100
DQB302	6.1	0	0	NT		12.9	2.27	(0.39-14.09)	0.522	5.1	0.94	(0.17-5.63)	0.747

freq - frequency NT - not tested
OR - Odds Ratio *p* - Chi-square test probability
* - Attributable risk = 49.4, 49.3% and 49.4% respectively for TT, LL and leprosy in general

Association of HLA-DQ and serum antibody levels in healthy subjects

In TABLE 6, the mean antibody levels of IgM anti PGL-I and IgG anti 36 kD antibodies are related to various HLA-DQ alleles. Alleles of DQA101 and DQB501 correlated significantly with IgM anti PGL-I antibody. A higher level of IgM anti PGL-I antibody was found in DQA101 negative compared to DQA101 positive and DQB501 negative individuals had a higher level of IgM anti PGL-I antibody compared to -DQB501 positive individuals. This was not true for IgG anti 36 kD antibody level, which was higher in DQA101 or DQB501 positive individuals than in DQA101 or DQB501 negative individuals, but neither were statistically significant.

DISCUSSION

Genetic susceptibility to leprosy or leprosy infection has been extensively investigated in some different populations (ethnic groups). In Indonesia, leprosy is an endemic infectious disease, however, so far no study related to the susceptibility to this disease has been done. Therefore, as a continuation of the previous study, this study may be the first in attempting to determine whether susceptibility to leprosy (or subclinical *M. leprae* infection) in the Indonesian population is also influenced by genetic factors, particularly HLA-related genes. As shown in the previous studies, the HLA is a genetic marker closely related to leprosy susceptibility, and particularly in Indonesia, HLA-DR2 and -DR12 are associated with leprosy. Among HLA antigens, HLA Class II antigens are directly related to the disease susceptibility, whereas HLA Class I antigens are not.[8,20] The previous study, has demonstrated that either HLA-DR2 or HLA-DR12 are associated with leprosy in Javanese population[12]. Therefore this study was focused on the HLA-DQ specificities, because the genetic markers in this loci have been demonstrated to be associated with leprosy in the largest number of studies[8]. In this study, a new method of HLA typing called the oligonucleotide typing technique was used as in the previous studies[12,13].

Subclinical infection with *M. leprae* can be demonstrated by the presence of specific antibody against *M. leprae* antigens in clinically healthy individuals[21,22,23,24]. The PGL-I antigen was used in this study because it had been standardized by W.H.O., and this antigen demonstrated positive responses in the majority of leprosy patients[25]. The 36 kD protein antigen used in this study is a membrane protein antigen identified by Klatser *et al.*[26,27] which has not yet been studied extensively so far.

TABLE 2. – Association between HLA-DQ1 specificities and leprosy

Groups	N	HLA-DQ1				
		freq.(%)	OR*	(95% CI)	χ^2	*p*
Control	50	61.2				
Leprosy						
TT	41	78.9	2.38	(0.82- 7.90)	3.10	0.08
LL	38	93.5	9.18	(1.89-86.30)	8.65	0.003
Total	79	85.5	3.83	(1.61- 9.10)	9.04	0.003

* Odds Ratio of leprosy (types) to control group

TABLE 3. – Association between HLA-DQ5 and -DQ6 with leprosy

Groups	N	DQ5					DQ5				
		freq. (%)	OR*	(95% CI)	χ^2	*p*	freq. (%)	OR*	(95% CI)	χ^2	*p*
Control	50	48.9									
Leprosy											
TT	41	71.0	2.56	(0.95- 6.95)	4.25	0.039	18.4	1.00	(0.95-6.95)	0.00	0.995
LL	38	77.4	3.57	(1.18-11.18)	6.23	0.012	19.3	1.07	(0.29-3.83)	0.01	0.913
Total	79	73.9	2.95	(1.27- 6.93)	7.63	0.006	18.8	1.03	(0.37-2.93)	0.02	0.993

* Odds Ratio of leprosy (types) to control group

TABLE 4. – Correlation of serum antibody level (IgM anti PGL-I and IgG anti 36 kD *M. leprae*) and HLA-DQ alleles in leprosy patients

Allele		n	IgM anti-PGL1 (OD)	*p*	IgG anti 36 kD (%-INH)	*p*
DQA101	pos	35	0.7330	0.792	54.88	0.717
	neg	41	0.6900		56.17	
DQA102	pos	24	0.6888	0.861	57.97	0.286
	neg	52	0.7195		54.47	
DQA103	pos	9	0.8069	0.662	51.22	0.368
	neg	67	0.6967		56.16	
DQA201	pos	6	0.3700	0.011	50.55	0.407
	neg	70	0.7389		56.01	
DQA301	pos	8	0.7555	0.847	62.26	0.032
	neg	68	0.7044		54.79	
DQA401	pos	5	0.2874	0.000	53.84	0.548
	neg	79	0.7395		55.70	
DQA501	pos	1	0.2780	NT	23.69	NT
	neg	75	0.7155		56.00	
DQA601	pos	46	0.7026	0.913	54.59	0.491
	neg	30	0.7209		57.09	
DQB501	pos	49	0.7199	0.796	56.56	0.419
	neg	20	0.6718		53.15	
DQB502	pos	5	0.7390	0.909	64.48	0.203
	neg	65	0.7021		55.13	
DQB503	pos	4	0.8573	0.565	62.63	0.378
	neg	66	0.6967		55.15	
DQB601	pos	9	0.7817	0.728	53.48	0.673
	neg	60	0.6946		55.88	
DQB602	pos	3	0.5350	0.666	58.84	0.716
	neg	66	0.7138		55.42	
DQB603	pos	1	0.4340	NT	54.24	NT
	neg	68	0.7100		55.59	
DQB604	pos	0	0.0000	NT	00.00	NT
	neg	69	0.7060		55.57	
DQB201	pos	2	0.3620	0.480	37.49	0.100
	neg	67	0.7163		56.11	
DQB301	pos	39	0.7397	0.649	55.89	0.845
	neg	30	0.6622		55.14	
DQB302	pos	4	1.1803	0.160	67.26	0.001
	neg	65	0.6768		54.85	
DQB303	pos	0	0.0000	NT	00.00	NT
	neg	69	0.7060		55.57	
DQB401	pos	0	0.0000	NT	00.00	NT
	neg	69	0.7060		55.57	
DQB402	pos	0	0.0000	NT	00.00	NT
	neg	69	0.7060		55.57	

OD : serum IgM anti PGL-1 antibody level expressed in mean Optical Density
%-INH : serum IgG anti 36 kD level expressed in percentage of inhibition
NT : not tested
p : probability (Student's t-test)

As in the previous report[12], a case control study was used in which a group of cases were compared to controls in relation to their HLA-DQ types. The selected cases were only of TT/BT and LL/BL type. BB leprosy was excluded in this study because of the immune instability of this form. The controls were recruited from healthy household contact of the cases which were non-blood related spouses. Healthy blood donors were added for the control group to determine a cut-off value seropositivity.

This study demonstrated that only HLA-DQB501 allele was associated significantly with leprosy, either TT or LL (TABLE 2), with OR of 3.26 (95% CI 1.42-7.60) and AR 49.4%. This association was also true for HLA-DQ5 which was related to DQB501, DQB502 and DQB503 sequences (TABLE 3). The association was greater for lepromatous than tuberculoid leprosy (OR: 3.57 vs. 2.56; AR: 55.7 vs. 48.9). Based on serology or microlymphocytotoxicity HLA typing techniques, most studies of HLA associated

TABLE 5. – Correlation of HLA-DQ alleles and subclinical infections detected by ELISA (IgM anti PGL-I) and INHIBITION-ELISA (IgG anti 36 kD)

Freq. Alleles (%)	ELISA					INHIBITION-ELISA				
	posotive (n=10)	negative (n=40)	OR	(95%CI)	*p*	positive (n=15)	negative (n=35)	OR	(95%CI)	*p*
DQA101	20.0	30.8	0.56	(0.07-3.62)	0.779	14.3	34.3	0.412	(0.11-1.63)	0.294
DQA102	30.0	33.3	0.86	(0.15-4.66)	0.859	35.7	31.4	1.15	(0.46-2.86)	0.772
DQA103	0	7.7	NT			0	8.6	NT		
DQA201	20.0	12.8	1.70	(0.19-13.27)	0.942	21.4	11.4	1.64	(0.61-4.42)	0.651
DQA301	0	10.3	NT			7.1	8.6	0.87	(0.15-5.02)	0.679
DQA401	10.0	7.7	1.33	(0-18.01)	0.682	0	11.4	NT		
DQA501	0	0	NT			0	0	NT		
DQA601	60.0	76.9	0.45	(0.08-2.45)	0.496	71.4	74.3	0.90	(0.34-2.38)	0.878
DQB501	22.2	47.5	0.32	(0.04-2.01)	0.156	46.7	41.2	1.17	(0.50-2.71)	0.720
DQB502	11.1	7.5	1.54	(0-21.33)	0.508	6.7	8.8	0.80	(0.14-4.63)	0.755
DQB503	11.1	0	NT			6.7	0	NT		
DQB601	11.1	17.5	0.59	(0.02-6.26)	0.976	20.0	14.7	1.28	(0.47-3.53)	0.965
DQB602	0	0	NT			0	0	NT		
DQB603	0	2.5	NT			0	2.9	NT		
DQB604	0	0	NT			0	0	NT		
DQB201	33.3	2.5	5.63	(2.21-14.33)	0.017	6.7	8.8	0.80	(0.14-4.63)	0.755
DQB301	66.7	72.5	0.76	(0.13-4.69)	0.507	66.7	72.5	0.80	(0.33-1.92)	0.624
DQB302	0	7.5	NT			0	5.9	1.10	(0.21-5.75)	0.588
DQB303	0	0	NT			0	0	NT		
DQB401	0	0	NT			0	0	NT		
DQB402	0	0	NT			0	0	NT		

freq – frequency OR – Odds Ratio
NT – not tested *p* – Chi-square test probability

disease had reported that the only DQ specificity associated with leprosy was DQ1[9]. Therefore, the possible association of this specificity with leprosy was analyzed. In the present study, HLA-DQw1, which is comprised of DQB501, DQB502, DQB503, DQB601, DQB602, DQB603 and DQB 604, is highly associated with lepromatous leprosy was analyzed, but not with tuberculoid (TABLE 3). This is similar with the previous findings in different populations[7,9,28,29]. Hence, the conclusion that this marker is a universal marker for lepromatous leprosy may still be true[10,21]. The OR of 9.18 (95% confidence interval 1.89-86.30, p=0.003) and the AR of 83.3% support that this association is very significant in this population.

The presence of combined leprosy associated with alleles in a haplotype may increase or decrease the risk of getting leprosy or leprosy type. The increased risk of lepromatous leprosy is found if a haplotype carries of DR2 combined with either DQ1, DQ5 or DQB501, whereas the combinations of any of the alleles with DR12 will decrease the risk of leprosy or leprosy type (data not shown). The fact that a combination of DR2 and DQ1 alleles is commonly found in this population is also supported by another HLA associated tuberculosis study[30]. This finding may explain the high proportion of multibacillary cases in this population[2]. The analysis of anti *M. leprae* antibody levels in relation to HLA specificities in leprosy patients showed a higher IgG anti 36 kD antibody level in DQA102 positive compared with in DQA102 negative individuals. This finding suggests that regulation of specific IgG anti 36 KD of *M. leprae* in leprosy is partly controlled by the HLA-linked gene or closely related genes. Other alleles like DQA201, DQA301 and DQA401 which seem to demonstrate associations with antibody levels, needs to be validated by further studies, because of their scarcity.

Although a significant association was found between HLA-DQA101 and -DQB501 with the antibody level of IgM anti PGL-I in healthy individuals, these alleles were not associated with subclinical infection in this group, even when the DQ1 specificity was analyzed (data not shown). All HLA-DQ specificities had no association with either specific IgM or IgG anti *M. leprae*, nor

TABLE 6. – Association of the serum antibody levels (IgM anti PGL-I and IgG anti 36 kD *M. leprae*) and HLA-DQ alleles in healthy subjects

Allele		n	IgM anti-PGL1 (OD)	*p*	IgG anti 36 kD (%-INH)	*p*
DQA101	pos	14	0.0993	0.158	30.98	0.804
	neg	35	0.1343		31.96	
DQA102	pos	16	0.1311	0.750	30.29	0.587
	neg	33	0.1210		32.36	
DQA103	pos	3	0.0717	0.361	25.77	0.397
	neg	46	0.1278		32.07	
DQA201	pos	7	0.1433	0.602	35.79	0.347
	neg	42	0.1212		30.99	
DQA301	pos	4	0.0485	0.003	21.97	0.362
	neg	45	0.1311		32.54	
DQA401	pos	4	0.1295	0.951	26.53	0.310
	neg	45	0.1239		32.14	
DQA501	pos	0	NT	NT	NT	NT
	neg	49	0.1243		31.68	
DQA601	pos	36	0.1207	0.684	31.65	0.981
	neg	13	0.1344		31.75	
DQB501	pos	21	0.0919	0.78	34.39	0.300
	neg	28	0.1387		30.65	
DQB502	pos	4	0.0943	0.613	28.07	0.486
	neg	45	0.1208		32.62	
DQB503	pos	1	1.1860	NT	49.82	NT
	neg	48	0.1173		31.82	
DQB601	pos	8	0.1234	0.885	34.45	0.588
	neg	41	0.1177		31.82	
DQB602	pos	0	NT	NT	NT	NT
	neg	49	0.1187		32.25	
DQB603	pos	1	0.0280	NT	33.87	NT
	neg	48	0.1205		32.22	
DQB604	pos	0	NT	NT	NT	NT
	neg	49	0.1187		32.25	
DQB201	pos	4	0.2005	0.085	32.23	0.997
	neg	45	0.1114		32.25	
DQB301	pos	35	0.1272	0.345	31.71	0.631
	neg	14	0.0973		33.62	
DQB302	pos	3	0.0627	0.002	25.16	0.310
	neg	46	0.1223		32.71	
DQB303	pos	0	NT	NT	NT	NT
	neg	49	0.1187		32.25	
DQB401	pos	0	NT	NT	NT	NT
	neg	49	0.1187		32.25	
DQB402	pos	0	NT	NT	NT	NT
	neg	49	0.1187		32.25	

OD : serum IgM anti PGL-1 antibody level expressed in mean Optical Density
%-INH : serum IgG anti 36 kD level expressed in percentage of inhibition
NT : not tested
p : probability (Students-t test)

with the subclinical infection. These findings support the argument that infection with *M. leprae* per se is not controlled by genetics or at least HLA-linked factors[4].

CONCLUSION

This study indicates that in addition to HLA-DR alleles, the susceptibility to (lepromatous) leprosy is also associated with HLA-DQ alleles specifically HLA-DQB501, HLA-DQ5 or HLA-DQ1. This also supports the previous findings that HLA-DQ1 is a universal marker for lepromatous leprosy; and that the infection with *M. leprae* per se is not controlled by HLA genetic factors.

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TABLE 7. – List of SSOs and the sequences used in the HHLA-DQ typing

SSO	Sequence 5' → 3'						Allele	Spesificity
3401	GAG	ATG	AGG	AGT	TCT	ACG	A101	–
3402	ATG	GAG	ATG	AGC	AGT	TCT	A102, 103, 501	–
2503	TCA	TGG	GTG	AAC	TGG	CCA	A103, 201, 601	–
2504	CTG	GCC	AGT	ACA	CCC	ATG	A101, 102, 401, 501	–
4101	ACC	TGG	AGA	GGA	AGG	AGA	A101,102, 02, 03	–
4102	ACC	TGG	AGA	AGA	AGG	AGA	A103	–
5501	TCA	GCA	AAT	TTG	GAG	GTT	A01	–
5502	CTG	TTC	CAC	AGA	CTT	AGA	A02	–
5503	CTG	TTC	CGC	AGA	TTT	AGA	A03(3011+3012+302)	–
5504	GTT	CTC	AGA	CAA	TTT	AGA	A401, 501, 601	–
6904	ATC	GCT	GTG	ACA	AAA	CAC	A401, 601	–
7404	CTT	GAA	CAG	TCT	GAT	TAA	A501	–
4000	TGG	GGA	GGA	AGG	AGA	CTG	all DQA	–
5DQAG	CCG	GGT	CGA	CTC	CCC	GCA	AGA 5'primer DQA	
3DQAG	TGC	TCT	AGA	GGG	CGA	CGA	CGC 3'perimer DQA	
4901	GGT	GTA	CCG	GGC	AGT	GAC	B501	DQ5(1)
5702	GCG	GCC	TAG	CGC	CGA	GTA	B502, 504	DQ5(1)
6703	GGC	GGC	CTG	ACG	CCG	AGT	B5031, 601	DQ5+DQ6(1)
5701	GCG	GCC	TGT	TGC	CGA	GTA	B501, 604, 605	DQ5+Dq6(1)
2601	CGG	GGT	GTG	ACC	AGA	CAC	B501, 502, 503	DQ5(1)
5704	GCG	GCC	TGC	TGC	CGA	GTA	B5032, 602, 603	DQ5+6(1)
3702	AGG	AGG	ACG	TAC	GCT	TCG	B601	DQ6(1)
7003	GAG	GGG	ACC	CGG	GCG	GAG	B602, 603	DQ6(1)
2604	CGT	CTT	GTA	ACC	AGA	CAC	B603, 604	DQ6(1)
2606	CGT	CTT	GTA	ACC	AGA	TAC	B605	DQ6(1)
3703	GAG	AAG	AGA	TCG	TGC	GCT	B201	DQ2
4501	GAC	GTG	GAG	GTG	TAC	CGG	B301	DQ7(3)
5707	GGC	CGC	CTG	CCG	CCG	AGT	B302	DQ8(3)
2301	GAC	CGA	GCT	CGT	GCG	GGG	B401	DQ4
2302	AAC	GGG	ACC	GAG	CGC	GTG	B3301, 402	DQ9(3)+4
2602	CGT	TAT	GTG	ACC	AGA	TAC	B301, 601	DQ7(3)+6(1)
2603	CGT	CTT	GTG	ACC	AGA	TAC	B302, 3031, 3032, 602	DQ8, 9, 6
5706	GGC	CGC	CTG	ACG	CCG	GTA	B301, 3031, 3032	DQ8, 9(3)
5708	GCG	GCT	TGA	CGC	CGA	GTA	B401, 402	DQ4
4201	CGC	TTC	GAC	AGC	GAC	GTG	all DQB	–
5DQBG	CTA	GTG	CTA	CTT	CAC	CAA	CGG 5'perimer DQC	
3DQBG	CTG	GTA	GTT	GTG	TCT	GCA	CAC 3'perimer DQB	

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