VI. RESULTS

A. Electrophoretic analysis of genomic DNA

In order to make sure that the genomic DNA prepared by the salting out method was of good quality and had evenly dissolved into the solution, 5 μl of the 100 μg/ml solution of each genomic DNA sample were electrophoresed on 0.7% agarose gel together with 500 ng of phage lambda DNA as control. After staining the agarose gel in ethidium bromide and washing in water, approximately equal amount of genomic DNA samples and phage lambda DNA were seen (Figure 5). This electrophoretic analysis demonstrated that the size and quantity of all genomic DNA samples were suitable and sufficient for the amplification by polymerase chain reaction.

B. Amplification of the HLA-DQA1 and HLA-DQB1 genes

In order to amplify the HLA-DQA1 and HLA-DQB1 genes in sufficient amount for hybridization with sequence-specific oligonucleotide probes, the PCR protocol suggested by the Eleventh International Histocompatibility Workshop was employed. The polymorphic second exon of HLA-DQA1 and HLA-DQB1 genes were amplified from genomic DNA samples of all leprosy patients and normal controls yielding the 229- and 214-bp fragments, respectively. To verify that the specifically amplified products were of the correct size and in sufficient quantity, a 3 μl-aliquot of the PCR products was analyzed on 1.5% agarose gel together with 1 μg of HaeIII-digested phi X 174 DNA as the size markers. As shown in Figures 6 and 7, PCR products of the HLA-DQA1 and HLA-DQB1 gene amplifications were detected with the expected sizes of 229 and 214 bp, respectively.

C. Identification of HLA-DQA1 and HLA-DQB1 genotypes

1. Identification of seven HLA-DQA1 alleles

In order to determine the genotype of the HLA-DQA1 locus of all subjects, 3 μl of the amplified products were dotted on the nylon membranes in a 96-dot

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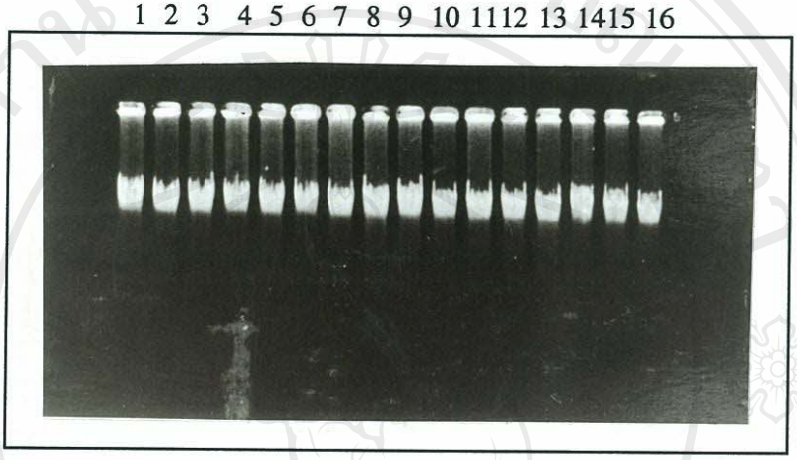

Figure 5. Agarose gel electrophoresis of genomic DNA samples. Five µl of 100 µl/ml solution of genomic DNA samples were run in 0.7% agarose gel (lanes 2-16) together with 500 ng of phage lambda DNA (lane 1).

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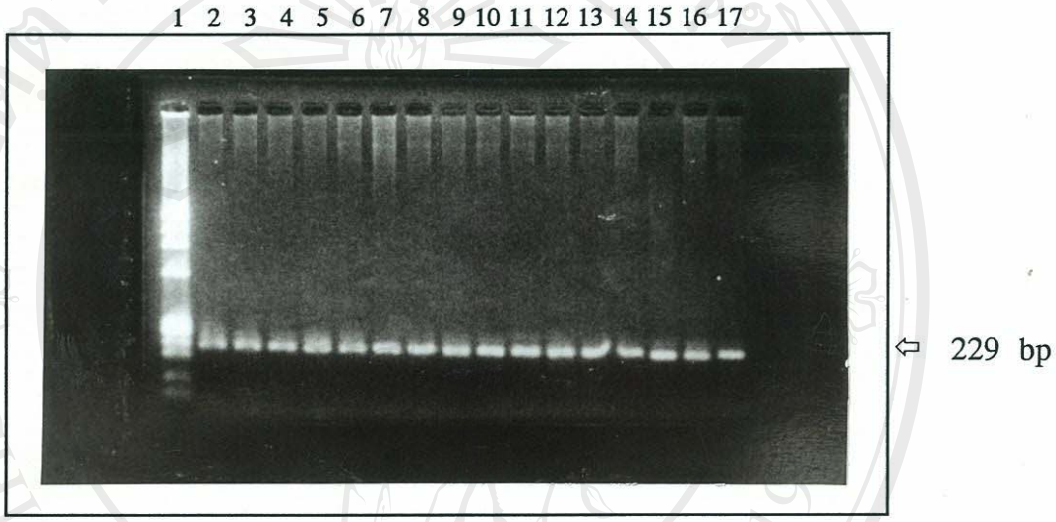

Figure 6. Agarose gel electrophoresis of the PCR products of HLA-DQA1 amplification. Genomic DNA samples were amplified for 30 cycles. Three μl of the PCR products were electrophoresed on 1.5% agarose gel. HaeIII-digested phi X 174 DNA was used as molecular weight markers.

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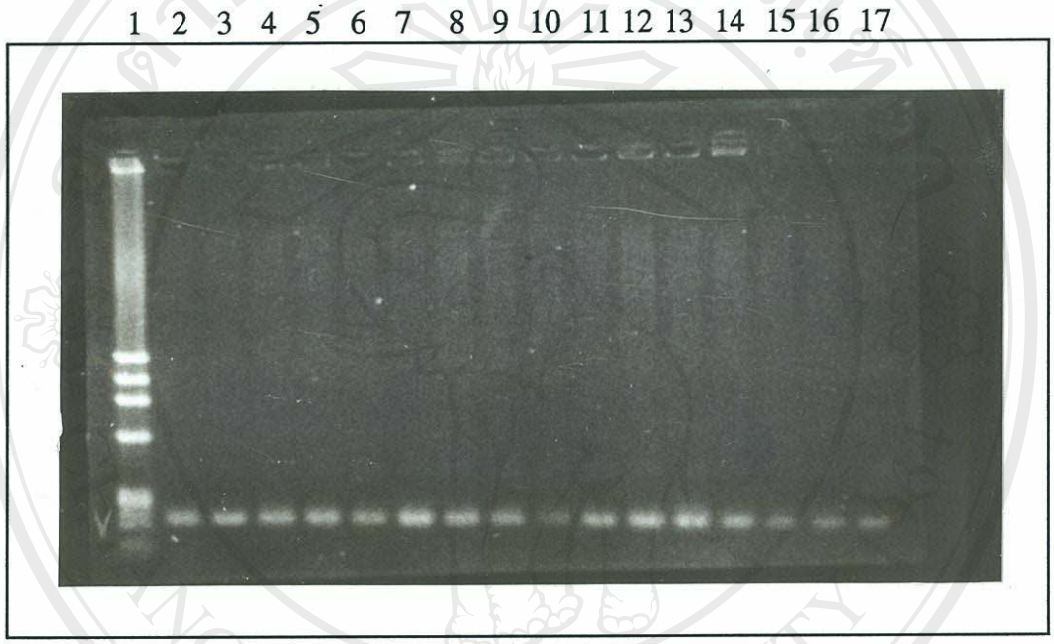

Figure 7. Agarose gel electrophoresis of the PCR products of HLA-DQB1 amplification. Genomic DNA samples were amplified for 30 cycles. Three µl of the PCR products were electrophoresed on 1.5% agarose gel. HaeIII-digested phi X 174 DNA was used as molecular weight markers.

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format and hybridized with ten HLA-DQA1 SSO probes. The hybridized probes were detected by DIG-anti-DIG chemiluminescent detection system. In the final step, the duration of autoradiography was adjusted to two hours to sixteen hours according to the difference in intensity between positive signal and non-specific background. An example of the hybridization signals was shown in Figure 8. In this example, the duration of exposure was adjusted such that both positive and negative signals were observed from the same membrane. The presence of both types of signal helped in the grading and designation of positive hybridization signals.

Of the possible nine alleles of the HLA-DQA1 locus, seven alleles were detected in both groups of subject. With an exception of four individuals, the use of ten HLA-DQA1 SSO probes allowed the determination of HLA-DQA1 genotype in all subjects. For N136, N140, N203 and L114, the positive hybridization results were obtained with the following four probes: DQA SSO 3402, 5503, 5504 and 6903. Such pattern of hybridization was compatible with the designation of both HLA-DQA1*03/*05 and HLA-DQA1*03012/*05. The ambiguity was resolved by hybridization with an additional probe, DQA SSO 6902 which helped to clearly identify the genotype of these exceptional subjects as HLA-DQA1*03/*05.

2. Identification of eleven HLA-DQB1 alleles

In order to determine the genotype of the HLA-DQB1 locus of all individuals, 3 µl of the PCR products were hybridized with seventeen DQB1 SSO probes. The conditions of hybridization and washing for DQB1 SSO probes were the same as those of DQA1 SSO probes. The positive and negative hybridization signals were obtained with fifteen DQB1 SSO probes. Even though the exposure time was extended to over 24 hours, the SSO 2606 and SSO 2302 probes did not yield any positive result. An example of the hybridization signals when reacted with an DQB1 SSO probe was shown in Figure 9.

Only eleven out of seventeen possible HLA-DQB1 alleles were detected in both groups. In three genomic DNA samples, N205, L90, L13, the hybridization results were suggestive of an unusual HLA-DQB1* allele (Table 7).

In the case of N205 and L13, the positive hybridization results were obtained with the DQB1 2601, 5702, 4901, 5701 and 2603 SSO probes. The

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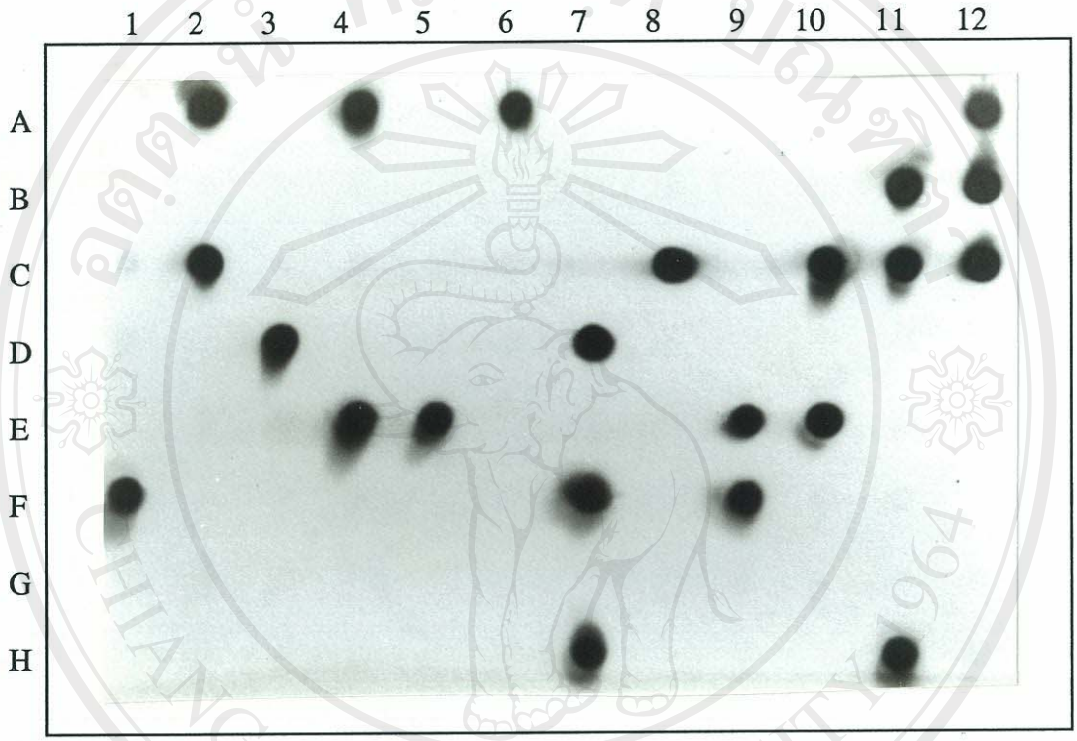

Figure 8. An example of the HLA-DQA1 allele hybridization signals: HLA-DQA1 amplified products hybridized with DIG-labeled DQA 3403 SSO probes.

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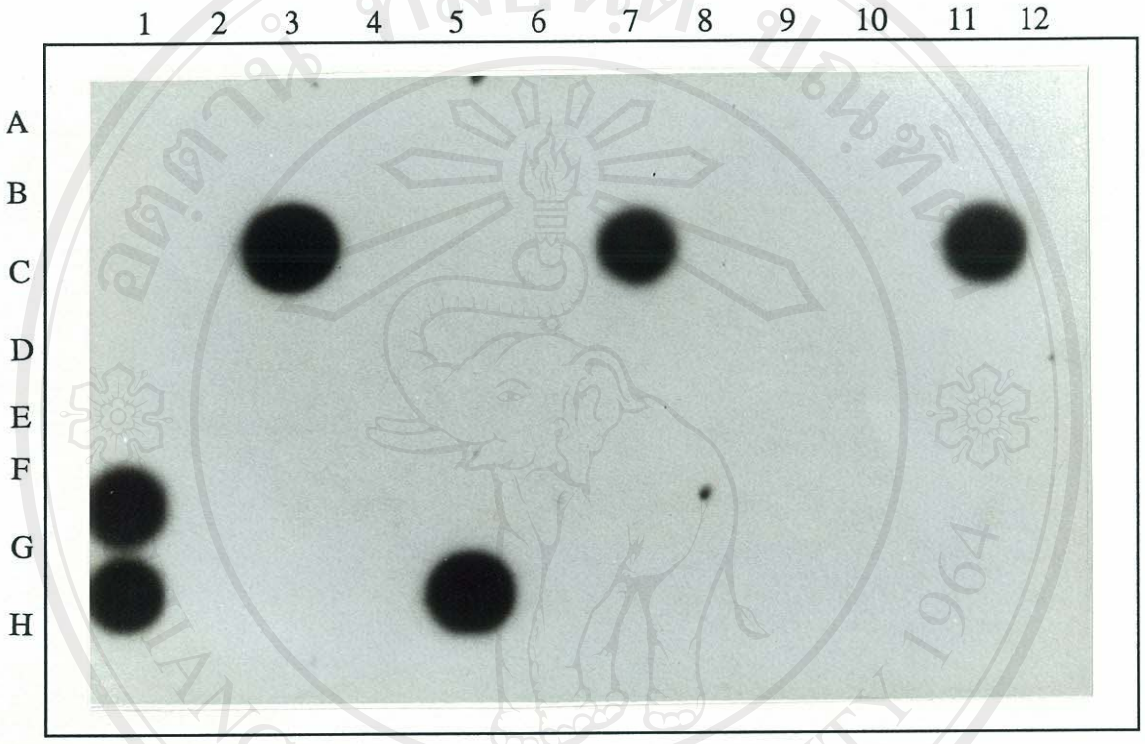

Figure 9. An example of the HLA-DQB1 allele hybridization signals: HLA-DQB1 amplified products hybridized with DIG-labeled DQB 0504 SSO probes.

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Table 9. Possible unusual HLA-DQB1 allele

DNA sample	HLA-DQB1 SSO probes yielded positive hybridization	Possible HLA-DQB1 allele
N205	2601, 5702, 4901, 5701, 2603	0501/0502*
L13	2601, 5702, 4901, 5701, 2603	0501/0502*
L90	2601, 5702, 2603	0502/0502*

* = unusual allele

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positive hybridizations with the first four probes were sufficient to assign their genotype as DQB1*0501/*0502. However, because they also hybridized with DQB1 SSO 2603, which recognized HLA-DQB1*0602, *0302, *03031 and *03032, either one or both of these two alleles was/were atypical. The reactivity with the DQB1 SSO 2603 probe was unlikely to be due to the presence of the alleles HLA-DQB1*0602, *0302, *03031 or *03032 in the subjects' genome nor to the contamination of the subjects' DNA samples because the DQB1 SSO probes 5704, 5706 and 5707 also gave negative results (Table 6). Quite similarly, L90 also hybridized with the DQB1 SSO 2601 and 5702 probes and should be assigned as HLA-DQB1*0502/*0502. One or both of the HLA-DQB1*0502 alleles in L90 was/were unusual because of the reactivity with the DQB1 SSO 2603 probe.

D. Distribution of the HLA-DQA1 and HLA-DQB1 alleles in normal northern Thais

The allele frequencies of the HLA-DQA1 locus in 119 normal northern Thais were obtained by direct counting (Table 8). Of all possible nine HLA-DQA1 alleles, the HLA-DQA1*03032 and -DQA1*0401 alleles were not found in this population. Among the seven alleles detected, the alleles HLA-DQA1*0102 and -DQA1*0101 were the most common alleles (28.8% and 25.8%, respectively). The alleles HLA-DQA1*0103 and -DQA1*0201 were rare (2.5% and 2.9%, respectively).

Among eleven HLA-DQB1 alleles found in the normal northern Thai group, HLA-DQB1*0502 was detected with highest frequency (35.4%) (Table 9). The second most frequent allele was -DQB1*0301 (15.8%). The HLA-DQB1*0602 and -DQB1*0603 alleles were rare (0.8%). The other six alleles (HLA-DQB1*05032, -DQB1*0504, -DQB1*0604, -DQB1*0605, -DQB1*03031 and -DQB1*0402) were not found.

E. Distribution of the HLA-DQA1 and HLA-DQB1 genotypes in normal northern Thais according to the Hardy-Weinberg analysis

In order to determine the nature of distribution of HLA-DQA1 and HLA-DQB1 genotypes among northern Thais, the Hardy-Weinberg analysis was

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Table 10. Antigen frequency and allele frequency of the HLA-DQA1 locus in 119 normal northern Thais.

DQA1 allele	Antigen frequency (%)	Allele frequency (%)
0101	45.8	25.8
0102	48.3	28.8
0103	4.2	2.5
0201	5.8	2.9
03	35	19.2
03012	0	0
0401	0	0
0501	17.5	9.2
0601	22.5	11.7

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Table 11. Antigen frequency and allele frequency of the HLA-DQB1 locus in 119 normal northern Thais

DQB1 allele	Antigen frequency (%)	Allele frequency (%)
0501	18.3	10.4
0502	60	35.4
05031	8.3	4.2
05032	0	0
0504	0	0
0601	11.7	5.8
0602	1.7	0.8
0603	1.7	0.8
0604	0	0
0605	0	0
0201	15.8	7.9
0301	30.8	15.8
0302	6.7	3.3
03031	0	0
03032	22.5	12.1
0304	0	0
0401	6.7	3.3
0402	0	0

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performed. The expected genotype frequencies were calculated from the allele frequencies and were then compared with the observed genotype frequencies to determine whether the distribution of the observed genotype frequency fitted the binomial distribution model in the same way as the expected genotype frequency. The chi-square test was employed for the determination of statistical significance.

For the HLA-DQA1 locus, the observed genotype frequency and expected genotype frequency were shown in Table 10. Among the twenty-eight possible HLA-DQA1 genotypes (expected frequency > 0), twenty-four genotypes (85.7%) were observed. The two most frequent HLA-DQA1 genotypes in normal northern Thais were HLA-DQA1*0102/03 and *0102/03 (15.1% and 11.8%, respectively). The observed and expected HLA-DQA1 genotype frequencies were very similar and theHardy-Weinberg analysis of HLA-DQA1 genotype revealed the chi-square value of 20.4 ($p > 0.05$, degree of freedom = 44). This results indicated that all observed genotypes of the HLA-DQA1 locus of this normal northern Thais group were in the Hardy-Weinberg equilibrium. Similar results were found for the leprosy patient group, the LL + BL group and the TT + BT group (Table 11).

Among sixty-six possible HLA-DQB1 genotypes (expected frequency > 0), only thirty-five (53%) genotypes were observed. The most common HLA-DQB1 genotypes among northern Thais were HLA-DQB1*0502/0301 (14.3%), HLA-DQB1*0502/*0502 (10.9%) and HLA-DQB1*0502/*03032 (10.9%). The observed and expected frequencies of each HLA-DQB1 genotype were very similar. The Hardy-Weinberg analysis indicated that the genotypes of HLA-DQB1 locus were in equilibrium in the normal northern Thai group ($p > 0.975$) (Table 12).

The distribution of HLA-DQB1 genotypes in the leprosy group was similar to those of normal controls. The genotype HLA-DQB1*0502/*0502 was the most common (11.9%), followed by DQB1*0502/*03032 (10.5%). In LL+BL group, the genotypes DQB1*0501/*0502 and DQB1*0502/*0301 were the most frequent (12%). In TT+BT patients, the genotype DQB1*0502/*0502 was again the most common one (17.6%). Analysis of the HLA-DQB1 genotype in the leprosy group, LL+BL group and TT+BT group indicated that this locus was also in Hardy-Weinberg equilibrium as in the normal control group (Table 13).

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Table 12. Hardy-Weinberg analysis of the HLA-DQA1 genotype of 119 normal northern Thais.

DQA1* allele	Expected frequency (n=119)	Observed frequency (n=119)	χ^2 (O-E) /E
0101/0101	7.92	7	0.10687
0101/0102	17.68	18	0.00579
0101/0103	1.54	1	0.18935
0101/0201	1.78	2	0.02719
0101/03	11.78	10	0.26896
0101/0501	5.65	5	0.07478
0101/0601	7.18	11	2.03237
0102/0102	9.87	11	0.12937
0102/0103	1.71	0	1.71000
0102/0201	1.99	2	0.00005
0102/03	13.16	14	0.05362
0102/0501	6.31	6	0.01523
0102/0601	8.02	6	0.50878
0103/0103	0.07	1	12.3557
0103/0201	0.17	0	0.17000
0103/03	1.14	2	0.64877
0103/0501	0.55	1	0.36818
0103/0601	0.70	0	0.70000
0201/0201	0.10	0	0.10000
0201/03	1.33	1	0.08188
0201/0501	0.63	1	0.21730
0201/0601	0.81	1	0.04457
03/03	4.39	4	0.03465
03/0501	4.20	5	0.15238
03/0601	5.35	6	0.07897
0501/0501	1.01	1	0.00010
0501/0601	2.56	2	0.12250
0601/0601	1.63	1	0.24350
		Chi-squre =	20.4409
		d.f. = 44	
		$p > 0.05$	

HLA-DQA1* alleles that expected frequencies is equal to zero, were not show

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Table 13. Hardy-Weinberg analysis of the HLA-DQA1 genotype of leprosy and two leprosy subtypes patients.

DQA1 allele	Exp freq (n=119) controls	Exp freq (n=143) controls	Obs freq (n=143) leprosy	$(O-E)^2/E$ leprosy	Obs freq. Lepro - matous. (n=76)	$(O-E)^2/E$ Lepro - matous.	Obs freq. Tuber- culoid (n=67)	$(O-E)^2/E$ Tuber- culoid
0101/0101	7	8.471	7	0.255	5	1.422	2	4.943
0101/0102	18	21.782	31	3.901	20	0.146	11	5.337
0101/0103	1	1.210	0	1.210	0	1.210	0	1.210
0101/0201	2	0.000	3	3.901	1	0.146	2	5.337
0101/03	10	12.101	14	0.298	8	1.390	6	3.076
0101/0501	5	6.050	8	0.628	4	0.695	4	0.695
0101/0601	11	13.311	8	2.119	5	5.189	3	7.987
0102/0102	11	13.311	11	0.401	2	9.611	9	1.396
0102/0103	0	0.000	0	0.000	0	0.000	0	0.000
0102/0201	2	2.420	6	5.295	2	0.073	4	1.031
0102/03	14	16.941	19	0.250	12	1.441	7	5.834
0102/0501	6	7.261	10	1.034	4	1.464	6	0.219
0102/0601	6	7.261	7	0.009	5	0.704	2	3.811
0103/0103	1	1.210	0	1.210	0	1.210	0	1.210
0103/0201	0	0.000	0	0.000	0	0.000	0	0.000
0103/03	2	2.420	1	0.833	0	2.420	1	0.833
0103/0501	1	1.210	2	0.516	1	0.036	1	0.036
0103/0601	0	0.000	0	0.000	0	0.000	0	0.000
0201/0201	0	0.000	0	0.000	0	0.000	0	0.000
0201/03	1	1.210	2	0.516	0	1.210	2	0.516
0201/0501	1	1.210	0	1.210	0	1.210	0	1.210
0201/0601	1	1.210	1	0.036	1	0.036	0	1.210
03/03	4	4.840	6	0.278	3	0.700	3	0.700
03/0501	5	6.050	3	1.538	1	4.216	2	2.712
03/0601	6	7.261	2	3.811	1	5.398	1	5.398
0501/0501	1	1.210	1	0.036	0	1.210	1	0.036
0501/0601	2	2.420	1	0.833	1	0.833	0	2.420
0601/0601	1	1.210	0	1.210	0	1.210	0	1.210
Chi-square			=	31.331		43.182		58.367

d.f. = 44

$p > 0.05$

HLA-DQA1* alleles that expected frequencies is equal to zero, were not shown

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Table 14. Hardy-Weinberg analysis of the HLA-DQB1 genotype of 119 normal northern Thais.

DQB1 genotype	Expected frequencies (n=119)	Observed frequencies (n=119)	$(O-E)^2/E$
0501/0501	1.29	3	2.267
0501/0502	8.76	8	0.066
0501/05031	1.04	1	0.002
0501/0601	1.44	2	0.218
0501/0602	0.2	0	0.200
0501/0603	0.2	0	0.200
0501/0201	1.96	3	0.552
0501/0301	3.91	1	2.166
0501/0302	0.82	0	0.820
0501/03032	2.99	1	1.324
0501/0401	0.82	2	1.698
0502/0502	14.91	13	0.245
0502/05031	3.54	3	0.082
0502/0601	4.89	5	0.002
0502/0602	0.67	1	0.163
0502/0603	0.67	1	0.163
0502/0201	6.66	8	0.270
0502/0301	13.31	17	1.023
0502/0302	2.78	2	0.219
0502/03032	10.19	13	0.775
0502/0401	2.78	1	1.140
05031/05031	0.21	0	0.210
05031/0601	0.58	2	3.477
05031/0602	0.08	0	0.080
05031/0603	0.08	0	0.080
05031/0201	0.79	0	0.790
05031/0301	1.58	2	0.112
05031/0302	0.33	1	1.360
05031/03032	1.21	0	1.210
05031/0401	0.33	0	0.330
0601/0601	0.4	0	0.400
0601/0602	0.11	0	0.110
0601/0603	0.11	0	0.110
0601/0201	1.09	0	1.090

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Table 14. (Continued).

DQB1 genotype	Expected frequencies (n=119)	Observed frequencies (n=119)	$(O-E)^2/E$
0601/0301	2.18	2	0.015
0601/0302	0.46	0	0.460
0601/03032	1.67	2	0.065
0601/0401	0.46	1	0.634
0602/0602	0.01	0	0.010
0602/0603	0.02	0	0.020
0602/0201	0.15	0	0.150
0602/0301	0.3	0	0.300
0602/0302	0.06	0	0.060
0602/03032	0.23	0	0.230
0602/0401	0.06	1	14.727
0603/0603	0.01	0	0.010
0603/0201	0.15	0	0.150
0603/0301	0.3	0	0.300
0603/0302	0.06	1	14.727
0603/03032	0.23	0	0.230
0603/0401	0.06	0	0.060
0201/0201	0.74	0	0.740
0201/0301	2.97	5	1.388
0201/0302	0.62	0	0.620
0201/03032	2.28	3	0.227
0201/0401	0.62	0	0.620
0301/0301	2.97	1	1.307
0301/0302	1.24	3	2.498
0301/03032	4.55	4	0.066
0301/0401	1.24	2	0.466
0302/0302	0.13	0	0.130
0302/03032	0.95	1	0.003
0302/0401	0.26	0	0.260
03032/03032	1.74	2	0.039
03032/0401	0.95	1	0.003
0401/0401	0.13	0	0.130

Chi-square = 40.643

d.f. = 170

$p > 0.05$

HLA-DQB1* alleles that expected frequencies is equal to zero, were not shown

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Table 15. Hardy-Weinberg analysis of the HLA-DQB1 genotype in leprosy and two leprosy subtypes patients.

DQB1 genotype	Exp freq (n=119) control	Exp freq (n=142) control	Obs freq (n=142) leprosy	$(O-E)^2/E$ leprosy	Obs freq Lepro-matous (n=75)	$(O-E)^2/E$ Lepro-matous	Obs freq Tuber-culoid (n=67)	$(O-E)^2/E$ Tuber-culoid
0501/0501	3	3.605	1	1.8824	0	3.6050	1	1.8824
0501/0502	8	9.613	14	2.0016	9	0.0391	5	2.2140
0501/05031	1	1.202	1	0.0338	1	0.0338	0	1.2017
0501/0601	2	2.403	4	1.0607	3	0.1481	1	0.8194
0501/0602	0	0.000	0	0.0000	0	0.0000	0	0.0000
0501/0603	0	0.000	0	0.0000	0	0.0000	0	0.0000
0501/0201	3	3.605	3	0.1015	1	1.8824	2	0.7146
0501/0301	1	1.202	4	6.5164	4	6.5164	0	1.2017
0501/0302	0	0.000	0	0.0000	0	0.0000	0	0.0000
0501/03032	1	1.202	4	6.5164	0	1.2017	4	6.5164
0501/0401	2	2.403	0	2.4034	0	2.4034	0	2.4034
0502/0502	13	15.622	17	0.1216	5	7.2222	12	0.8397
0502/05031	3	3.605	4	0.0433	4	0.0433	0	3.6050
0502/0601	5	6.008	2	2.6741	2	2.6741	0	6.0084
0502/0602	1	1.202	2	0.5304	1	0.0338	1	0.0338
0502/0603	1	1.202	0	1.2017	0	1.2017	0	1.2017
0502/0201	8	9.613	11	0.2000	4	3.2778	7	0.7105
0502/0301	17	20.429	13	2.7013	9	6.3936	4	13.2118
0502/0302	2	2.403	7	8.7915	6	5.3824	1	0.8194
0502/03032	13	15.622	15	0.0248	8	3.7187	7	4.7585
0502/0401	1	1.202	1	0.0338	0	1.2017	1	0.0338
05031/05031	0	0.000	0	0.0000	0	0.0000	0	0.0000
05031/0601	2	2.403	2	0.0677	1	0.8194	1	0.8194
05031/0602	0	0.000	0	0.0000	0	0.0000	0	0.0000
05031/0603	0	0.000	0	0.0000	0	0.0000	0	0.0000
05031/0201	0	0.000	0	0.0000	0	0.0000	0	0.0000
05031/0301	2	2.403	5	2.8055	2	0.0677	3	0.1481
05031/0302	1	1.202	1	0.0338	1	0.0338	0	1.2017
05031/03032	0	0.000	2	0.0000	2	0.0000	0	0.0000
05031/0401	0	0.000	0	0.0000	0	0.0000	0	0.0000
0601/0601	0	0.000	1	0.0000	0	0.0000	1	0.0000
0601/0602	0	0.000	3	0.0000	0	0.0000	0	0.0000
0601/0603	0	0.000	0	0.0000	0	0.0000	0	0.0000

Table 15. (Continued).

DQB1 genotype	Exp freq (n=119) control	Exp freq (n=142) control	Obs freq (n=142) leprosy	$(O-E)^2/E$ leprosy	Obs freq (n=75) Lepro-matous	$(O-E)^2/E$ Lepro-matous	Obs freq (n=67) Tuber-culoid	$(O-E)^2/E$ Tuber-culoid
0601/0301	2	2.403	1	0.8194	0	2.4034	1	0.8194
0601/0302	0	0.000	0	0.0000	0	0.0000	0	0.0000
0601/03032	2	2.403	6	5.3824	2	0.0677	4	1.0607
0601/0401	1	1.202	0	1.2017	0	1.2017	0	1.2017
0602/0602	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/0603	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/0201	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/0301	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/0302	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/03032	0	0.000	0	0.0000	0	0.0000	0	0.0000
0602/0401	1	1.202	0	1.2017	0	1.2017	0	1.2017
0603/0603	0	0.000	0	0.0000	0	0.0000	0	0.0000
0603/0201	0	0.000	0	0.0000	0	0.0000	0	0.0000
0603/0301	0	0.000	0	0.0000	0	0.0000	0	0.0000
0603/0302	1	1.202	0	1.2017	0	1.2017	0	1.2017
0603/03032	0	0.000	0	0.0000	0	0.0000	0	0.0000
0603/0401	0	0.000	0	0.0000	0	0.0000	0	0.0000
0201/0201	0	0.000	1	0.0000	0	0.0000	1	0.0000
0201/0301	5	6.008	2	2.6741	1	4.1748	1	4.1748
0201/0302	0	0.000	0	0.0000	0	0.0000	0	0.0000
0201/03032	3	3.605	2	0.7146	1	1.8824	1	1.8824
0201/0401	0	0.000	0	0.0000	0	0.0000	0	0.0000
0301/0301	1	1.202	1	0.0338	1	0.0338	0	1.2017
0301/0302	3	3.605	3	0.1015	1	1.8824	2	0.7146
0301/03032	4	4.807	2	1.6389	0	4.8067	2	1.6389
0301/0401	2	2.403	0	2.4034	0	2.4034	0	2.4034
0302/0302	0	0.000	0	0.0000	0	0.0000	0	0.0000
0302/03032	1	1.202	2	0.5304	1	0.0338	1	0.0338
0302/0401	0	0.000	0	0.0000	0	0.0000	0	0.0000
03032/03032	2	2.403	3	0.1481	1	0.8194	2	0.0677
03032/0401	1	1.202	2	0.5304	2	0.5304	0	1.2017
0401/0401	0	0.000	0	0.0000	0	0.0000	0	0.0000
Chi-square =				18.5821		22.6434		18.8042

d.f. = 170

$p > 0.05$

HLA-DQB1* alleles that expected frequencies is equal to zero, were not shown

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F. Linkage disequilibrium of the HLA-DQA1 and -DQB1 loci in normal northern Thais

In order to determine the association between HLA-DQA1 and HLA-DQB1 loci in normal northern Thais, the linkage disequilibrium of these two loci was analyzed. Linkage disequilibrium was defined as the tendency of specific combinations of two alleles at two or more linked loci to occur together on the same chromosome more frequently than would be expected by chance (Lewin and Benjamin, 1993). Since the family study was not performed, the chromosomal phase (actual linkage) of each individual was unknown. However, strong linkage of the HLA-DQA1 and -DQB1 alleles may be detected without performing actual family study. This was done by comparing, for a particular pair of HLA-DQA1 allele and HLA-DQB1 allele, the actual haplotype frequency (determined by direct counting) with the expected haplotype frequency (determined by mutiplying the allele frequencies of the two alleles). From such analysis, there were eleven HLA-DQA1 and -DQB1 haplotypes with significant linkage disequilibrium in the normal northern Thai group (Table 14). Four haplotypes that showed both strong association and high frequency were HLA-DQA1*0102-DQB1*0502, -DQA1*0101-DQB1*0501, -DQA1*0601-DQB1*0301 and -DQA1*03-DQB1*03032.

G. Equal distribution of the HLA-DQA1 alleles between leprosy patients and normal controls

In order to determine the association between leprosy, and two broad types of leprosy and the HLA-DQA1 allele in northern Thai population, the frequencies (antigen and allele) of nine HLA-DQA1 alleles derived from 143 leprosy patients, 76 patients with lepromatous form and 67 patients with tuberculoid form of leprosy were compared with those of 120 normal controls (Tables 15, 16, 17 and 18). The allele frequency was obtained by direct counting the actual number of the allele carried in each individual, presuming that all individuals were diploid with regards to the two loci tested, whereas the antigen frequency was obtained by counting of number of individuals who carried the allele. Thus, a homozygote would contribute

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Table 16. The HLA-DQA1-DQB1 haplotypes with significant linkage disequilibrium among 119 normal northern Thais.

DQA1-DQB1 haplotype	Observed frequency	Expected frequency	delta (%)	chi-square	p value
0101-0501	0.067	0.027	4	30.69	$<10^{-7}$
0101-05031	0.023	0.011	1.2	11.72	0.0006184
0102-0502	0.172	0.102	7	38.42	$<10^{-7}$
0103-0601	0.008	0.001	0.7	11.7	0.0006256
0201-0201	0.015	0.002	1.3	39.14	$<10^{-7}$
03-0302	0.017	0.006	1.1	15.72	0.0000733
03-03032	0.074	0.023	5.1	64.03	$<10^{-7}$
03-0401	0.019	0.006	1.3	15.72	0.0000733
0501-0201	0.029	0.007	2.2	40.11	$<10^{-7}$
0501-0301	0.027	0.015	1.2	8.08	0.0044832
0601-0301	0.063	0.018	4.5	77.4	$<10^{-7}$

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Table 17. Comparison of the antigen frequency of HLA-DQA1 alleles between leprosy patients and normal controls.

DQA1* alleles	normal controls (n=120)		Leprosy (n=143)		Relative risk	p	Pc
	n	%	n	%			
0101	55	45.8	71	49.3	1.15	0.574	5.166
0102	58	48.3	84	58.7	1.52	0.092	0.828
0103	5	4.2	3	2.1	0.49	0.325	2.925
0201	7	5.8	12	8.3	1.47	0.434	3.906
03	42	35	47	32.6	0.9	0.686	6.174
03012	0	0	0	0	ND	ND	ND
0401	0	0	0	0	ND	ND	ND
0501	21	17.5	25	17.4	0.99	0.976	8.784
0601	27	22.5	19	13.2	0.52	0.047	0.423

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Table 18. Comparison of the antigen frequency of HLA-DQA1 alleles between tuberculoid and lepromatous leprosy patients with normal controls.

DQA1* allele	normal controls	Tuberculoid group (n=67)				Lepromatous group (n=76)			
	%	%	R.R.	p	Pc	%	R.R.	p	Pc
0101	45.8	41.2	0.83	0.537	4.833	56.6	1.54	0.143	1.287
0102	48.3	58.2	1.49	0.195	1.755	59.2	1.55	0.137	1.233
0103	4.2	2.9	0.67	0.67	6.03	1.3	0.31	0.259	2.331
0201	5.8	11.8	2.15	0.149	1.341	5.3	0.90	0.865	7.785
03	35	32.4	0.14	0.713	6.417	32.9	0.91	0.762	6.858
03012	0	0	ND	ND	ND	0	ND	ND	ND
0401	0	0	ND	ND	ND	0	ND	ND	ND
0501	17.5	20.6	1.22	0.601	5.409	14.5	0.80	0.576	5.184
0601	22.5	8.8	0.33	0.018	0.162	17.1	0.71	0.361	3.249

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Table 19. Comparison of the allele frequencies of HLA-DQA1 alleles between leprosy patients and normal controls.

DQA1* alleles	control group (total no.of allele = 240)		leprosy group (total no.of allele = 288)		Relative risk	p	Pc
	number	%	number	%			
0101	62	25.8	78	27.1	1.07	0.746	6.714
0102	69	28.8	95	33.2	1.23	0.271	2.439
0103	6	2.5	3	1.0	0.41	0.312	2.808
0201	7	2.9	12	4.2	1.47	0.444	3.996
03	46	19.2	53	18.4	0.95	0.823	7.407
03012	0	0	0	0	ND	ND	ND
0401	0	0	0	0	ND	ND	ND
0501	22	9.2	26	9.0	0.98	0.956	8.604
0601	28	11.7	19	6.6	0.53	0.042	0.378

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Table 20. Comparison of the allele frequency of HLA-DQA1 alleles between tuberculoid and lepromatous leprosy patients with normal controls.

DQA1* allele	normal controls	Tuberculoid group (n=134)					Lepromatous group (n=152)				
	%	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0101	25.8	30	22.1	0.81	0.413	3.717	48	31.6	1.33	0.217	1.953
0102	28.8	48	35.8	1.38	0.157	1.413	47	30.9	1.11	0.646	5.814
0103	2.5	2	1.5	0.58	0.399	3.591	1	0.7	0.26	0.256	2.304
0201	2.9	8	5.9	2.08	0.158	1.422	4	2.6	0.90	1.000	9
03	19.2	25	18.4	0.95	0.852	7.668	28	18.4	0.95	0.854	7.686
03012	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0401	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0501	9.2	15	11.0	1.23	0.560	5.04	11	7.2	0.77	0.503	4.527
0601	11.7	6	4.4	0.35	0.018	0.162	13	8.6	0.71	0.326	2.934

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two units to the allele frequency, but yielded only one unit to the antigen frequency.

None of the seven HLA-DQA1 alleles was significantly increased or decreased in leprosy patients when both of the antigen and allele frequencies were compared with normal controls ($p > 0.05$). When the leprosy patients were divided into lepromatous (LL + BL) group and tuberculoid (TT + BT) group, comparison of the HLA-DQA1 antigen frequencies between each of the two types of leprosy with normal controls revealed that initially the HLA-DQA1*0601 allele was decreased in tuberculoid pole leprosy patients ($p = 0.018$), but this difference failed to reach significance after the p value was corrected by seven, the number of alleles of the HLA-DQA1 locus compared. There was no significant difference in the HLA-DQA1 allele frequencies between each of the two types of leprosy and normal controls.

In order to examine whether the HLA-DQA1 and HLA-DQB1 alleles were associated with any of the four different types of leprosy, we further divided the tuberculoid leprosy patients into 22 TT and 45 BT patients. Similarly, the patients belonging to the lepromatous pole were segregated into 28 LL and 48 BL patients. Comparison of the HLA-DQA1 antigen frequencies between each of the four types of leprosy and normal controls disclosed that no significant difference with regards to all seven HLA-DQA1 alleles (Table 19). Thus, none of the HLA-DQA1 alleles was significantly increased or decreased in leprosy or subtypes of leprosy when compared with normal controls.

H. Equal distribution of the HLA-DQB1 alleles between leprosy patients and normal controls

In order to determine the association between leprosy or the subtypes of leprosy with the HLA-DQB1 alleles, the antigen frequencies and allele frequencies of 11 HLA-DQB1 alleles were compared. In this study, 142 leprosy patients, 75 lepromatous leprosy patients, 67 tuberculoid leprosy patients and 120 normal controls were included. Comparisons of the HLA-DQB1 antigen frequencies and allele frequencies between leprosy or both types of leprosy patients with normal controls also revealed that none of 11 HLA-DQB1 alleles was significantly increased or decreased in all patient groups (Tables 20, 21, 22 and 23). When the

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Table 21. Comparison of the antigen frequency of HLA-DQA1 alleles between TT, BT, BL and LL leprosy patients and normal controls.

DQA1	controls (n=120)	TT (n=22)					BT (n=45)				
	(%)	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0101	46	11	50.0	1.18	0.719	6.471	17	37.8	0.72	0.353	3.177
0102	48	12	54.5	1.28	0.592	5.328	27	60.0	1.60	0.182	1.638
0103	4	0	0.0	0.00	1.000	9.000	2	4.4	1.07	1.000	9.000
0201	6	4	18.2	1.57	0.493	4.437	4	8.9	3.59	0.069	0.621
03	35	7	31.8	0.87	0.773	6.957	15	33.3	0.93	0.841	7.569
03012	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0401	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0501	18	5	22.7	1.39	0.555	4.995	9	20.0	1.18	0.885	7.965
0601	23	2	9.1	0.34	0.248	2.232	4	8.9	0.34	0.462	4.158

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Table 21. (continued).

DQA1	controls (n=120)	BL (n=28)					LL (n=48)				
	(%)	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0101	46	17	60.7	1.83	0.156	1.404	26	54.2	1.40	0.329	2.961
0102	48	12	42.9	0.80	0.601	5.409	33	68.8	2.35	0.164	1.476
0103	4	1	3.6	0.85	1.000	9.000	0	0.0	0.00	0.323	2.907
0201	6	2	7.1	1.24	0.679	6.111	2	4.2	0.70	1.000	9.000
03	35	11	39.3	1.20	0.670	6.030	14	29.2	0.76	0.469	4.221
03012	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0401	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0501	18	5	17.9	1.02	1.000	9.000	6	12.5	0.67	0.425	3.825
0601	23	6	21.4	0.94	0.902	8.118	7	14.6	0.59	0.249	2.241

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Table 22. Comparison of the antigen frequency of HLA-DQB1 alleles between leprosy patients and normal controls

DQB1* alleles	normal controls (n=120)		Leprosy (n=142)		Relative risk	p	Pc
	n	%	n	%			
0501	22	18.3	31	21.7	1.23	0.500	8.5
0502	72	60	86	60.1	1.02	0.926	15.742
05031	10	8.3	15	10.5	1.29	0.553	9.401
05032	0	0	0	0	ND	ND	ND
0504	0	0	0	0	ND	ND	ND
0601	14	11.7	19	13.3	1.17	0.677	11.509
0602	2	1.7	2	1.4	0.84	1.000	17
0603	2	1.7	0	0	0.8	0.207	3.519
0604	0	0	0	0	ND	ND	ND
0605	0	0	0	0	ND	ND	ND
0201	19	15.8	22	15.4	0.97	0.920	15.64
0301	37	30.8	31	21.7	0.79	0.091	1.547
0302	8	6.67	13	9.1	1.4	0.470	7.99
03031	0	0	0	0	ND	ND	ND
03032	27	22.5	38	26.6	1.25	0.446	7.582
0304	0	0	0	0	ND	ND	ND
0401	8	6.7	3	2.1	0.3	0.065	1.105
0402	0	0	0	0	ND	ND	ND

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Table 23. Comparison of the antigen frequency of HLA-DQB1 allele between tuberculoid and lepromatous leprosy patients with normal controls

DQB1* allele	normal controls	Tuberculoid group (n=67)				Lepromatous group (n=75)			
	%	%	R.R.	p	Pc	%	R.R.	p	Pc
0501	18.3	19.1	1.05	0.894	15.198	24.0	1.44	0.340	5.78
0502	60	56.7	0.87	0.662	11.254	64.0	1.19	0.576	9.792
05031	8.3	5.9	0.69	0.539	9.163	14.7	1.89	0.165	2.805
05032	0	0	ND	ND	ND	0	ND	ND	ND
0504	0	0	ND	ND	ND	0	ND	ND	ND
0601	11.7	13.4	1.17	0.724	12.308	13.3	1.16	0.730	12.41
0602	1.7	1.5	0.88	1.000	17	1.3	0.80	1.000	17
0603	1.7	0	0	0.536	9.112	0	0	0.377	6.409
0604	0	0	ND	ND	ND	0	ND	ND	ND
0605	0	0	ND	ND	ND	0	ND	ND	ND
0201	15.8	19.1	1.26	0.565	9.605	12.0	0.72	0.458	7.786
0301	30.8	19.1	0.53	0.081	1.377	24.0	0.71	0.302	5.134
0302	6.67	5.9	0.88	1.000	17	12.0	1.91	0.199	3.383
03031	0	0	ND	ND	ND	0	ND	ND	ND
03032	22.5	30.9	1.54	0.205	3.485	22.7	1.01	0.978	16.626
0304	0	0	ND	ND	ND	0	ND	ND	ND
0401	6.7	1.5	0.21	0.109	1.853	2.7	0.38	0.322	5.474
0402	0	0	ND	ND	ND	0	ND	ND	ND

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Table 24. Comparison of the allele frequency between HLA-DQB1 alleles of leprosy patients and normal controls

DQB1* alleles	Control group (total no.of allele=240)		Leprosy group (total no.of allele=240)		Relative risk	p	Pc
	number	%	number	%			
0501	25	10.4	32	11.2	1.13	0.656	11.152
0502	85	35.4	103	36.3	1.04	0.84	14.28
05031	10	4.2	15	5.2	1.27	0.563	9.571
05032	0	0	0	0	ND	ND	ND
0504	0	0	0	0	ND	ND	ND
0601	14	5.8	20	7	1.44	0.308	5.236
0602	2	0.8	2	0.7	0.84	1.000	17
0603	2	0.8	0	0	0	0.208	3.536
0604	0	0	0	0	ND	ND	ND
0605	0	0	0	0	ND	ND	ND
0201	19	7.9	23	8.0	1.02	0.958	16.286
0301	38	15.8	32	11.2	0.67	0.118	2.006
0302	8	3.3	13	4.5	1.38	0.479	8.143
03031	0	0	0	0	ND	ND	ND
03032	29	12.1	41	14.3	1.22	0.570	9.69
0304	0	0	0	0	ND	ND	ND
0401	8	3.3	3	1.0	0.31	0.068	1.156
0402	0	0	0	0	ND	ND	ND

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Table 25. Comparison of the allele frequency of HLA-DQB1 alleles between tuberculoid and lepromatous leprosy patients with normal controls

DQB1* allele	normal controls	Tuberculoid group (total no. of allele=134)					Lepromatous group (total no. of allele=150)				
	%	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0501	10.4	14	10.3	0.99	0.970	16.49	18	12	1.17	0.627	10.659
0502	35.4	50	37.3	1.09	0.714	12.138	53	35.3	1	0.987	16.779
05031	4.2	4	2.9	0.70	0.546	9.282	11	7.3	1.82	0.178	3.026
05032	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0504	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0601	5.8	10	7.5	1.53	0.316	5.372	10	6.7	1.15	0.739	12.563
0602	0.8	1	0.7	0.92	1.000	17	1	0.7	0.80	1.000	17
0603	0.8	0	0	0.00	0.537	9.129	0	0	0.00	0.525	8.925
0604	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0605	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0201	7.9	14	10.3	1.33	0.434	7.378	9	6	0.74	0.476	8.092
0301	15.8	13	9.6	0.86	0.628	10.676	19	12.7	0.77	0.389	6.613
0302	3.3	4	2.9	0.88	0.835	14.195	9	6	1.85	0.210	3.57
03031	0	0	0	ND	ND	ND	0	0	ND	ND	ND
03032	12.1	23	16.9	1.48	0.193	3.281	18	12	0.99	0.980	16.66
0304	0	0	0	ND	ND	ND	0	0	ND	ND	ND
0401	3.3	1	0.7	0.21	0.165	2.805	2	1.3	0.39	0.190	3.23
0402	0	0	0	ND	ND	ND	0	0	ND	ND	ND

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Table 26. Comparison of the antigen frequency of HLA-DQB1 alleles between TT, BT, BL and LL leprosy patients with normal controls.

DQB1	controls (n=120)	TT (n=23)					BT (n=44)				
	(%)	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0501	18	6	26.1	1.57	0.397	6.749	7	15.9	0.84	0.718	12.206
0502	60	15	65.2	1.25	0.639	10.863	23	52.3	0.73	0.374	6.358
05031	8	1	4.3	0.50	1.000	17.000	3	6.8	0.80	1.000	17.000
05032	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0504	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0601	12	2	8.7	0.72	1.000	17.000	7	15.9	1.43	0.471	8.007
0602	2	0	0.0	1.37	1.000	17.000	1	2.3	0.00	1.000	17.000
0603	2	0	0.0	0.00	1.000	17.000	0	0.0	0.00	1.000	17.000
0604	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0605	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0201	16	5	21.7	1.48	0.543	9.231	8	18.2	1.18	0.719	12.223
0301	31	5	21.7	0.62	0.380	6.460	8	18.2	0.50	0.108	1.836
0302	7	0	0.0	0.00	0.355	6.035	4	9.1	1.40	0.735	12.495
03031	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
03032	23	8	34.8	1.84	0.209	3.553	13	29.5	1.44	0.352	5.984
0304	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0401	7	0	0.0	0.00	0.355	6.035	1	2.3	0.33	0.447	7.599
0402	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND

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Table 26. (continued).

DQB1	controls (n=120)	BL (n=28)					LL (n=47)				
	(%)	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
0501	18	6	21.4	1.21	0.707	12.019	12	25.5	1.53	0.299	5.083
0502	60	16	57.1	0.89	0.782	13.294	32	68.1	1.42	0.332	5.644
05031	8	4	14.3	1.83	0.304	5.168	7	14.9	1.92	0.255	4.335
05032	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0504	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0601	12	4	14.3	1.26	0.749	12.733	6	12.8	1.11	0.844	14.348
0602	2	0	0.0	0.00	1.000	17.000	1	2.1	1.28	1.000	17.000
0603	2	0	0.0	0.00	1.000	17.000	0	0.0	0.00	1.000	17.000
0604	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0605	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0201	16	5	17.9	1.16	0.779	13.243	4	8.5	0.49	0.217	3.689
0301	31	7	25.0	0.75	0.543	9.231	11	23.4	0.69	0.340	5.780
0302	7	5	17.9	3.04	0.072	1.224	4	8.5	1.30	0.741	12.597
03031	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
03032	23	7	25.0	1.15	0.777	13.209	10	21.3	0.93	0.864	14.688
0304	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND
0401	7	1	3.6	0.52	1.000	17.000	1	2.1	0.30	0.447	7.599
0402	0	0	0.0	ND	ND	ND	0	0.0	ND	ND	ND

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leprosy patients were subdivided into 23 TT patients, 44 BT patients, 28 LL pateients and 47 BL patients, again none of the HLA-DQB1 alleles was significantly associated with any of the leprosy subtypes (Table 24).

I. Lack of association between deduced HLA-DQ antigens with leprosy patients

In the previous study, Schauf et al. (1985) found that the association between HLA-DR2 and HLA-DQw1 antigens with tuberculoid leprosy in the northern Thai population. In order to investigate whether such association of leprosy with HLA-DQ antigen was still true in our larger samples, the patients and controls in this study were reclassified into three corresponding serologic groups of HLA-DQ antigens. Because serological classification of the HLA-DQ molecules primarily reflects difference of the beta subunit of the DQ molecules, only the information derived from HLA-DQB1 typing were used for the designation of corresponding HLA-DQ molecules. According to previous findings from homozygous cell lines derived largely from Caucasian population, HLA-DQB1*0501, DQB1*0502, DQB1*05031, DQB1*05032, DQB1*0504, DQB1*0601, DQB1*0602, DQB1*0603 and DQB1*0604 alleles corresponded to HLA-DQw1 antigen; HLA-DQB1*0201 allele to HLA-DQw2 antigen; and HLA-DQB1*0301, DQB1*0302, DQB1*03031, DQB1*03032 allele to HLA-DQw3 antigen, respectively (Nepom and Erlich, 1991).

Comparison of the frequency of deduced HLA-DQ antigens between leprosy patients or two broad types of leprosy with normal controls revealed no statistical significant association between any of the three HLA-DQ antigens with leprosy or the tuberculoid and lepromatous subtypes of leprosy in these groups of subject (Tables 25 and 26).

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Table 27. Comparison of the frequency of deduced HLA-DQ antigens between leprosy patients and normal controls.

HLA-DQ antigen	normal		leprosy		R.R.	p	Pc
	n	%	n	%			
DQw1	122	55.2	153	58.8	1.14	0.477	1.431
DQw2	19	8.6	22	8.5	1.03	0.939	2.817
DQw3	72	32.6	82	31.5	1.31	0.158	0.474

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Table 28. Comparison of the frequency of deduced HLA-DQ antigens between tuberculoid and lepromatous leprosy patients with normal controls.

HLA-DQ	normal		Tuberculoid group					Lepromatous group				
antigen	n	%	n	%	R.R.	p	Pc	n	%	R.R.	p	Pc
DQw1	122	55.2	65	55.6	1.06	0.785	2.355	88	61.5	1.21	0.379	1.137
DQw2	19	8.6	13	11.1	1.36	0.408	1.224	9	6.3	0.74	0.476	1.428
DQw3	72	32.6	38	32.5	1.28	0.29	0.87	46	32.2	1.33	0.203	0.609

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