IV. RESULTS

A. Identification of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles by hybridization and non-isotopic detection

A.1 HLA-DQA1 locus

Eleven allelic forms of the HLA-DQA1 locus have been identified in the 11th International Histocompatibility Workshop (Kimura et al., 1991). In this study, ten probes were chosen from the 11th reference protocol and two new probes were designed to help discriminate the alleles HLA-DQA1*0502 and *0501 (Table 5). Thus, the 12 SSO probes that we employed could distinguish upto nine allelic forms of the HLA-DQA1 locus. Seven alleles were indeed detected in this population of northern Thais with Graves' disease: HLA-DQA1*0101/0104, *0102, *0103, *0201, *03, *0501/0503 and *0601 (Table 16). Two other alleles, HLA-DQA1*0401 and *0502 were absent.

A.2 HLA-DQB1 locus

Seventeen allelic forms of HLA-DQB1 locus had been detected by the 11th International Histocompatibility Workshop protocol (Kimura et al., 1991). They were: HLA-DQB1*0501, *0502, *0503, *0504, *0601, *0602, *0603, *0604, *0605, *0201, *0301, *0302, *0303, *0401 and *0402. The alleles HLA-DQB1*0503 and *0303 have now been subdivided into two alleles each, HLA-DQB*05031 or *05032 and *03031 and *03032, respectively (Marsh and Bodmer, 1995). By using 17 DQB SSO probes capable of distinquising all 17 alleles, we detected only 13 alleles in Graves' disease patients (Table 18). The alleles HLA-DQB1*0504, *0603, *0605 and *03031 were absent. Unexpectedly, the sample number G006 did not hybridize with any probes.

A.3 HLA-DRB1 generic group

For the typing of HLA-DRB locus, all samples were amplified first with the generic DRB1 primer pair. Hybridization with the probes DRB SSO 1001 to 1008N probes allowed the assignment of the subjects into one or two following eigth DR groups which corresponded with the HLA-DR serological specificities (Table 15).

Subjects with serological specificities DR7, DR9 and DR10 were positive with the probes 1006, 1007 and 1008N probes, respectively. Among these individuals, the HLA-DRB1 alleles that could be typed directly following the generic DRB amplification procedure were HLA-DRB1*07, *0901 and *1001 (corresponding to DR7, DR9 and DR10, respectively). The number of Graves' patients bearing these three alleles is shown in Table 27 and 28. Although the HLA-DRB1*07 can be subdivided into two alleles (HLA-DRB1*0701 and *0702), these two alleles had the same hybridization pattern (Kimura et al., 1991) because they were identical in the second exon and were different in the third exon (Marsh and Bodmer, 1995). There were 5 HLA-DRB1*07 positive subjects in the patient group.

The DR9 specificity is encoded by two allelic forms (HLA-DRB1*09011 and *09012) of the HLA-DRB1 gene. Using only the probe 5705 which reacts with the HLA-DRB1*09012 allele, but not *09011 allele, we could distinguish these two allelic forms only when they were not inherited simultaneously (ie. in heterozygous state). In Graves' patients, 50 out of 62 DR9 positive subjects were HLA-DRB1*09012 heterozygotes; the remaining 12 were HLA-DRB1*09 homozygotes (Table 27). Among the latter, some may be heterozygous for the HLA-DRB1*09011/*09012 alleles, or homozygous for the HLA-DRB1*09012 allele eventhough the probe 5705 gave positive result.

The HLA-DRB1*1001 was the only allele found in patients with DR10 serological specificity (Marsh and Bodmer, 1995). There was four individuals who were positive for the HLA-DRB1*1001.

A.4 Other groups of the HLA-DRB1 locus

A.4.1 DR1 and DR4 groups

The serological specificities DR1 and DR4 correspond to the HLA-DRB1*01 allele and HLA-DRB1*04 allele, respectively (Table 15). Recently, the HLA-DRB1*01 allele have been subdivided into four distinct allelic forms: HLA-DRB1*0101-*0104 (Marsh and Bodmer, 1995). In this study, all Graves' patients were negative with DRB SSO 1001 (which was positive with the allele DRB1*01). There were 17 individuals who showed positive result with the probe 1004. They were all HLA-DRB1*04 heterozygotes. By this molecular typing method, the HLA-DRB1*04 can be further classified into 22 alleles (HLA-DRB1*0401-*0422) (Marsh and Bodmer, 1995). However, we did not

attempt at further subtyping because they represented only a small proportion (4.8%) of the patient group.

A.4.2 DR2 group

Seven DRB probes (1002, 2813, 7002, 7003, 7011, 8601 and 8603) were used to identify five alleles (out of eleven) in the DR2 group (Table 9). Among 130 patients in the DR2 group, four alleles (HLA-DRB1*1501, *1502, *1504 and *1602) were identified (Table 27 and 28). The DRB1*1602 allele was found in 59 subjects; it represented the largest subgroup (Table 20 and 21).

A.4.3 DR52 associated DRB1 group

This group consisted of HLA-DR3, HLA-DR5, HLA-DR6 and HLA-DR8 specificities (Fig 3). The DRB SSO probes 1003 and 1005 were used to distinguish the DR52 associated DRB1 group. There were 98 patients who were positive with the DRB SSO 1003 and/or DRB SSO 1005 probes. Following the second amplification with the group specific primer pair, 14 additional probes were used for detecting DR52 associated alleles (DRB SSO 2813, 5701, 5702, 5703, 5704, 5708, 7002, 7003, 7004, 7007, 7010, 8601, 8602 and 8603). Among 25 alleles that were reported in the 11th Workshop, 11 alleles (HLA-DRB1*0301, *1101, *1106, *1201, *1202, *1302, *1303, *1401, *1403, *1404 and *1405) were found in this group of patients (Table 20 and 21). They were further subdivided into 3 groups in accordance with the serological specificities. The HLA-DRB1*0301 allele was grouped as HLA-DR3. The HLA-DR5 group was consisted of the HLA-DRB1*1101, *1106, *1201 and *1202 alleles. The last group, HLA-DR6, was composed of HLA-DRB1*1302, *1303, *1401, *1403 and *1404 alleles. The HLA-DRB1*1401 allele constituted the biggest group. There were 4 samples (G043, G071, G079 and G172) that were problematic; their hybridization signals could not be interpreted.

B. <u>Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles in male and female Graves' patients.</u>

B.1. HLA-DQA1 locus

Among seven HLA-DQA1 alleles detected, the HLA-DQA1*0102 allele was the most frequent allele in both male and female patients (Table 16 and 17). The frequency of HLA-DQA1*03 allele was comparable to that of HLA-DQA1*0102 allele only among male patients. Two other alleles, HLA-DQA1*0101 and *0501, were found less frequently and the rarest allele was the HLA-DQA1*0103 allele. A new HLA-DQA1*0502 allele was not found in this population.

There was a significant negative association between the HLA-DQA1*0601 allele with Graves' disease (RR = 0.21, *pc* = 0.00007). The preventive fraction (PF) confered by the allele HLA-DQA1*0601 was 9%. This negative association was detected in both the male and female subgroups (Table 16 and 17). The distribution of HLA-DQA1 alleles in patients who were subgrouped according to the onset of disease was similar to that of total Graves' patients (data not shown).

B.2 HLA-DQB1 locus

The allele HLA-DQB1*0502 was the most frequent allele in both of the patient and the control groups (Table 18 and 19) (Wongkuttiya, 1994). Other alleles, HLA-DQB1*05032, *0604 and *0402, were quite infrequent; the cumulative allele frequency of these three alleles was less than 1%. The distribution of these HLA-DQB1 alleles was not different between male and female patient groups.

No significant association between any HLA-DQB1 alleles and Graves' disease was detected. The HLA-DQB1*03032 allele showed high relative risk (RR = 1.88, EF = 9.5 %) but this did not reach statistically significant level. On the other hand, when the patients were divided according to the age of onset, the HLA-DQB1*03032 was significantly associated with Graves' disease in the younger patient group (the age of onset of disease below 31 years) (RR = 2.28, *pc* = 0.03, EF = 13.3 %).

B.3 HLA-DRB1 locus

The alleles HLA-DRB1*09012 and *1602 were the most frequent alleles in this population (Table 20 and 21). HLA-DRB1*1501 and *1401 alleles were also found at high, but lesser, frequencies. When the patients were classified according to HLA-DR serological specificities, the HLA-DR2 group was the largest group. Within this DR2

group, the HLA-DRB1*1602 allele was the most frequent allele. The second most common serological specificities in Graves' patients was the specificity HLA-DR9. The third group was the specificity HLA-DR6 and, in this group, the allele HLA-DRB1*1401 was detected at the highest frequency. The smallest group was HLA-DR10 with only the allele HLA-DRB1*1001 found.

The distribution of the HLA-DR alleles in male patients differs from those of the female group. In male patients, the first and second most commonly found alleles were: HLA-DRB1*09012 and *1602. In the female patients, however, the alleles *1501 and *1502 were the third and fourth most common alleles. In contrary, the third and fourth were the alleles *1401 and *1501 in male patients.

The HLA-DR5 serological specificity showed a statistically significant negative association with Graves' disease (RR = 0.41, *pc* = 0.005, PF = 10 %). The allele HLA-DRB1*1202 was the most common allele in HLA-DR5 group; this allele gave the relative risk of 0.23 (*pc* = 0.00006). The preventive fraction from the HLA-DRB1*1202 allele was 11%. On the contrary, the HLA-DRB1*1101 allele appear to result in the highest risk of developing Graves' disease (RR = 3.11) but again this association did not reach statistically significant level.

C. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles among patients with exophthalmos

Exophthalmos was found in 58% (101 out of 177) of this group of Graves' patients. Comparison of the patients with exophthalmos with the control group revealed significant decrease of the alleles HLA-DQA1*0601 and HLA-DRB1*1202 alleles in the patients with exophthalmos (RR = 0.22, *pc* = 0.003, PF = 9.6 % and RR = 0.25, *pc* = 0.005, PF = 6.1 % respectively) (Table 22, 25 and 26); however, similar reduction was also observed in patients without exophthalmos. No association of any HLA-DQB1 alleles with the presence of exophthalmos was detected. A tendency for patients with the HLA-DQA1*0501 allele (RR = 1.84, *pc* = 0.45), HLA-DQB*03032 allele (RR = 1.86, *pc* = 0.54) and HLA-DRB1*09012 allele (RR = 1.65, *pc* = 1.75) with exophthalmos was noted but did not reach statistically significant level. Similar tendency was also observed in patients without exophthalmos.

When patients with/without exophthalmos were further subdivided into two groups according to the age of onset, the HLA-DQB1*03032 allele was significantly

increased in the group of early-onset patients with exophthalmos (RR = 2.83, *pc* = 0.007, EF = 17.9 %).

D. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles among patients with relapse/exacerbation

Eighty-one patients were classified as having relapse/exacerbation. In 6 patients relapse was diagnosed only on the clinical group, but in 48 there was laboratory confirmation. Twenty-seven patients were regarded as having disease exacerbation. When the two clinical courses were considered together, Graves' disease relapse/exacerbation was associated with the HLA-DQB1*03032 allele (RR = 2.33, *pc* = 0.02, EF = 13.8 %) and the HLA-DRB1*09012 allele (RR = 2.20, *pc* = 0.04, EF = 13.6 %) (Table 30 and 31). However, when further subdivided, only the exacerbation group was associated with HLA-DQB1*03032 allele (RR = 2.83, *pc* = 0.04, EF = 17.9 %) and the HLA-DRB1*09012 allele (RR = 3.67, *pc* = 0.05, EF = 20.9 %). The HLA-DQB1*03032 was also significant associated with the laboratory proven group (RR = 2.98, *pc* = 0.03, EF = 19.1 %) and the exacerbate group (RR = 3.48, *pc* = 0.05, EF = 22.9 %) with the early age of onset of disease (Table 37). If only the antigen frequency was taken into account, the HLA-DRB1*1303 allele showed a significant association with relapsing disease.

E. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles in Graves' patients with uncommon manifestations

Periodic paralysis, myopathy and myasthenia gravis were uncommonly associated with Graves' disease. The antigen and allele frequencies of HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes in the subgroup of patients who were presented with these manifestations are shown in Table 38. The estimation of the relative risk was not done because of a small number of subjects in each group (10 patients with periodic paralysis, 12 with myopathy, and 3 with myasthenia gravis). The distribution of alleles tended to be quite similar to those of regular Graves' patients. Patients with periodic paralysis mostly inherited the HLA-DQA1*03, HLA-DQB1*0502, HLA-DRB1*1401 and *09012 alleles. The alleles HLA-DQA1*0102 and HLA-DQB*0502 and HLA-DRB1*09012 were also found in patients with myopathy.

F. Comparison of the frequency of polymorphic amino acid residues in HLA-DQA1 and DQB1 molecules.

Among patients with insulin-dependent diabetes mellitus there is a strongly significant association between the absence of aspartic acid residue at the position 57 of the HLA-DQB molecule and insulin-dependent diabetes mellitus (Todd et al, 1987; Badenhoop et al, 1995). Similar association has been detected in celiac disease and Graves' disease (Paulsen et al., 1995). In our study, the frequency of Graves' patients who had non-Asp DQβ57 allele was 52.8% compared with 56.8% in normal subjects; this difference is not significant statistically. Our finding and some of other studies in Oriental Graves' patients did not follow the DQβ57 pattern (Inoue et al., 1992; Cavan et al., 1994).

In the three-dimensional structure of the extracellular portion of HLA-DR1, certain amino acid positions were involved in forming the antigen binding groove (Brown et al., 1993). Comparison of the crystal structure of the HLA-DR molecule with polymorphic amino acid positions which were found in different HLA-DQA and -DQB molecules revealed at least 6 polymorphic positions of the HLA-DQA molecule and 11 polymorphic positions of the HLA-DQB molecule which lined the peptide binding groove. Comparison of the frequency of the alleles that beared each polymorphic amino acid positions between Graves' disease patients and normal subjects revealed that 95.1% of all HLA-DQA alleles found in Graves' disease patients contained tyrosine residue at the position 25 (alleles HLA-DQA1*0501, *0301, *0101 and *0102) compared with 82.9% of normal subjects (RR = 4.11, p = 0.0000007, EF = 71.9 %). Similarly, 39.5 % of all HLA-DQA allele of Graves' disease patients had leucine at the position 69 (alleles HLA-DQA1*0501, *0301, and *0201) compared with 30.8 % of normal subjects (RR = 1.48, p = 0.028, EF = 12.8 %). No association between alleles that contained specific amino acid residues of the HLA-DQB1 molecule with Graves' disease was detected.

G. Distribution of HLA class II haplotypes in Graves' disease patients

Certain alleles of the HLA-DQA, HLA-DQB and HLA-DRB loci tend to occurred together more frequently than would be expected by chance. Such linkage was detected in this study by direct observation (counting). Among linked alleles of two loci, many were observed at quite high frequency. The most common putative DRB1-DQA1 haplotypes were DRB1*1602-DQA1*0102 and DRB1*09012-DQA1*03. For the DRB1-DQB1 loci

the putative haplotypes DRB1*1602-DQB1*0502 and DRB1*09012-DQB1*03032 were most frequent whereas those of the DQA1-DQB1 loci were DQA1*0102-DQB1*0502 and DQA1*03-DQB1*03032 (Table 39). Comparison of the calculated haplotype frequency with those derived from direct counting revealed strongly linked alleles; these are shown in Table 40. When the HLA-DQA, -DQB, and -DRB loci were considered together, the two most common putative haplotypes were DRB1*1602-DQA1*0102-DQB1*0502 and DRB1*09012-DQA1*03-DQB1*03032.

An uncommon haplotype HLA-DRB1*1202-DQA1*0601-DQB1*0301 was significantly decreased in Graves' disease patients (RR = 0.24, *pc* = 0.002, PF = 8.4 %). On the contrary, the haplotype HLA-DRB1*1303-DQA1*0501-DQB1*0301 was detected in 15 Graves' disease patients, but none of the control group (*pc* = 0.03). Collectively, these two haplotypes, however, were presented only 2.9% and 4.3%, respectively, of all the patients.

Table 15 The serological specificities of DRB SSO corresponded to HLA-DRB1 alleles.

DRBSSO	Serological specificity	HLA-DRB1 allele
1001	HLA-DR1	HLA-DRB1*01
1002N	HLA-DR2	HLA-DRB1*15, HLA-DRB1*16
1003	HLA-DR3*	HLA-DRB1*03
	HLA-DR5*	HLA-DRB1*11
	HLA-DR6*	HLA-DRB1*13, HLA-DRB1*14
	HLA-DR8*	HLA-DRB1*08
1004	HLA-DR4	HLA-DRB1*04
1005	HLA-DR5*	HLA-DRB1*12, HLA-DRB1*1404
1006	HLA-DR7	HLA-DRB1*07
1007	HLA-DR9	HLA-DRB1*09
1008	HLA-DR10	HLA-DRB1*10

* They were grouped in HLA-DR52 associated-DRB1 group.

Table 16 The antigen frequency of HLA-DQA1 gene of Graves' male and female patients comparing with normal control subjects.

HLA-DQA1 allele	Normal Control (n=117)		Graves' disease											
			Total (n=178)				Male (n=70)				Female (n=108)			
	number	Ag freq.	number	Ag freq.	R.R.	Pc	number	Ag freq.	R.R.	Pc	number	Ag freq.	R.R.	Pc
0101	54	46.2	74	41.5	0.83	3.06	30	42.8	0.88	4.62	44	40.7	0.80	2.89
0102	58	49.6	102	57.3	1.37	1.34	37	52.8	1.14	4.64	65	60.1	1.54	0.77
0103	4	3.4	2	1.1	0.32	1.20	1	1.4	0.41	2.90	1	0.9	0.26	1.43
0201	7	6.0	5	2.8	0.45	1.24	3	4.3	0.70	4.32	2	1.8	0.15	0.29
03	40	34.2	73	41.0	1.34	1.67	32	45.7	1.62	0.81	41	37.9	1.18	3.89
0501	20	17.1	41	23.0	1.45	1.52	17	24.3	1.56	1.62	24	22.2	1.39	2.32
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	**27**	**23.1**	**10**	**5.6**	**0.20**	**0.00007**	**2**	**2.9**	**0.10**	**0.002**	**8**	**7.4**	**0.27**	**0.008**

Table 17 The allele frequency of HLA-DQA1 gene of Graves' male and female patients comparing with control subjects.

HLA-DQA1 allele	Normal Control (n= 234)		Graves' disease											
			Total (n= 356)				Male (n= 140)				Female (n= 216)			
	number	Allele freq.	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc
0101	60	25.6	80	22.5	0.84	2.63	32	22.9	0.86	3.82	48	22.2	0.38	2.77
0102	69	29.5	123	34.5	1.26	1.39	43	30.7	1.06	5.61	80	37.0	1.41	0.62
0103	5	2.1	2	0.6	0.26	0.59	1	0.7	0.33	2.02	1	0.5	0.21	0.85
0201	7	3.0	5	1.4	0.46	1.27	3	2.1	0.71	4.35	2	0.9	0.30	0.82
03	44	18.8	92	25.8	1.50	0.33	42	30.0	1.85	0.09	50	23.1	1.30	1.80
0501	21	9.0	44	12.3	1.43	1.39	17	12.1	1.40	2.28	27	12.5	1.45	1.58
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	**28**	**12.0**	**10**	**2.8**	**0.21**	**0.00007**	**2**	**1.4**	**0.11**	**0.002**	**8**	**3.7**	**0.28**	**0.009**

Table 18 The antigen frequency of HLA-DQB1 gene of Graves' disease patients compare with control subjects

HLA-DQB1 allele	Normal Control (n=117)		Graves' disease											
			Total (n=177)				Male (n=69)				Female (n=108)			
	number	Ag freq.	number	Ag freq.	R.R.	Pc	number	Ag freq.	R.R.	Pc	number	Ag freq.	R.R.	Pc
0501	20	17.1	24	13.6	0.76	5.27	8	11.6	0.64	4.04	16	14.8	0.84	8.33
0502	71	60.7	103	58.2	0.90	8.71	40	57.9	0.89	9.30	63	58.3	0.91	9.35
05031	9	7.7	12	6.8	0.87	9.96	1	1.4	0.18	0.89	11	10.2	1.36	6.64
05032	0	0	1	0.6	ND	ND	0	0	ND	ND	1	0.9	ND	ND
0601	14	12.0	23	13.0	1.10	10.32	10	14.5	1.25	8.05	13	12.0	1.01	12.81
0602	2	1.7	3	1.7	0.99	12.90	1	1.4	0.85	13.00	2	1.9	1.08	13.00
0604	0	0	1	0.6	ND	ND	1	1.4	ND	ND	0	0	ND	ND
0201	19	16.2	18	10.2	0.58	1.61	6	8.7	0.49	1.89	12	11.1	0.64	3.43
0301	36	30.8	38	21.5	0.62	0.94	16	23.2	0.68	3.45	22	20.4	0.58	0.96
0302	7	6.0	10	5.6	0.94	11.75	6	8.7	1.50	6.28	4	3.7	0.60	5.56
03032	26	22.2	62	35.0	1.89	0.25	27	39.1	2.25	0.18	35	32.4	1.68	1.11
0401	8	6.8	11	6.2	0.90	10.81	3	4.3	0.62	6.33	8	7.4	1.09	11.28
0402	0	0	1	0.6	ND	ND	1	1.4	ND	ND	0	0	ND	ND

Table 19 The allele frequency of HLA-DQB1 gene of Graves' male and female patients compare with control subjects

HLA-DQB1 allele	Normal Control (n=234)		Graves' disease											
			Total (n=354)				Male (n=138)				Female (n=216)			
	number	Allele freq.	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc
0501	23	9.8	25	7.1	0.70	2.99	8	5.8	0.56	2.26	17	7.9	0.78	6.05
0502	84	35.9	134	37.9	1.09	8.19	40	37.6	0.78	2.22	82	38.0	1.09	8.45
05031	9	3.8	15	4.2	1.11	10.59	1	0.7	ND	ND	14	6.5	1.70	2.87
05032	0	0	1	0.3	ND	ND	0	0	ND	ND	1	0.5	ND	ND
0601	14	6.0	23	6.5	ND	ND	10	7.2	ND	ND	13	6.0	ND	ND
0602	2	0.9	3	0.8	ND	ND	1	0.7	ND	ND	2	0.9	ND	ND
0604	0	0	1	0.3	ND	ND	1	0.7	ND	ND	0	0	ND	ND
0201	19	8.1	18	5.1	0.61	1.78	6	4.3	0.51	2.08	12	5.6	0.67	3.68
0301	37	15.8	40	11.3	0.60	0.55	16	12.3	0.70	3.38	23	10.6	0.63	1.39
0302	7	3.0	10	2.8	ND	ND	6	4.3	1.47	6.39	4	1.9	0.61	5.64
03032	28	12.0	72	20.3	1.88	0.11	27	23.2	1.79	0.60	40	18.5	1.67	0.68
0401	8	3.4	11	3.1	0.91	10.85	3	2.2	0.63	6.42	8	3.7	ND	ND
0402	0	0	1	0.3	ND	ND	1	0.7	ND	ND	0	0	ND	ND

Table 20 The antigen frequency of HLA-DRB1 gene between patients with Graves' disease and normal controls

HLA-DRB1 allele	Control (n=118)		Graves' disease											
			Total (n=174)				Male (n= 69)				Female (n= 105)			
	number	Ag freq.	number	Ag freq.	R.R.	Pc	number	Ag freq.	RR	Pc	number	Ag freq.	R.R.	Pc
0301	12	10.2	11	6.3	0.60	4.39	2	2.9	0.26	1.29	9	8.6	0.83	12.92
1501	28	23.7	37	21.3	0.87	11.76	13	18.8	0.75	8.27	24	22.8	0.95	16.66
1502	24	20.3	28	16.1	0.75	6.69	7	10.1	0.44	1.33	21	20.0	0.98	18.03
1504	-	-	6	3.5	ND	ND	1	1.4	ND	ND	5	4.8	ND	ND
1602	30	25.4	59	33.9	1.50	2.32	22	31.9	1.37	6.49	37	35.2	1.60	2.10
1101	2	1.7	9	5.2	3.16	2.38	4	5.8	3.57	2.36	5	4.8	2.90	3.59
1106	-	-	4	2.3	ND	ND	2	2.9	ND	ND	2	1.9	ND	ND
1201	1	0.8	1	0.6	ND	ND	1	1.4	ND	ND	0	0	ND	ND
1202	**32**	**27.1**	**13**	**7.5**	**0.22**	**0.00009**	**5**	**7.2**	**0.21**	**0.0002**	**8**	**7.6**	**0.22**	**0.003**
1302	0	0	1	0.6	ND	ND	1	1.4	ND	ND	0	0	ND	ND
1303	3	2.5	15	8.6	3.62	0.64	8	11.6	5.03	0.21	7	6.7	2.74	2.60
1401	24	20.3	35	20.1	0.99	18.29	20	28.9	1.60	3.39	15	14.3	0.65	4.45
1403	0	0	1	0.6	ND	ND	0	0	ND	ND	1	0.9	ND	ND
1404	9	7.6	7	4.0	0.51	3.50	1	1.4	0.18	1.33	6	5.7	0.73	10.81
1405	1	0.8	3	1.7	2.05	10.01	0	0	ND	ND	3	2.9	3.44	4.92
04	13	11.0	17	9.8	0.87	13.87	7	10.1	0.91	16.19	10	9.5	0.85	13.57
07	7	5.9	5	2.9	0.47	3.72	3	4.3	0.72	12.20	2	1.9	0.31	2.41
09012	29	24.6	62	35.6	1.70	0.86	28	40.6	2.10	0.41	34	32.4	1.47	3.72
1001	2	1.7	4	2.3	1.36	13.69	2	2.9	1.73	11.07	2	1.9	1.13	17.10

Table 21 The allele frequency of HLA-DRB1 gene between Graves' male and female patients compared with normal controls

HLA-DRB1 allele	Normal Control (n=236)		Graves' disease											
			Total (n=348)				Male (n=138)				Female (n=210)			
	number	Allele freq.	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc
0301	12	5.1	11	3.2	0.61	4.56	2	1.4	0.27	1.39	9	4.3	0.84	13.11
1501	32	13.6	37	10.6	0.76	5.36	13	9.4	0.66	4.47	24	11.4	0.82	9.44
1502	25	10.6	31	8.9	0.83	7.46	7	5.1	0.45	1.24	24	11.4	1.09	14.78
1504	-	-	6	1.7	ND	ND	1	0.7	ND	ND	5	2.4	ND	ND
1602	31	13.1	71	20.4	1.70	0.44	25	18.1	1.46	3.66	46	21.9	1.85	0.27
1101	2	0.8	9	2.6	3.11	2.45	4	2.9	3.49	2.41	5	2.4	2.85	3.67
1106	-	-	4	1.1	ND	ND	2	1.4	ND	ND	2	0.9	ND	ND
1201	1	0.4	1	0.3	ND	ND	1	0.7	ND	ND	0	0	ND	ND
1202	**34**	**14.4**	**13**	**3.7**	**0.23**	**0.00006**	**5**	**3.6**	**0.22**	**0.02**	**8**	**3.8**	**0.24**	**0.002**
1302	0	0	1	0.3	ND	ND	1	0.7	ND	ND	0	0	ND	ND
1303	3	1.3	15	4.3	3.50	0.70	8	5.8	4.78	0.24	7	3.3	2.68	2.70
1401	25	10.6	38	10.9	1.03	17.10	21	15.2	1.51	3.59	17	8.1	0.74	6.97
1403	0	0	1	0.3	ND	ND	0	0	ND	ND	1	0.5	ND	ND
1404	9	3.8	7	2.0	0.52	3.61	1	0.7	0.18	1.39	6	2.9	0.74	10.94
1405	1	0.4	3	0.9	2.04	10.03	0	0	ND	ND	3	1.4	3.41	6.57
04	13	5.5	17	4.8	0.88	14.00	7	5.1	0.92	16.27	10	4.8	0.86	13.70
07	7	3.0	5	1.4	0.48	3.80	3	2.2	0.73	12.27	2	0.9	0.31	2.49
09012	31	13.1	74	21.3	1.79	0.23	35	25.4	2.25	0.05	39	18.6	1.51	2.19
1001	2	0.8	4	1.1	1.36	13.71	2	1.4	1.72	11.11	2	0.9	1.13	17.21

Table 22 The antigen and allele frequencies of the HLA-DQA1 locus of Graves' patients with and without exophthalmos

HLA-DQA1 allele	Normal control (n= 117)		Graves' disease							
			with exophthalmos (n=102)				without exophthalmos (n=75)			
	number	Ag freq.	number	Ag freq.	RR	Pc	number	Ag freq.	RR	Pc
0101	54	46.2	39	38.2	ND	ND	37	49.3	ND	ND
0102	58	49.6	55	53.9	ND	ND	44	58.7	ND	ND
0103	4	3.4	1	0.9	ND	ND	1	1.3	ND	ND
0201	7	6.0	2	2.0	ND	ND	3	4.0	ND	ND
03	40	34.2	41	40.2	ND	ND	32	42.7	ND	ND
0501	20	17.1	28	27.5	1.84	0.45	13	17.3	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND
0601	**27**	**23.1**	**6**	**5.9**	**0.21**	**0.003**	**4**	**5.3**	**0.19**	**0.008**

HLA-DQA1 allele	Normal control (n= 234)		Graves' disease							
			with exophthalmos (n=204)				without exophthalmos (n=150)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	42	20.6	ND	ND	40	26.6	ND	ND
0102	69	29.5	69	33.8	ND	ND	50	33.3	ND	ND
0103	5	2.1	1	0.5	ND	ND	1	0.7	ND	ND
0201	7	3.0	2	1.0	ND	ND	3	2.0	ND	ND
03	44	18.8	54	26.5	ND	ND	38	25.3	ND	ND
0501	21	9.0	30	14.7	ND	ND	14	9.3	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND
0601	**28**	**12.0**	**6**	**2.9**	**0.22**	**0.003**	**4**	**2.7**	**0.20**	**0.009**

Table 23 The antigen frequency of HLA-DQB1 alleles of Graves' patients with and without exophthalmos

HLA-DQB1 allele	Normal control (n=117)		Graves' disease							
			with exophthalmos (n=101)				without exophthalmos (n=75)			
	number	Ag freq.	number	Ag freq.	RR	Pc	number	Ag freq.	RR	Pc
0501	20	17.1	9	8.9	0.47	0.99	17	21.3	ND	ND
0502	71	60.7	57	56.4	ND	ND	43	57.5	ND	ND
05031	9	7.7	8	7.9	ND	ND	4	5.3	ND	ND
05032	0	0	0	0	ND	ND	1	1.3	ND	ND
0601	14	12.0	11	10.9	ND	ND	12	16.0	ND	ND
0602	2	1.7	3	2.9	ND	ND	0	0	ND	ND
0604	0	0	1	1.0	ND	ND	0	0	ND	ND
0201	19	16.2	11	10.9	ND	ND	6	8.0	0.45	1.27
0301	36	30.8	25	24.8	ND	ND	13	17.3	0.47	0.48
0302	7	6.0	7	6.9	ND	ND	3	4.0	ND	ND
03032	26	22.2	35	34.7	1.86	0.54	27	36.0	1.97	0.48
0401	8	6.8	6	5.9	ND	ND	3	4.0	ND	ND
0402	0	0	1	1.0	ND	ND	0	0	ND	ND

Table 24 The allele frequency of HLA-DQB1 alleles of patients with and without exophthalmos

HLA-DQB1 allele	Normal control (n=234)		Graves' disease							
			with exophthalmos (n=202)				without exophthalmos (n=150)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0501	23	9.8	9	4.5	ND	ND	18	11.3	ND	ND
0502	84	35.9	74	36.6	ND	ND	56	37.3	ND	ND
05031	9	3.8	11	5.4	ND	ND	4	2.7	ND	ND
05032	0	0	0	0	ND	ND	1	0.7	ND	ND
0601	14	6.0	11	5.4	ND	ND	12	8.0	ND	ND
0602	2	0.9	3	1.5	ND	ND	0	0	ND	ND
0604	0	0	1	0.5	ND	ND	0	0	ND	ND
0201	19	8.1	11	5.4	ND	ND	6	4.0	ND	ND
0301	37	15.8	26	12.9	ND	ND	14	9.3	ND	ND
0302	7	3.0	7	3.5	ND	ND	3	2.0	ND	ND
03032	28	12.0	40	19.8	ND	ND	31	20.7	ND	ND
0401	8	3.4	6	3.0	ND	ND	3	2.0	ND	ND
0402	0	0	1	0.5	ND	ND	0	0	ND	ND

Table 25 The antigen frequency of HLA-DRB1 alleles of patients with Graves' disease with and without exophthalmos

HLA-DRB1 allele	Normal control (n=118)		Graves' disease							
			with exophthalmos (n=100)				without exophthalmos (n=73)			
	number	Ag freq.	number	Ag freq.	RR	Pc	number	Ag freq.	RR	Pc
0301	12	10.2	8	8.0	ND	ND	3	4.1	ND	ND
1501	28	23.7	17	17.0	ND	ND	21	28.8	ND	ND
1502	24	20.3	14	14.0	ND	ND	13	17.8	ND	ND
1504	-	-	3	3.0	ND	ND	3	4.1	ND	ND
1602	30	25.4	34	34.0	1.51	3.14	24	32.8	ND	ND
1101	2	1.7	5	5.0	ND	ND	4	5.5	ND	ND
1106	-	-	2	2.0	ND	ND	2	2.7	ND	ND
1201	1	0.8	1	1.0	ND	ND	0	0	ND	ND
1202	**32**	**27.1**	**8**	**8.0**	**0.23**	**0.005**	**4**	**5.5**	**0.16**	**0.004**
1302	0	0	1	1.0	ND	ND	0	0	ND	ND
1303	3	2.5	11	11.0	ND	ND	4	5.5	ND	ND
1401	24	20.3	20	20.0	ND	ND	15	20.5	ND	ND
1403	0	0	1	1.0	ND	ND	0	0	ND	ND
1404	9	7.6	4	4.0	ND	ND	3	4.1	ND	ND
1405	1	0.8	2	2.0	ND	ND	1	1.4	ND	ND
04	13	11.0	11	11.0	ND	ND	6	8.2	ND	ND
07	7	5.9	2	2.0	ND	ND	3	4.1	ND	ND
09012	29	24.6	35	35.0	1.65	1.75	27	36.9	1.80	1.27
1001	2	1.7	1	1.0	ND	ND	3	4.1	ND	ND

Table 26 The allele frequency of HLA-DRB1 alleles of Graves' patients with and without exophthalmos

HLA-DRB1 allele	Normal control (n=236)		Graves' disease							
			with exophthalmos (n=200)				without exophthalmos (n=146)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0301	12	5.1	8	4.0	ND	ND	3	2.0	ND	ND
1501	32	13.6	17	8.5	ND	ND	21	14.4	ND	ND
1502	25	10.6	15	7.5	ND	ND	15	10.3	ND	ND
1504	-	-	3	1.5	ND	ND	3	2.0	ND	ND
1602	31	13.1	43	21.5	ND	ND	27	18.5	ND	ND
1101	2	0.8	5	2.5	ND	ND	4	2.7	ND	ND
1106	-	-	2	1.0	ND	ND	2	1.4	ND	ND
1201	1	0.4	1	0.5	ND	ND	0	0	ND	ND
1202	**34**	**14.4**	**8**	**4.0**	**0.25**	**0.005**	**4**	**2.7**	**0.17**	**0.004**
1302	0	0	1	0.5	ND	ND	0	0	ND	ND
1303	3	1.3	11	5.5	ND	ND	4	2.7	ND	ND
1401	25	10.6	22	11.0	ND	ND	16	10.9	ND	ND
1403	0	0	1	0.5	ND	ND	0	0	ND	ND
1404	9	3.8	4	2.0	ND	ND	3	2.0	ND	ND
1405	1	0.4	2	1.0	ND	ND	1	0.7	ND	ND
04	13	5.5	11	5.5	ND	ND	6	4.1	ND	ND
07	7	3.0	2	1.0	ND	ND	3	2.0	ND	ND
09012	31	13.1	43	21.5	ND	ND	31	21.2	ND	ND
1001	2	0.8	1	0.5	ND	ND	3	2.0	ND	ND

Table 27 The antigen frequency of HLA-DR serological specificity of patients with Graves' disease

HLA-DRB1 serological specificity	Control (n=118)		Graves' disease											
			Total (n=174)				Male (n= 69)				Female (n= 105)			
	number	Ag freq.	number	Ag freq.	R.R.	Pc	number	Ag freq.	RR	Pc	number	Ag freq.	R.R.	Pc
DR2	82	69.5	130	74.7	ND	ND	43	62.3	ND	ND	87	82.8	2.12	0.12
DR3	12	10.2	11	6.3	ND	ND	2	2.9	ND	ND	9	8.6	ND	ND
DR4	13	11.0	17	9.7	ND	ND	7	10.1	ND	ND	10	9.5	ND	ND
DR5	38	32.2	**27**	**15.6**	**0.44**	**0.03**	12	17.3	0.50	0.49	**15**	**14.3**	**0.40**	**0.047**
DR6	29	24.6	54	31.0	ND	ND	29	41.9	2.22	0.10	25	23.9	ND	ND
DR7	7	5.9	5	2.9	ND	ND	3	4.3	ND	ND	2	1.9	ND	ND
DR9	29	24.6	62	35.6	ND	ND	28	40.6	ND	ND	34	32.4	ND	ND
DR10	2	1.7	4	2.3	ND	ND	2	2.9	ND	ND	2	1.9	ND	ND

Table 28 The allele frequency of HLA-DR depending on the serological specificity of patients with Graves' disease

HLA-DRB1 serological specificity	Normal Control (n=236)		Graves' disease											
			Total (n=348)				Male (n=138)				Female (n=210)			
	number	Allele freq.	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc	number	Allele freq.	R.R.	Pc
DR2	88	37.3	145	41.7	1.20	2.29	46	33.3	0.84	3.52	99	47.1	1.50	0.28
DR3	12	5.1	11	3.2	ND	ND	2	1.4	ND	ND	9	4.3	ND	ND
DR4	13	5.5	17	4.9	ND	ND	7	5.1	ND	ND	10	4.8	ND	ND
DR5	40	16.9	**27**	**7.8**	**0.41**	**0.005**	12	8.7	0.47	0.21	**15**	**7.1**	**0.38**	**0.01**
DR6	30	12.7	57	16.4	ND	ND	30	21.7	1.91	0.17	27	12.9	ND	ND
DR7	7	3.0	5	1.4	ND	ND	3	2.2	ND	ND	2	1.0	ND	ND
DR9	31	13.1	74	21.3	ND	ND	35	25.4	ND	ND	39	18.6	ND	ND
DR10	2	0.8	4	1.1	ND	ND	2	1.5	ND	ND	2	1.0	ND	ND

Table 29 The alelle frequency of HLA-DQA1 alleles of patients with Graves' disease with relapse/exacerbation

HLA-DQA1 allele	Normal control (n= 234)		Graves' disease with relapse															
			Total (n=164)				Clinical (n=12)				Laboratory proven (n=98)				Exacerbate (n=54)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	34	20.7	ND	ND	0	0	ND	ND	23	23.5	ND	ND	11	20.4	ND	ND
0102	69	29.5	51	31.1	ND	ND	6	50.0	2.39	0.93	31	31.6	ND	ND	14	25.9	ND	ND
0103	5	2.1	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	7	3.0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
03	44	18.8	48	29.3	1.79	0.10	2	16.7	ND	ND	28	28.6	1.73	0.34	18	33.3	2.16	0.13
0501	21	9.0	24	14.6	ND	ND	2	16.7	ND	ND	13	13.3	ND	ND	9	16.7	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	7	4.6	0.33	0.05	2	16.7	ND	ND	3	3.1	0.23	0.08	2	3.7	0.28	0.51

Table 30 The allele frequency of HLA-DQB1 alleles of patients with Graves' disease with relapse/exacerbation.

HLA-DQB1 allele	Normal control (n=234)		Graves' disease with relapse															
			Total (n=162)				Clinical (n=12)				Laboratory proven (n=96)				Exacerbate (n=54)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0501	23	9.8	9	5.6	ND	ND	0	0	ND	ND	6	6.3	ND	ND	3	5.6	ND	ND
0502	84	35.9	61	37.7	ND	ND	5	41.7	ND	ND	39	40.6	ND	ND	17	31.5	ND	ND
05031	9	3.8	5	3.1	ND	ND	0	0	ND	ND	3	3.1	ND	ND	2	3.7	ND	ND
05032	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	14	6.0	7	4.3	ND	ND	1	8.3	ND	ND	4	4.2	ND	ND	2	3.7	ND	ND
0602	2	0.9	2	1.2	ND	ND	0	0	ND	ND	1	1.0	ND	ND	1	1.9	ND	ND
0604	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	19	8.1	6	3.7	ND	ND	0	0	ND	ND	5	5.2	ND	ND	1	1.9	ND	ND
0301	37	15.8	26	16.0	ND	ND	4	33.3	2.66	1.46	12	12.5	ND	ND	10	18.5	ND	ND
0302	7	3.0	2	1.2	ND	ND	1	8.3	ND	ND	0	0	ND	ND	1	1.9	ND	ND
03032	28	12.0	**39**	**24.1**	**2.33**	**0.02**	1	8.3	ND	ND	23	23.9	2.32	0.08	**15**	**27.7**	**2.83**	**0.04**
0401	8	3.4	4	2.5	ND	ND	0	0	ND	ND	3	3.1	ND	ND	1	1.9	ND	ND
0402	0	0	1	0.6	ND	ND	0	0	ND	ND	0	0	ND	ND	1	1.9	ND	ND

Table 31 The allele frequency of HLA-DRB1 alleles of patients with Graves' disease with relapse/exacerbation.

HLA-DRB1 allele	Normal control (n=236)		Graves' disease with relapse															
			total (n=160)				Clinical (n=12)				Laboratory proven (n=96)				Exacerbate (n=52)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0301	12	5.1	4	2.5	ND	ND	0	0	ND	ND	3	3.1	ND	ND	1	1.9	ND	ND
1501	32	13.6	13	8.3	ND	ND	1	8.3	ND	ND	7	7.3	ND	ND	5	9.6	ND	ND
1502	25	10.6	15	9.4	ND	ND	0	0	ND	ND	11	11.4	ND	ND	4	7.7	ND	ND
1504	-	-	3	1.9	ND	ND	0	0	ND	ND	2	2.1	ND	ND	1	1.9	ND	ND
1602	31	13.1	31	19.4	ND	ND	4	33.3	3.31	0.95	20	20.8	ND	ND	7	13.5	ND	ND
1101	2	0.8	4	2.5	ND	ND	0	0	ND	ND	2	2.1	ND	ND	2	3.4	ND	ND
1106	-	-	2	1.3	ND	ND	1	8.3	ND	ND	1	1.0	ND	ND	0	0	ND	ND
1201	1	0.4	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
1202	34	14.4	9	5.6	0.35	0.11	3	25.0	1.98	5.98	4	4.2	0.26	0.15	2	3.4	0.24	0.70
1302	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
1303	3	1.3	11	6.9	ND	ND	1	8.3	ND	ND	6	6.3	ND	ND	4	7.7	ND	ND
1401	25	10.6	17	10.6	ND	ND	0	0	ND	ND	11	11.4	ND	ND	6	11.5	ND	ND
1403	0	0	1	0.6	ND	ND	0	0	ND	ND	0	0	ND	ND	1	1.9	ND	ND
1404	9	3.8	2	1.3	ND	ND	0	0	ND	ND	1	1.0	ND	ND	1	1.9	ND	ND
1405	1	0.4	1	0.6	ND	ND	0	0	ND	ND	1	1.0	ND	ND	0	0	ND	ND
04	13	5.5	7	4.4	ND	ND	0	0	ND	ND	4	4.2	ND	ND	3	5.8	ND	ND
07	7	3.0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
09012	31	13.1	**40**	**25.0**	**2.20**	**0.04**	2	16.7	ND	ND	23	24.0	2.08	0.29	**15**	**28.8**	**3.67**	**0.05**
1001	2	0.8	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND

Table 32 The allele frequency of HLA-DQA1 gene of Graves' patients belonging to the age of onset

HLA-DQA1 allele	Normal control (n= 234)		Below the median* of the age of onset											
			Total (n=152)				Male (n=38)				Female (n=114)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	39	25.7	ND	ND	11	31.6	ND	ND	27	23.7	ND	ND
0102	69	29.5	45	29.6	ND	ND	10	26.3	ND	ND	35	30.7	ND	ND
0103	5	2.1	1	0.7	ND	ND	0	0	ND	ND	1	0.9	ND	ND
0201	7	3.0	1	0.7	ND	ND	0	0	ND	ND	1	0.9	ND	ND
03	44	18.8	43	28.3	1.70	0.21	12	31.6	1.99	0.49	31	27.2	1.61	0.52
0501	21	9.0	17	11.2	ND	ND	3	7.9	ND	ND	14	12.3	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	6	3.9	0.30	0.046	1	2.6	0.20	0.59	5	4.4	0.34	0.16
HLA-DQA1 allele	Normal control (n= 234)		Above the median* of the age of onset											
			Total (n=152)				Male (n=72)				Female (n=80)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	28	18.2	ND	ND	11	15.3	0.52	0.48	17	21.3	ND	ND
0102	69	29.5	62	40.8	1.65	0.15	26	36.1	ND	ND	36	45.0	1.96	0.07
0103	5	2.1	1	0.7	ND	ND	1	1.4	ND	ND	0	0	ND	ND
0201	7	3.0	2	1.3	ND	ND	1	1.4	ND	ND	1	1.3	ND	ND
03	44	18.8	35	23.0	ND	ND	20	27.8	1.66	0.71	15	18.8	ND	ND
0501	21	9.0	20	13.2	ND	ND	12	16.7	ND	ND	8	10.0	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	4	2.6	0.20	0.008	1	1.4	0.10	0.05	3	3.8	0.29	0.23

* Median = 31 years

Table 33 The distribution of the HLA-DQB1 alleles of patients with Graves' disease according to the age of onset of disease

HLA-DQB1 allele	Normal Control (n=234)		Early onset (below the median* of the age of onset)												Late onset (above the median* of the age of onset)									
			Total (n=152)				Male (n=38)				Female (n=114)				Total (n=150)				Male (n=70)				Female (n=80)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0501	23	9.8	11	7.2	ND	ND	2	5.3	ND	ND	9	7.9	ND	ND	10	6.7	ND	ND	3	4.3	ND	ND	7	8.8	ND	ND
0502	84	35.9	57	37.5	ND	ND	17	44.7	1.45	3.84	40	35.0	ND	ND	59	39.3	ND	ND	24	34.3	ND	ND	35	43.8	ND	ND
05031	9	3.8	7	4.6	ND	ND	0	0	ND	ND	6	6.1	ND	ND	7	4.7	ND	ND	1	1.4	ND	ND	6	7.5	ND	ND
05032	0	0	1	0.7	ND	ND	0	0	ND	ND	1	0.9	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	14	6.0	7	4.6	ND	ND	2	5.3	ND	ND	5	4.3	ND	ND	12	8.0	ND	ND	7	10.0	ND	ND	5	6.3	ND	ND
0602	2	0.9	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	2	1.3	ND	ND	1	1.4	ND	ND	1	1.3	ND	ND
0604	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	19	8.1	5	3.3	ND	ND	0	0	ND	ND	5	4.3	ND	ND	9	6.0	ND	ND	3	4.3	ND	ND	6	7.5	ND	ND
0301	37	15.8	17	11.2	ND	ND	4	10.5	ND	ND	13	11.4	ND	ND	18	12.0	ND	ND	11	15.7	ND	ND	7	8.8	ND	ND
0302	7	3.0	7	4.6	ND	ND	3	7.9	ND	ND	4	3.5	ND	ND	1	0.7	ND	ND	1	1.4	ND	ND	0	0	ND	ND
03032	28	12.0	36	23.7	2.28	0.03	10	26.3	2.63	0.23	26	22.8	2.17	0.11	27	18.0	ND	ND	17	24.2	2.36	0.14	10	12.5	ND	ND
0401	8	3.4	4	2.6	ND	ND	0	0	ND	ND	4	11.8	ND	ND	4	2.7	ND	ND	1	1.4	ND	ND	3	3.8	ND	ND
0402	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	1	0.7	ND	ND	1	1.4	ND	ND	0	0	ND	ND

* Median = 31 years

Table 34 The distribution of the HLA-DQA1 alleles of patients with Graves' disease with and without exophthalmos according to the age of onset

HLA-DQA1 allele	Normal control (n= 234)		Below the median* of the age of onset (n=152)								Above the median* of the age of onset (n=152)							
			With exophthalmos (n=90)				Without exophthalmos (n=62)				With exophthalmos (n=86)				Without exophthalmos (n=66)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	18	20.0	ND	ND	22	38.7	1.59	0.86	17	19.8	ND	ND	10	15.2	0.52	0.53
0102	69	29.5	29	32.2	ND	ND	16	25.8	ND	ND	33	38.4	ND	ND	30	45.5	1.99	0.10
0103	5	2.1	0	0	ND	ND	1	1.6	ND	ND	1	1.2	ND	ND	0	0	ND	ND
0201	7	3.0	0	0	ND	ND	1	1.6	ND	ND	1	1.2	ND	ND	1	1.5	ND	ND
03	44	18.8	29	32.2	2.05	0.06	13	20.9	ND	ND	17	19.8	ND	ND	17	25.8	ND	ND
0501	21	9.0	10	11.1	ND	ND	7	11.3	ND	ND	15	17.4	2.14	4.63	6	9.1	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	4	4.4	0.34	0.89	2	3.2	0.25	0.29	2	2.3	0.18	0.06	2	3.0	0.23	0.23

* Median = 31 years

Table 35 The distribution of the HLA-DQB1 alleles belonging to the age of onset among patients with and without exophthalmos

HLA-DQB1 allele	Normal control (n=234)		Early onset (below the median* of the age of onset)								Late onset (above the median* of the age of onset)							
			with exophthalmos (n=90)				without exophthalmos (n=62)				with exophthalmos (n=84)				without exophthalmos (n=66)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0501	23	9.8	3	3.3	ND	ND	8	12.9	ND	ND	5	5.9	ND	ND	5	7.6	ND	ND
0502	84	35.9	36	40.0	ND	ND	21	33.9	ND	ND	28	33.3	ND	ND	31	47.0	1.58	1.33
05031	9	3.8	4	4.4	ND	ND	3	4.8	ND	ND	6	7.2	ND	ND	1	1.5	ND	ND
05032	0	0	0	0	ND	ND	1	1.6	ND	ND	0	0	ND	ND	1	1.5	ND	ND
0601	14	6.0	2	2.2	ND	ND	5	8.1	ND	ND	8	9.5	ND	ND	4	6.0	ND	ND
0602	2	0.9	0	0	ND	ND	0	0	ND	ND	2	2.4	ND	ND	0	0	ND	ND
0604	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	19	8.1	3	3.3	ND	ND	1	1.6	ND	ND	5	5.9	ND	ND	4	6.0	ND	ND
0301	37	15.8	10	11.1	ND	ND	8	12.9	ND	ND	13	15.5	ND	ND	5	7.6	0.44	1.15
0302	7	3.0	4	4.4	ND	ND	3	4.8	ND	ND	1	1.2	ND	ND	0	0	ND	ND
03032	28	12.0	25	27.8	2.83	0.007	11	17.7	ND	ND	13	15.5	ND	ND	13	19.7	ND	ND
0401	8	3.4	3	3.3	ND	ND	1	1.6	ND	ND	2	2.4	ND	ND	2	3.0	ND	ND
0402	0	0	0	0	ND	ND	0	0	ND	ND	1	1.2	ND	ND	0	0	ND	ND

* Median = 31 years

Table 36 The distribution of the HLA-DQA1 alleles of patients with Graves' disease with relapse/exacerbation according to the age of onset of disease.

HLA-DQA1 allele	Normal control (n= 234)		Early onset (below the median* of the age of onset, n=90)											
			Clinical relapse (n=10)				Laboratory proven (n=52)				Exacerbate (n=28)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	0	0	ND	ND	15	28.8	ND	ND	5	17.9	ND	ND
0102	69	29.5	6	50.0	3.59	0.28	12	23.1	ND	ND	7	25.0	ND	ND
0103	5	2.1	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	7	3.0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
03	44	18.8	1	10.0	ND	ND	16	30.8	1.92	0.39	11	39.2	2.79	0.08
0501	21	9.0	2	20.0	2.54	1.69	8	15.4	ND	ND	4	14.3	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	2	20.0	1.84	3.14	1	1.9	0.14	0.21	1	3.6	0.27	1.27
HLA-DQA1 allele	Normal control (n= 234)		Late onset (above the median* of the age of onset, n=72)											
			Clinical relapse (n=2)				Laboratory proven (n=46)				Exacerbate (n=24)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0101	60	25.6	0	0	ND	ND	7	15.2	0.52	0.90	5	20.8	ND	ND
0102	69	29.5	1	50.0	ND	ND	20	43.5	1.84	0.43	7	29.2	ND	ND
0103	5	2.1	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	7	3.0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
03	44	18.8	1	50.0	ND	ND	11	23.9	ND	ND	7	29.2	1.78	1.57
0501	21	9.0	0	0	ND	ND	6	13.0	ND	ND	4	16.7	ND	ND
0502	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	28	12.0	0	0	ND	ND	2	4.3	ND	ND	1	4.2	ND	ND

* Median = 31 years.

Table 37 The distribution of the HLA-DQB1 alleles according to the age of onset among the patients with relapse/exacerbation

HLA-DQB1 allele	Normal Control (n=234)		Early onset (below the median* of the age of onset)												Late onset (above the median* of the age of onset)											
			Clinical relapse (n=10)				Laboratory proven (n=52)				Exacerbate (n=28)				Clinical relapse (n=2)				Laboratory proven (n=44)				Exacerbate (n=24)			
	number	Allele freq.	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc	number	Allele freq.	RR	Pc
0501	23	9.8	0	0	ND	ND	4	7.7	ND	ND	0	0	ND	ND	0	0	ND	ND	2	4.5	ND	ND	2	8.3	ND	ND
0502	84	35.9	4	40.0	ND	ND	20	38.5	ND	ND	9	32.1	ND	ND	1	50.0	ND	ND	19	43.2	ND	ND	8	29.2	ND	ND
05031	9	3.8	0	0	ND	ND	2	3.8	ND	ND	2	7.1	ND	ND	0	0	ND	ND	1	2.3	ND	ND	0	0	ND	ND
05032	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0601	14	6.0	1	10.0	ND	ND	1	1.9	ND	ND	1	3.6	ND	ND	0	0	ND	ND	3	6.8	ND	ND	1	4.2	ND	ND
0602	2	0.9	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	1	2.3	ND	ND	1	4.2	ND	ND
0604	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
0201	19	8.1	0	0	ND	ND	2	3.8	ND	ND	0	0	ND	ND	0	0	ND	ND	2	4.5	ND	ND	1	4.2	ND	ND
0301	37	15.8	4	40.0	3.55	0.59	7	13.5	ND	ND	5	17.9	ND	ND	0	0	ND	ND	6	13.6	ND	ND	4	16.7	ND	ND
0302	7	3.0	1	10.0	ND	ND	0	0	ND	ND	1	3.6	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND
03032	28	12.0	0	0	ND	ND	**15**	**28.8**	**2.98**	**0.03**	**9**	**32.1**	**3.48**	**0.05**	1	50.0	ND	ND	8	18.2	ND	ND	6	13.6	ND	ND
0401	8	3.4	0	0	ND	ND	1	1.9	ND	ND	1	3.6	ND	ND	0	0	ND	ND	2	4.5	ND	ND	0	0	ND	ND
0402	0	0	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	0	0	ND	ND	1	4.2	ND	ND

* Median = 31 years.

Table 38 The antigen and allele frequencies of Graves' patients with uncommon manifestations

HLA allele	Graves' disease with uncommon manifestations											
	Periodic paralysis (n=10)				Myopathy (n=12)				Myasthenia gravis (n=3)			
	number	Ag freq.	number	Allele freq.	number	Ag freq.	number	Allele freq.	number	Ag freq.	number	Allele freq.
HLA-DQA1												
0101	6	60.0	6	30.0	5	41.7	5	20.8	2	66.7	2	33.3
0102	4	40.0	4	20.0	8	66.7	10	41.7	2	66.7	2	33.3
03	5	50.0	7	35.0	4	33.3	5	20.8	2	66.7	2	33.3
0501	3	30.0	3	15.0	4	41.7	4	16.7	0	0	0	0
HLA-DQB1												
0501	0	0	0	0	2	16.7	2	8.3	1	33.3	1	16.7
0502	7	70.0	7	35.0	8	66.7	11	45.8	3	100.0	3	50.0
0601	2	20.0	2	10.0	2	16.7	2	8.3	0	0	0	0
0602	1	10.0	1	5.0	0	0	0	0	0	0	0	0
0301	3	30.0	3	15.0	4	33.3	4	16.7	0	0	0	0
0302	1	10.0	1	5.0	0	0	0	0	0	0	0	0
03032	4	40.0	5	25.0	4	33.3	5	20.8	2	66.7	2	33.3
0401	1	10.0	1	5.0	0	0	0	0	0	0	0	0
HLA-DRB1												
1501	2	20.0	2	10.0	4	33.3	4	16.7	0	0	0	0
1502	2	20.0	2	10.0	3	25.0	3	12.5	1	33.3	1	16.7
1602	1	10.0	1	5.0	3	25.0	4	16.7	2	66.7	2	33.3
1101	2	20.0	2	10.0	1	8.3	1	4.2	0	0	0	0
1106	1	10.0	1	5.0	1	8.3	1	4.2	0	0	0	0
1202	0	0	0	0	2	16.7	2	8.3	0	0	0	0
1303	0	0	0	0	2	16.7	2	8.3	0	0	0	0
1401	5	50.0	5	25.0	2	16.7	2	8.3	1	33.3	1	16.7
04	2	20.0	2	10.0	0	0	0	0	0	0	0	0
09012	4	40.0	5	25.0	4	33.3	5	20.8	2	66.7	2	33.3

Table 39 The linkage disequilibrium of HLA-DRB1-DQA1, HLA-DRB1-DQB1 and HLA-DQB1-DQA1 which were presented by haplotype frequencies and delta

HLA haplotype DRB1-DQA1	Haplotype freq. (%)	Expected haplotype freq. (%)	Delta	P value
1501-0102	10.6	3.7	0.04	0.0022
1602-0102	20.2	7.1	0.08	0.0000
04-03	4.6	1.3	0.02	0.0148
1202-0601	2.8	0.9	0.01	0.0014
1303-0501	4.3	0.5	0.02	0.0014
1401-0101	10.9	2.5	0.05	0.0000
09012-03	21.4	5.6	0.1	0.0000
DRB1-DQB1				
1501-0601	5.8	0.7	0.03	0.0001
1602-0502	18.8	7.7	0.07	0.0000
1202-0301	2.8	0.4	0.02	0.0062
1303-0301	4.3	0.4	0.02	0.0014
1401-0502	10.9	4.2	0.04	0.0010
09012-03	20.5	4.5	0.1	0.0000
DQB1-DQA1				
0301-0501	7.2	1.3	0.03	0.0000
03032-03	21.4	5.6	0.1	0.0000
0501-0101	6.9	1.6	0.03	0.0000
0502-0102	27.5	13.3	0.1	0.0000
05031-0101	4.3	0.9	0.02	0.0041
0601-0102	5.8	2.2	0.02	0.0310
0201-0501	3.5	0.5	0.02	0.0069

Table 40 The haplotype frequencies of HLA-DRB1-DQA1-DQB1 haplotypes of Graves' patients compared with those of normal control from Chiang Mai and Bangkok

HLA haplotype	Haplotype frequency (%)				
	Graves' patients	Normal subjects			
DRB1-DQA1-DQB1		Chiang Mai	Pc	Bangkok	Pc
1202-0601-0301	2.9	11.1	0.0016	13.0	0.0000
1303-0501-0301	4.3	0	0.0341	0	0.0932
1602-0102-0502	18.8	12.8	1.7688	2.0	0.0000
09012-03-03032	20.6	12.4	0.0053	9.0	0.0147