The Effect of HLA-B Allele on the IDDM Risk Defined by DRB1*04 Subtypes and DQB1*0302

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The genes encoding the HLA-DQ heterodimer molecules, DQB1 and DQA1, have been found to have the strongest association with IDDM risk, although there is cumulative evidence for the effect of other gene loci within the major histocompatibility complex gene region. After the HLA-DQ locus, the HLA-DR locus has been suggested most often as contributing to the disease susceptibility. In this study we analyzed at the population level the effect of DR4 subtypes and class I, HLA-B alleles, on IDDM risk when the influence of the DQ locus was stratified. In all three populations studied (Estonian, Latvian, and Russian), DQB1*0302 haplotypes most frequently carried DRB1*0401 or DRB1*0404. DRB1*0401 was the most prevalent subtype in IDDM patients, whereas DRB1*0404 was decreased in frequency. DRB1*0402 was also prevalent among Russian haplotypes, but was not associated with IDDM risk. When HLA-B alleles were analyzed, strong associations between the presence of specific B alleles and DRB1*04 subtypes were detected. The HLA-B39 allele was found significantly more often in DRB1*0404-DQB1*0302–positive patients than in healthy control subjects positive for this haplotype: 27 of 54 (50%) vs. 4 of 49 (8.2%) ($P < 0.0001$). The results demonstrate that DQ and DR genes cannot explain all of the HLA-linked susceptibility to IDDM, and that the existence of a susceptibility locus telomeric to DR is probable. *Diabetes* 46:1888–1892, 1997

The genetic component in the susceptibility to IDDM is well recognized, as is the localization of the major susceptibility loci within the HLA gene region (1). Recent genome-wide studies have confirmed the importance of HLA, but have also brought evidence for more than 10 other chromosomal loci affecting disease risk (2). The genes coding for HLA-DQ heterodimer molecules have been found to be most strongly associated with IDDM susceptibility or protection against it, suggesting a direct role of these molecules in disease pathogenesis (3).

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PCR, polymerase chain reaction.

IDDM risk can be defined by the presence of particular DQ alleles. In most of the North European populations, DQB1*0302, which is known to carry the strongest susceptibility to IDDM, is highly prevalent, whereas DQB1*0201 is more prevalent in South European populations. DQB1*0602, *0603, and *0301 have been shown to be protective alleles in most populations (4). However, several studies have suggested participation of other HLA gene loci in IDDM pathogenesis, although, because of the strong linkage disequilibrium, it is difficult to estimate their separate role. The DRB1 gene is the main candidate for an additional susceptibility locus, because DQB1*0302 haplotypes with different DR4 subtypes confer an unequal risk for the disease (5).

To clarify the question of susceptibility loci in addition to DQ, we investigated DR4 subtypes in patients and control subjects matched for the DQB1*0302 allele in Russian, Estonian, and Latvian populations, which so far are poorly defined for DR4 subtypes. Further, we studied the effect of class I HLA B gene alleles on the IDDM risk defined by combinations of specific DRB1 subtypes with DQB1*0302.

RESEARCH DESIGN AND METHODS

Subjects. Individuals from three Baltic populations—Estonians, Latvians, and Russians from St. Petersburg—were studied. Ethnic Estonians and Latvians from these two countries with sizable Russian minorities were selected for the study. The IDDM patients were unrelated subjects; in the Estonian and Latvian populations, age at diagnosis of disease was <15 years; the St. Petersburg population included some young adults. The mean age (± SD) at diagnosis of Estonian patients was 7.4 ± 3.8; of Latvian patients, 8.2 ± 3.8; and of Russian patients, 11.4 ± 6.2 years. Female patients made up 54, 51, and 60% of the Estonian, Latvian, and Russian patient populations, respectively. Control samples were obtained from healthy blood donors or university students. EDTA blood was collected, and DNA was extracted using a standard salting out method.

Study design. In the first phase, 210 IDDM and 402 control subjects from the Russian population, 97 IDDM and 269 control subjects from the Estonian population, and 136 IDDM and 182 control subjects from the Latvian population were studied for HLA-DQB1 markers associated with IDDM risk using a rapid screening method based on lanthanide-labeled probes and time-resolved fluorometry (6). All subjects positive for DQB1*0302 were selected for the further DR4 subtyping, which was carried out by genomic amplification in two polymerase chain reactions (PCRs), with the primer sequences as defined by Olerup and Zetterquist (7). The following primers were used:

5′04 5′-GTT TCT TGG AGC AGG TTA AAC A-3′,
3′047 5′-CTG CAC TGT GAA GCT CTC AC-3′,
and 3′048 5′-CTG CAC TGT GAA GCT CTC CA-3′.

Primers were specific for DRB1*0401,*0405,*0407,*0408,*0409 (5′04–3′047) and *0402,*0403,*0404,*0406,*0410,*0411 (5′04–3′048). The alleles were further distinguished by dot blot hybridization of the amplification product using the following ^{32}P-or digoxygenin-labeled sequence specific oligonucleotides:

5′-GGT GTC CAC CTC GGC CCG CC-3′	DRB1*0403, *0406, *0407, *0411
5′-GCA GAG GCG GGC CGC GGT-3′	DRB1*0404, *0405, *0408, *0410
5′-CGG CCT AGC GCC GAG TAC-3′	DRB1*0405, *0409, *0410, *0411
5′-GAA GAC GAG CGG GCC GCG-3′	DRB1*0402
5′-GAG CAG AAG CGG GCC GCG-3′	DRB1*0401, *0409

TABLE 1
Frequencies (%) of HLA-DRB1*04 subtypes in DQB1*0302-positive patients and control subjects

	Estonians			Latvians		Russians			Combined		
Allele	IDDM (n = 62)	Control (n = 47)	P	IDDM (n = 76)	Control (n = 13)	IDDM (n = 148)	Control (n = 75)	P	IDDM (n = 286)	Control (n = 135)	P
0401	72.6	25.5	<0.0001	60.5	38.5	57.4	37.3	0.007	61.5	33.3	<0.0001
0402	1.6	8.5		11.8	0	18.9	25.3		13.3	17.0	
0403/6	0	6.4		0	0	0	1.3		0	3.0	0.017
0404	29.0	53.2	0.018	26.3	53.8	24.3	32.0		25.9	41.5	0.0018
0405	3.2	2.1		0	0	2.7	1.3		2.1	1.5	
0408	0	6.4		2.6	0	2.0	0		1.7	2.2	
x*	0	4.3		6.6	7.7	2.0	6.7		2.8	5.9	

*Nontypable.

The DRB1*04 alleles were distinguished by combining results of specific amplification and dot blot hybridization. DQB1*0302–positive samples were also analyzed for the presence of nine HLA-B alleles (B*07, 62, 60, 35, 56, 51, 27, 39, and 44). These alleles were chosen for the study because they are known to be in linkage disequilibrium with DR4 in other Caucasian populations (8); or in particular, they are known to characterize IDDM-associated DR4 haplotypes in the neighboring Finnish population (9,10). HLA-B typing was done by means of PCR with sequence-specific primers according to the method described by Bunce et al. (11).

Statistical analysis. The level of significance was assessed by χ^2 test in comparisons between different groups. Odd ratios were calculated according to the formula $(a \times d)/(b \times c)$, where a and b are the numbers of the IDDM patients that were positive and negative for the marker, respectively, and c and d, the respective numbers of control subjects.

RESULTS

The frequency of HLA DQB1*0302 was highly increased in IDDM patients in all populations, but DRB1*04 subtyping revealed an unequal risk of IDDM when DQB1*0302 was found in context of distinct DRB1-DQB1 haplotypes (Table 1). DRB1*0401 was associated with IDDM in all populations, whereas DQB1*0404 was decreased among IDDM patients, although this effect was not significant among the Russian patients. These two alleles were the most prevalent subtypes in all three populations. Only in the Russian patients was DQB1*0402 found in a considerable proportion of DQB1*0302 haplotypes, but without any association with IDDM. DQB1*0403/6 alleles were not found in any of the patients, and although also rare in control samples, the decrease among IDDM patients was significant in the combined data.

HLA-B typing of selected alleles was performed only in Estonian and Russian subjects because of the small number of Latvian control subjects with various DR4 subtypes. The analysis of all DQB1*0302–positive subjects without taking DR4 subtypes into account showed an association of B*39 with the disease. B*7 and B*51 were significantly decreased in the patients in the combined series (Table 2).

Analysis of B alleles associated with different DR4-DQB1*0302 haplotypes revealed ordinary allelic associations as well as some differences between IDDM and control subjects (Tables 3–5). The B*62 allele was significantly more common in DRB1*0401-DQB1*0302–positive than in DRB1*0404-DQB1*0302–positive subjects among both IDDM (P = 0.002) and control subjects (P = 0.018). DRB1*0402-DQB1*0302, which is prevalent in Russian subjects, was frequently associated with B*51, and this allele was significantly more common in DRB1*0402-DQB1*0302–positive than in DRB1*0401-DQB1*0302–positive patients (P = 0.0035).

The most conspicuous difference between IDDM patients and control subjects was found among DRB1*0404-DQB1*0302–positive subjects. B*39 was found in half of both Estonian and Russian IDDM patients, compared with only a few cases among control subjects. The difference was significant

TABLE 2
Frequencies (%) of HLA-B alleles in HLA DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians			Russians		Combined		
Allele	IDDM (n = 58)	Control (n = 46)	P	IDDM (n = 146)	Control (n = 75)	IDDM (n = 204)	Control (n = 121)	P
62	34.5	28.3		24.7	17.3	27.5	21.5	
39	25.9	2.2	0.0023	14.4	6.7	17.6	5.0	0.0018
35	15.5	19.6		13.0	16.0	13.7	17.4	
44	5.2	10.9		16.4	18.7	13.2	15.7	
7	12.1	21.7		11.6	20.0	11.8	20.7	0.045
27	13.8	19.6		10.3	13.3	11.3	15.7	
51	3.4	10.9		10.3	20.0	8.3	16.5	0.039
60	6.9	4.3		5.5	14.7	5.9	10.7	
56	5.2	4.3		0.7	0	2.0	1.7	
xx*	8.6	13.0		17.8	12.0	15.2	12.4	

*None of the above studied alleles.

TABLE 3
Frequencies (%) of HLA-B alleles in HLA-DRB1*0401-DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians		Russians			Combined		
Allele	IDDM (n = 44)	Control (n = 14)	IDDM (n = 85)	Control (n = 28)	P	IDDM (n = 129)	Control (n = 42)	P
62	43.2	35.7	37.6	39.3		39.5	38.1	
39	15.9	0	5.9	3.6		9.3	2.4	
35	18.2	14.3	23.5	10.7		21.7	11.9	
44	6.8	21.4	16.5	21.5		13.2	21.4	
7	6.8	14.3	11.8	14.3		10.1	14.3	
27	13.6	14.3	9.4	10.7		10.9	11.9	
51	0	14.3	5.9	14.3		3.9	14.3	0.043
60	4.5	0	2.4	21.4	0.0028	3.1	14.3	0.021
56	6.7	7.1	1.2	0		3.1	2.4	
xx*	9.1	14.3	14.1	10.7		12.4	11.9	

*None of the above studied alleles.

in both populations, and highly significant in the combined series at the level of $P < 0.0001$, even after multiplication by the number of tested HLA-B alleles (Table 4). Calculations of relative risk estimates or odds ratios for DRB1*0404-DQB1*0302 with and without B*39 allele demonstrated that the combination with B*39 is a marker associated with a higher risk than the DRB1*0401-DQB1*0302 haplotype, whereas without B*39, this haplotype carried no susceptibility (Table 6).

The decrease of B*51 among IDDM patients that was observed when comparing all DQB1*0302–positive subjects was further found in those with the DRB1*0401-DQB1*0302 haplotype (Table 3). B*60 was decreased in Russian and combined IDDM patients with DRB1*0401-DQB1*0302, whereas B*35 was decreased in DRB1*0402-DQB1*0302–positive Russian IDDM patients compared with healthy subjects with this haplotype. On the other hand, the number of subjects without any of the tested B alleles was increased among DRB1*0402-DQB1*0302 patients (Table 5).

The age at diagnosis or proportion of male to female patients did not differ between patients with various HLA-DR4 subtypes. Neither were differences found when those with the combination of HLA-DQB1*0404 and B*39 allele were compared with other patients (data not shown).

DISCUSSION

The results described in this study confirm the presence of several gene loci within the HLA region responsible for the susceptibility to IDDM. The risk defined by the strongest known single risk allele, DQB1*0302, is dependent on the DR4 subtype. Amino acid residues differing between DR4 alleles participate in the formation of the peptide binding groove and have functional effects on peptide binding and antigen presentation (12). If the disease susceptibility and protection associated with DQ molecules is mediated by their role in antigen presentation, this might in fact be more precisely defined by the combination of different class II alleles, DQ and DR molecules together. However, some findings contradict this hypothesis. The hierarchy of DR4 alleles in the definition of susceptibility that has been built in accordance with several studies (5) is not a common rule. In the present study, DRB1*0402, which has been found in some populations to be the strongest DR4 risk factor, did not differ in frequency between Russian patients and control subjects. Also, the populations with the highest IDDM incidence (13)—Finns and Scandinavians—practically lack those DR4 subtypes reported to be associated with the highest risk, 0402 and 0405 (14–16). The effect of

TABLE 4
Frequencies (%) of HLA-B alleles in HLA-DRB1*0404-DQB1*0302–positive IDDM patients and healthy control subjects

	Estonians			Russians			Combined		
Allele	IDDM (n = 17)	Control (n = 25)	P	IDDM (n = 37)	Control (n = 24)	P	IDDM (n = 54)	Control (n = 49)	P
62	11.7	28.0		16.2	0		14.8	14.3	
39	52.9	4.0	0.001	48.6	12.5	0.0086	50.0	8.2	<0.0001
35	11.7	16.0		5.4	16.7		7.4	16.3	
44	0	4.0		13.5	16.7		9.3	10.2	
7	23.5	24.0		16.2	29.2		18.5	26.5	
27	17.6	28.0		10.8	12.5		13.0	20.4	
51	5.9	0		5.4	16.7		5.6	8.2	
60	5.9	8.0		13.5	16.7		11.1	12.2	
56	0	4.0		0	0		0	2.0	
xx*	5.9	12.0		2.7	12.5		3.7	12.2	

*None of the above studied alleles.

TABLE 5
Frequencies (%) of HLA-B alleles in Russian HLA-DRB1*0402-DQB1*0302–positive IDDM patients and healthy control subjects

Allele	IDDM (n = 28)	Control (n = 19)	P
62	3.6	10.5	
39	3.6	5.3	
35	0	21.4	0.045
44	21.4	15.8	
7	0	10.5	
27	10.7	15.8	
51	28.6	31.6	
60	3.6	5.3	
56	0	0	
xx*	42.9	10.5	0.04

*None of the above studied alleles.

DRB1*0404 varies in its protective effect in different populations, from strong to weak or lacking (14,15,17,18), as in the present report.

Our study also suggested a strong specific heterogeneity in the effect of DRB1*0404-DQB1*0302 haplotype, depending on the presence or absence of B*39 allele, with a strong disposition being conferred in the former and no disposition being conferred in the latter. This result might be attributable to a haplotypic effect, but this cannot be confirmed in a population study. In a Finnish family study, B39 divides DRB1*0404-DQB1*0302 haplotypes similarly in terms of IDDM risk (19). In the Finnish population, B39 is also strongly associated with A24, with A24 (9), B39 (16), DR4 being one of the most common DR4 haplotypes found in IDDM patients (9,10,20). A24, B39, and DR4 haplotypes have not been reported as susceptibility haplotypes elsewhere, but A24 has been recently found to be associated with an earlier age at diagnosis and with a more rapid progression to IDDM in an Australian family series (21). A recent study from St. Petersburg also reported the increase of both A24 and B16 alleles among Russian IDDM patients (22).

Other deviations in B allele frequencies between DR-DQ–matched IDDM and control subjects found in the present study also support the role of class I genes. The decreased B7 frequency among IDDM patients was shown only when all DQB1*0302–positive subjects were compared. This decrease may be secondary to the decrease of linked DRB1*15-DQB1*0602 alleles protecting against IDDM, and may reflect the genotype effect of the other chromosome. The decreased frequency of B51 and B60, instead, was seen only in DRB1*0401–positive subjects, and that of B35, only in DRB1*0402–positive subjects. However, these associations were detected for the first time and were of low statistical significance, thus requiring further confirmation.

The results of the present study suggest that class I alleles have a separate role in IDDM in addition to DR and DQ. Another possibility is that the association of IDDM with certain DR4 subtypes appears to be attributable to the linkage disequilibrium with an unidentified gene in the HLA region. The hypothesis of an additional susceptibility locus within the major histocompatibility complex region was supported by recent molecular findings (23,24), as well as by analogy with the NOD mouse model (25). The role and localization of these genetic elements remain to be clarified.

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TABLE 6
Combinations of HLA-B, DRB1, and DQB1 as risk markers for IDDM among Estonians and Russians

Marker combination	Odds ratio (95% CI) Estonians	Russians	Combined
DQB1*0302	8.9 (5.1–15.5)	12.0 (7.9–18.2)	10.9 (7.8–15.1)
DQB1*0302-DRB1*0401	15.8 (7.7–32.7)	9.1 (5.5–15.0)	11.0 (7.4–15.5)
DQB1*0302-DRB1*0404	2.2 (1.1–4.5)	3.4 (1.9–6.0)	2.8 (1.8–4.3)
DQB1*0302-DRB1*0404-B*39	27.4 (3.5–126.2)	12.5 (3.4–34.8)	16.1 (5.3–36.7)
DQB1*0302-DRB1*0404-X†	0.92 (0.36–2.2)	1.8 (0.90–3.6)	1.3 (0.79–2.3)

†B*39 lacking.

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