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HLA Genotype Studies in Juvenile Insulin-dependent Diabetes

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Summary. HLA genotypes were ascertained in 53 French Caucasian families, comprising 68 juvenile onset insulin-dependent diabetic siblings. Among the 49 alleles detected at different loci in the HLA complex (A, C, B, Bf, DR) 4 appeared to occur at a significantly higher frequency among the 53 index cases than in a control series of 116 healthy individuals: HLA-B18 ($p < 10^{-3}$), DRw3, DRw4 and BfF1 ($p < 10^{-6}$). The excess of HLA identical affected siblings confirms genotype disequilibrium and supports the hypothesis of an HLA-linked gene(s) conferring susceptibility. There was no increase of homozygosity for HLA DRw3 and DRw4 whereas there was a marked excess heterozygosity for HLA DRw3/DRw4 in diabetic patients (32% versus 0% in the control series, $p < 0.001$). These data provide evidence for the existence of two cooperating genes, linked to each of the HLA DR alleles.

Key words: Insulin dependent diabetes, HLA complex, DR, Bf, DRw3/4 heterozygosity.

Amongst the associations observed between insulin-dependent diabetes (IDD) and some alleles of the HLA system, those most frequently reported are HLA-B8 [1–13], B15 [1–6, 13], B18 [5, 9–12], Cw3 [6], DRw3, DRw4 [4, 12, 14–17] and BfF1 [18].

The variability of these associations is probably due to different ethnic influences [19]. Evidence for linkage between the susceptibility gene(s) for IDD and the HLA system has emanated from studies in families with two or more affected siblings who show an increased incidence of HLA-identity [1, 6, 20–22].

Other findings such as an increased prevalence of recombination [23], a higher frequency of homozygosity [21] or heterozygosity of certain alleles [5, 10, 13, 24, 25] are still debated.

The aim of the present study was to contribute to a better understanding of these questions by investigating the association of five different loci of the HLA region with IDD, in 53 French families with a single diabetic child or diabetic multiplex kindreds (including 8 families previously described [10] and retyped).

Patients and Methods

HLA genotyping was performed in 53 families comprising one or more children with juvenile onset, insulin-dependent diabetes (IDD) after obtaining their informed consent. All were Caucasians. Although they were living near Paris, many had origins in other parts of France.

Sixty-eight diabetic children of these families (mean age at onset of diabetes 10.4 ± 0.9 years) were HLA-typed.

In 41 families, only one child was diabetic. There were 2 diabetic children in 10 families, and 3 and 4 in two others. From these families, the index case was chosen randomly. Forty seven healthy siblings were also HLA-typed. Forty-five siblings, 2 diabetic and 43 healthy ones, were not available for HLA-typing.

116 unrelated healthy HLA-genotyped individuals, living in the same geographic region served as controls. Haplotypes were obtained unequivocally in both patients and controls.

Immunogenetic Studies

Forty-nine alleles at 5 loci of the HLA complex including the properdin factor B (Bf) were tested (see Table 1). HLA A, B and C antigens were determined by microcytotoxicity [26] on peripheral lymphocytes. HLA DR specificities were recognised on B lymphocytes which were separated by a rosetting technique [27]. Bf polymorphism was studied following the technique of Alper et al. [28].

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Table 1. Gene frequencies (%) of HLA antigens in IDD children and normal controls

Antigens	Patients (53)	Controls (116)	RR
A1	12	10	
A2	35	23	
A3	6	9	
A11	2	8	0.2
Aw23	4	6	
Aw24	12	10	
A25	2	2	
A26	2	7	0.3
A28	6	4	
A29	4	4	
Aw30	7	5	
Aw31	2	1	
Aw32	6	4	
Aw33	0	0	
Blank	1	7	
B5	3	9	0.3
B7	6	11	0.5
B8	13	8	1.7
B12	8	14	
B13	1	1	
B14	4	4	
B15	11	5	2.4
B17	0	1	
B18	20[a]	8	2.9
Bw21	9	5	
Bw22	1	2	
B27	5	5	
Bw35	6	10	
B37	1	2	
Bw38	3	2	
Bw39	4	3	
B40	4	4	
Bw41	0	1	
Blank	1	5	
Cw1	3	6	
Cw2	4	5	
Cw3	17[a]	8	2.4
Cw4	9	13	
Cw5	19[a]	9	2.4
Cve	16	16	
Blank	32	43	
DRw1	11	6	
DRw2	5	14	0.3
DRw3	31[b]	10	4.0
DRw4	31[b]	7	6.0
DRw5	8	17	0.4
DRw6	4	6	
DRw7	7	11	
Blank	3	29	
BfS	66	79	
S1	4	1	4.1
F	11	16	
F1	19[b]	4	5.6
Blank	0	0	

RR = relative risk, [a] $p < 0.001$, [b] $p < 10^{-6}$

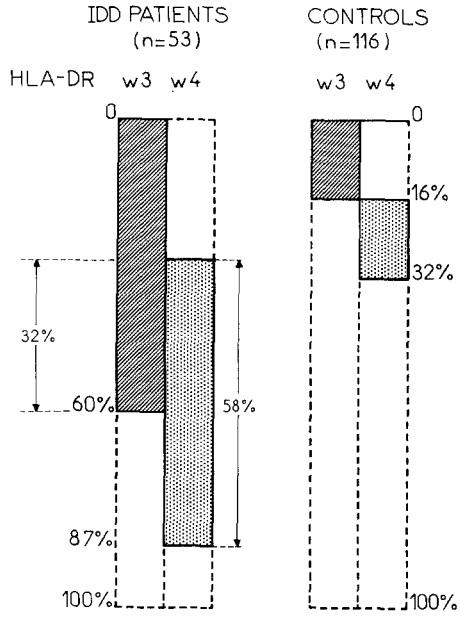

Fig. 1. Phenotype frequencies (%) of DRw3 and DRw4 in 53 IDD patients and 116 controls. In IDD patients, 60% carry the DRw3 and 58% the DRw4 allele, and 32% carry both of them, whereas in controls 16% carry DRw3 or DRw4, respectively, and none, both

Only the HLA specificities defined at the VIIth Histocompatibility Workshop Nomenclature Committee [29] were used. However a new specificity, Cve [30] recognised as an allele at the C locus was also tested both in patients and controls.

The statistical significance of the associations was tested by chi-square analysis and the p value was corrected (pc) multiplying the obtained value by the number of tested alleles, except in the case of confirmation of previously observed data.

The relative risk (RR) was calculated according to Svejgaard et al. [31].

Results

Gene Frequencies (Table 1)

The frequencies of the HLA markers DRw3 and DRw4 and of the properdin factor B allele F1 were observed to be highly significantly increased among the IDD patients ($p < 10^{-6}$). HLA-B18, Cw3 and Cw5 were also found at higher frequencies ($p < 10^{-3}$). HLA-B8, B15 and BfS1 were increased but not significantly.

The following HLA antigens were less frequent in IDD patients: A11, A26, B5, B7 DRw2 and DRw5.

DRw3/Drw4 Heterozygosity

HLA DRw3 and DRw4 phenotype frequencies are shown in Figure 1, both for the diabetic and the control groups. Whereas 32% of the diabetics carried both alleles, none of the normal subjects did, though 1% was expected. When using this theoretical value, calculated from the gene frequencies of HLA-DRw3 and DRw4 in the controls, a relative risk for HLA-DRw3/DRw4 heterozygotes of 46.6 was obtained.

The Hardy-Weinberg equilibrium applied to the 53 index cases indicated significant imbalance in favour of heterozygosity at the DR locus. For HLA DRw3 and DRw4, a deficit of homozygosity associated with a significant excess of heterozygosity was observed (χ 2 = 12.85, $p < 10^{-4}$). One patient was HLA DRw3 homozygous and one HLA DRw4 homozygous instead of five expected in each combination. In marked contrast 17 heterozygous HLA DRw3/DRw4 patients were observed, instead of 10 expected.

Recombinations

There were two recombinations in the total offspring of the 53 families: 230 meioses. Both were of maternal origin, one between the A and C loci in a healthy child, the other between the A and B loci in a diabetic child. In the last case, the B–D end of the recombined haplotype was identical with that carried by the three other diabetic children of this family.

Diabetic versus Healthy Siblings

The comparison of the diabetic children with their healthy siblings showed a frequency of HLA DRw3/DRw4 heterozygotes of 10% among the healthy siblings, which was significantly lower than that observed in the affected sibs (37%) ($p < 0.001$).

In 20 informative families, a significant genotype disequilibrium was observed in the diabetic children concerning HLA DRw3/DRw4 heterozygosity (Table 2), while the distribution was near that expected in the healthy siblings.

HLA Identity in Multiple Kindreds

The observed HLA genotypes compared with the theoretically expected random distribution, showed an excess of HLA identical affected siblings, since among the randomly chosen pairs of diabetic siblings (one from each of 12 families), 9 were identical, 3 were haploidentical and none was HLA-different. This distribution is significantly different from the expected ratio; 3: 6: 3 ($p < 0.001$).

Table 2. DRw3-DRw4 segregation among siblings in informative families. The families were selected as being informative for heterozygosity, when one parent carried DRw3/x and the other DRw4/x (x being a recognised DR specificity other than w3 or w4), and for homozygosity, when both parents carried DRw3/x or DRw4/x respectively. NS = not significant

Informative families		Number of diabetic siblings	Number of healthy siblings	p value
DRw3/4 heterozygosity	3/4	15	4	
13 families	3/x	2	6	<0.001
	4/x	0	3	
	x/x	0	7	
DRw3 homozygosity	3/3	2	5	
5 families	3/x	6	3	NS
	x/x	0	1	
DRw4 homozygosity	4/4	1	0	
2 families	4/x	1	4	NS
	x/x	0	2	

Discussion

The results of the present study add further support to the hypothesis that within the HLA complex, the alleles HLA DRw3 and DRw4 are the most strongly associated with the susceptibility gene(s) of IDD. This has been suggested by earlier observations [12, 14–17, 24, 32]. The associations with certain alleles of the B and C loci are therefore secondary, due to linkage disequilibrium within the HLA system [25, 33]. The variations of gene frequencies and gene associations according to geographical and ethnic background account for the differences observed between several countries or regions [19].

The strong association with HLA B18 confirms that observed already in France [10–12], and to a lesser degree in Sardinia [9] and England [5].

The Bf locus allele BfF1 has been suggested to be an important marker for IDD, since in a Boston series of patients [18] BfF1 was found in 22.6% of cases (1.9% in controls). This has not been confirmed by other groups [34]. In our series, the phenotype frequency of BfF1 was 33% in IDD patients compared with 6% in the control population (RR = 5.6). This high frequency of BfF1 is due to its strong linkage disequilibrium with HLA-B18 and DRw3 [33, 35]. BfS1 is also in excess, yet without any linkage disequilibrium with other markers.

The increase of HLA DRw3 (RR = 4) is related to its linkage disequilibrium with HLA B8 and B18. The frequency of HLA DRw4 however greatly exceeds that of HLA B15, which are also in linkage disequilibrium.

These data, together with the findings of others [15, 17] provide evidence that there is a strong association of IDD with the HLA-DRw4 allele as well as HLA-DRw3 and HLA-Dw3.

The high frequency of HLA-DRw3/DRw4 heterozygotes in our diabetic children, compared with their absence in the controls and their low frequency in the healthy sibs, indicates the highest relative risk of developing diabetes for carriers of this combination.

The excess of HLA-DRw3/DRw4 heterozygotes in the diabetics, as compared to the number expected on the basis of the frequencies of both genes, combined with no increase in homozygotes, is a strong argument in favour of the hypothesis of two diabetogenic genes associated with the two HLA-DR alleles [15, 24, 32].

The HLA relationships among affected siblings of the diabetic multiplex families show, in agreement with other family studies [1, 6, 15, 20–22], an excess of HLA identity, which supports the linkage of the disease susceptibility gene with HLA genes.

While a simple true recessive model of inheritance seems unlikely from the lod score data [36], our results are compatible with a more complex "pseudo-recessive" mode of transmission. Overdominance or epistasis of two complementary acting genes, closely linked within the HLA regions, are two of the mechanisms which at present receive most consideration [33].

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