Originals

Trans-racial studies implicate HLA-DQ as a component of genetic susceptibility to Type 1 (insulin-dependent) diabetes

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Summary. Type 1 (insulin-dependent) diabetic patients and control subjects of Afro-Caribbean Negroid racial origin were investigated by serological HLA-DR-typing and restriction fragment length polymorphism analysis using DNA probes corresponding to the DQα, DQβ and DRβ chain genes. Combined analysis indicated that four DR antigens are positively associated with the condition in Negroid subjects – DR3, 4, 7 and w9. DR3 and 4 are also associated in Caucasians, but the relative risk for DR3 is lower in Negroid subjects. The DR7 association is specific for the Negroid race, and DRw9 is only weakly associated in Caucasoid subjects. Restriction fragment length polymorphism analysis demonstrated a DQβ restriction pattern in Negroid subjects which is absent from Caucasoid subjects. This pattern was associated with DRw9 and a subset of DR7, and was markedly increased in frequency in diabetic patients compared with control subjects (48.7% vs 10.4%, respectively; $p < 10^{-4}$). In the absence of this pattern, DR7 showed no positive association. DR3 in Negroid subjects was associated with two distinct DQα-DQβ patterns, only one of which was positively associated with diabetes. A DQβ pattern, in linkage disequilibrium with different DR antigens in different races, conferred a consistent protective effect against the development of Type 1 diabetes. Trans-racial genetic analysis thus supports a primary role for DQ in susceptibility to Type 1 diabetes.

Key words: Type 1 (insulin-dependent) diabetes, HLA-DQ, racial studies.

The association of Type 1 (insulin dependent) diabetes with certain DR antigens indicates that a major part of its genetic susceptibility is located close to, or within, the HLA-D (class II HLA) region. Recent studies have pointed to the DQ subregion as a potential determinant of disease predisposition [1–9]. Identification of the primary susceptibility locus (or loci) is complicated, however, by linkage disequilibrium. This refers to the strong tendency of HLA alleles to co-associate non-randomly to form specific haplotype combinations. HLA linkage relationships vary in different races, and, thus, comparison of HLA-disease associations in distinct ethnic populations may define the primary site of genetic predisposition [10].

The relative risk for Type 1 diabetes conferred by certain DR antigens shows racial variation. DRw9, for example, is increased in frequency in diabetic patients of Japanese origin [11], but the relative risk for this antigen in Caucasoid subjects is close to one [12]. This variation suggests that the serologically-defined DR antigens do not correspond directly to disease susceptibility alleles, but it may help to identify these factors. If an antigen shows a race-specific association with Type 1 diabetes, the question arises "what allele is found on haplotypes bearing this antigen in the race(s) in which it is diabetes-associated, which is absent from haplotypes bearing the same antigen in other populations?". The genetic locus encoding this allele will be a candidate for a primary determinant of genetic susceptibility to Type 1 diabetes.

In an attempt to define race-specific disease associations, we have serologically DR-typed Negroid patients with Type 1 diabetes and racially-matched control subjects. Associations thus defined were further investigated by restriction fragment length polymorphism (RFLP) analysis using DNA probes corresponding to the DQα, DQβ and DRβ chain genes.

Subjects and methods

Subjects

Type 1 diabetic patients and control subjects were UK residents of Afro-Caribbean (predominantly Jamaican) Negroid racial origin, with no known Caucasoid ancestry. Control subjects were healthy un-

Table 1. Clinical characteristics of diabetic patients

Non-obese
Acute onset of symptoms
Under 30 years of age at diagnosis
Frank clinical ketoacidosis or heavy non-fasting ketonuria ($+ + +$ on
  stick testing) at presentation
Absolute requirement for insulin from diagnosis

Table 2. HLA-DR antigen frequencies in Negroid Type 1 diabetic patients and control subjects

HLA-DR antigen	Patients ($n=39$)	Control subjects		
		($n=80$)[a]	RR	p_c
1	3 (7.7)	11 (13.8)	0.58	NS
2	2 (5.1)	24 (30.0)	0.15	<0.025
3	15 (38.5)	21 (26.3)	1.8	NS
4	15 (38.5)	2 (2.5)	20	$<10^{-4}$
5	3 (7.7)	34 (42.5)	0.13	<0.002
w 6	8 (20.5)	22 (34.4)	0.51	NS
7	11 (28.2)	9 (11.3)	3.0	NS
w 8	4 (10.3)	6 (9.4)	1.1	NS
w 9	9 (23.1)	3 (4.7)	5.5	NS
w10	0 (0)	1 (1.6)	0.54	NS

This table shows the numbers (percentages) of patients and control subjects with each DR antigen. n = total number of subjects in each group. [a] for DRw6, w8, w9 and w10, $n=39$ for the patient group, and $n=64$ for the control subjects. RR = relative risk; p_c = corrected significance level; NS = non-significant

related individuals with no personal or family history of diabetes. All patients fulfilled the classic clinical criteria for insulin-dependence (see Table 1).

Methods

Patients and control subjects were studied by: (1) serological HLA-DR-typing (39 patients and 80 control subjects). (2) DQα, DQβ and DRβ RFLP analysis (39 patients and 77 control subjects).

DR-typing was by a standard microlymphocytotoxicity method using commercially prepared typing trays (Biotest, Birmingham, UK). These trays are in routine clinical use in the United Kingdom for tissue typing prior to bone marrow transplantation. DR1-5 & 7 were determined in all subjects. DRw6, w8, w9 and w10 were defined in all 39 patients and in 64 control subjects. The typing trays included the following numbers of specific antisera: DR1 - four; DR2 - five; DR3 - five; DR4 - five; DR5 - five; DR7 - five; DRw8 - two; DRw10 - two. For DRw6, five antisera were included, two of which were cross-reactive with DR3, and three of which cross-reacted with the other DQw1-related antigens DR1, 2 and w10. For DRw9, three antisera were employed, two of which cross-reacted with DR4 and one of which cross-reacted with DR1 and w10. DR antigen assignments were confirmed by DRβ probing (see below "Restriction fragment length polymorphism analysis").

For the RFLP studies, DNA was isolated from peripheral blood. Ten μg DNA were digested with restriction enzymes BamHI, Eco RI or TaqI under conditions recommended by the manufacturer (BRL, Glasgow, UK). Digested DNA was separated by electrophoresis in 0.7% agarose at 50 V, 25 mA for 18 h (TaqI and BamHI digests) or 22 h (EcoRI digests) and Southern blotted onto nylon filters (Hybond-N, Amersham Int., Amersham, UK) [13]. Three cDNA probes were used: (1) the HindIII/PstI insert of pIIβ1 (full-length DQβ chain); (2) the ApaI fragment of pIIα5 (full-length DQα chain); (3)

the 500 base pair PstI fragment of pIIβ4 (corresponding to the second domain, transmembrane, cytoplasmic and 3'-untranslated portions of DRβ chain). The restriction enzymes used were BamHI (DQβ), EcoRI (DQβ) and TaqI (DQβ, DQα and DRβ). Probes were labelled to a specific activity of 10^9 cpm/μg DNA by the oligonucleotide primer method. Prehybridisation and hybridisation were performed at 65°C in 6× standard sodium citrate solution (SSC; 1× SSC = 0.15 mol/l NaCl, 0.015 mol/l Na citrate), 5× Denhardt's solution, 0.5% sodium dodecyl sulphate (SDS) with 250 μg/ml denatured salmon sperm DNA. Ten percent dextran sulphate was used in the hybridisation solution. After hybridisation, filters were rinsed in 2× SSC, 0.1% SDS, twice, at room temperature, then washed in the same solution at 65°C for 30 min, in 0.5× SSC, 0.1% SDS at 65°C for 60 min, followed by four 15-min washes in 0.1× SSC, 0.1% SDS at 65°C. After air drying, autoradiography was performed for 3 days at −70°C.

Statistical analysis

Statistical analysis of associations of DR antigens and DQ RFLPs with Type 1 diabetes was by the X^2 test, or Fisher's exact test, as appropriate. Corrected significance levels (p_c) werer calculated by multiplying the "p value" by the number of statistical comparisons performed at each locus. Relative risk (RR) estimates are given with 95% confidence intervals in brackets. RR estimates, 95% confidence intervals for RR, combined RR risk estimates, and significance levels for RR estimates were determined by the method of Woolf with the Haldane modification for small numbers [14-16].

Results

HLA-DR serological analysis

The DR antigen frequencies in Negroid diabetic patients and control subjects are shown in Table 2. DR4 was markedly increased in frequency in the patients, but was rare in the control population (38.5% vs 2.5%; RR = 20 (5.4-74); $p_c < 10^{-4}$). DR3 was non-significantly increased in frequency in patients compared with control subjects (38.5% vs 26.3%; RR = 1.8 (0.81-4.0)), as were DR7 (28.2% vs 11.3%; RR = 3.0 (1.2-7.7)) and DRw9 (23.1% vs 4.7%; RR = 5.5 (1.6-19)).

DR3/4 heterozygosity was non-significantly increased in frequency in patients compared with control subjects (10.3% vs 0%). DR3, DR4, or both antigens, were found in 66.7% patients and 28.8% control subjects (RR = 4.8 (2.2-11); $p_c < 0.001$). DR3, DR4, DR7 or DRw9 (or any two of these antigens) were found in 36/39 (92.3%) patients and 28/64 (43.8%) control subjects (RR = 13 (4.3-42); $p_c < 10^{-5}$).

DR2 was reduced in frequency in patients vs control subjects (5.1% vs 30.0%; RR = 0.15 (0.04-0.55); $p_c < 0.025$), as was DR5 (7.7% vs 42.5%; RR = 0.13 (0.04-0.40); $p_c < 0.002$).

Combined analysis

In view of the increased frequency (non-significant) of DR3, DR7 and DRw9 in this study, combined analysis of these data with those of all previously published

studies of DR antigens in Negroid subjects with Type 1 diabetes [17–23] was performed, to determine whether there were significant associations with these markers (only four published studies tested for DRw9 [20–23]).

The combined RR estimate for DR3 was 1.94 (1.48–2.53); this was significantly different from one (X^2 (1 df) = 23.5; $p_c < 0.001$). Further analysis indicated significant variation (heterogeneity) among RR estimates for DR3 (X^2 (7 df) = 18.8; $p < 0.01$). Removal of a data set derived from a study in American Negroid patients [17] (which gave a relatively high RR estimate for DR3 (5.9)) from the analysis gave non-significant heterogeneity ($p > 0.05$), and a combined RR value for DR3 of 1.71 (1.29–2.27). This was significantly different from one ($p_c < 0.002$).

The combined RR estimate for DR7 was 1.71 (1.25–2.35). This was significantly different from one (X^2 (1 df) = 11.1; $p_c < 0.01$). The RR estimates showed significant variation (X^2 (7 df) = 17.3; $p < 0.025$). Removal of a data set derived from a study of Ethiopian Negroid subjects [23] (in which DR7 was reduced in diabetic patients) from the analysis gave non-significant heterogeneity ($p > 0.2$), and a combined RR value for DR7 of 1.95 (1.41–2.70). This was significantly different from one ($p_c < 0.001$).

The combined RR estimate for DRw9 was 4.53 (2.23–9.19) (X^2 (1 df) = 17.5; $p_c < 0.001$). The RR estimates were not significantly heterogeneous (X^2 (4 df) = 2.2; $p > 0.5$).

Restriction fragment length polymorphism analysis

DQβ RFLPs were the same as those previously described in Caucasoid subjects [10, 24, 25], apart from a novel pattern, found only in Negroid subjects, designated DQβVIIc. Table 3 shows the restriction fragments for each DQβ RFLP. Correlations between the DQβ DNA fragment patterns obtained with the three restriction enzymes (BamHI, TaqI and EcoRI) were based on (1) published studies which analysed DQβ polymorphism with these three restriction enzymes in homozygous typing cells [25] and in pedigrees [3], and (2) the empirical observation that the correlations defined in these studies were also seen in Afro-Caribbean patients and control subjects. DQβVIIc has not been previously recognised, but the other RFLPs listed in Table 3 are known to be DQβ-specific [3].

BamHI fragments were the same for DQβVIIb and DQβVIIc. The distinction was made by a TaqI 4 kb fragment in DQβVIIc, instead of the 8 kb fragment characteristic of DQβVIIb (see Fig. 1). The DQ-specificity of DQβVIIc was supported by the observation that the 4 kb fragment gave a strong hybridisation signal, and that it was not visible on filters rehybridised with DRβ probe. EcoRI fragments also varied, with an 18 kb fragment in DQβVIIb and a 13 kb fragment in DQβVIIc.

Table 3. DNA restriction fragments characteristic of each DQβ restriction fragment length polymorphism

DQβ Pattern	DNA restriction fragments (kb) BamHI	TaqI	EcoRI
I	6.2 + 3.2	5.5	13
III	7.0 + 4.0	2.5 + 1.5	13
IV	12	1.8	18 + 5.0
V	7.0 + 3.7	4.6	13 + 5.0
VIa	7.0 + 3.0	3.2	9 + 2.2
VIb	7.0	5.5	9 + 2.2
VIIb	4.0 + 3.2	8.0 + 2.5 + 1.5	18 + 7.5
VIIc	4.0 + 3.2	4.0 + 2.5 + 1.5	13 + 7.5

kb = DNA fragment size in kilobases
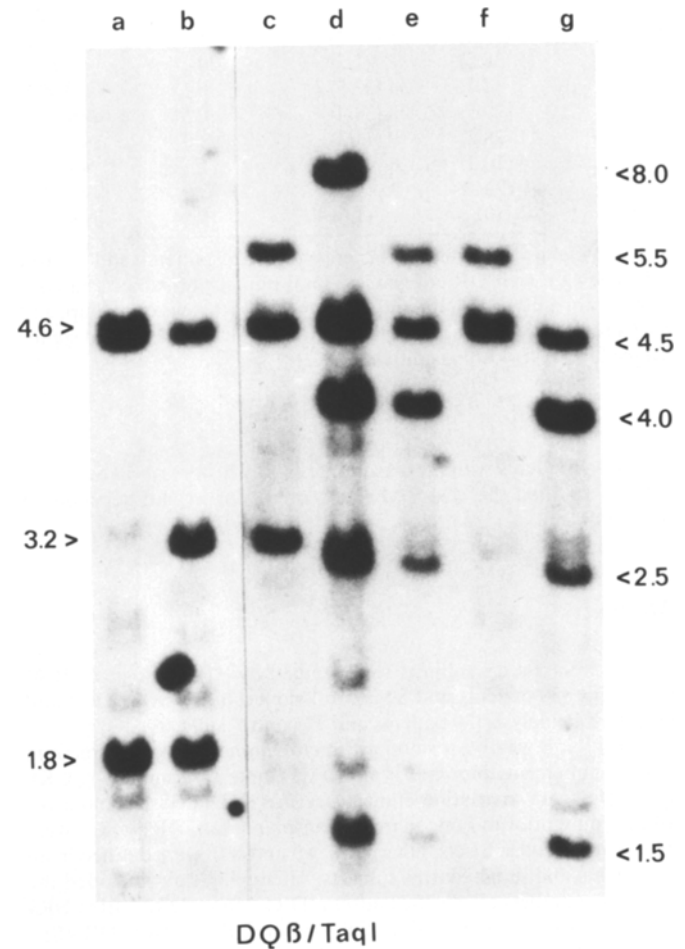
Fig. 1. DNA restriction fragment patterns obtained with DQβ probe and restriction enzyme TaqI. Tracks d, e, and g show the 4.0 kb fragment characteristic of DQβVIIc. Track d also shows the 8.0 kb fragment characteristic of DQβVIIb

The frequencies of the DQβ RFLPs are shown in Table 4. DQβVIIc was markedly increased in frequency in patients compared with control subjects (48.7% vs 10.4%; RR = 7.8 (3.1–20); $p_c < 10^{-4}$). DQβVIIb showed no association. DQβIII was also increased in patients vs control subjects (35.9% vs 13.0%; RR = 3.7 (1.5–9.1); $p_c < 0.04$). DQβIV was non-significantly increased in

Table 4. DQβ RFLP frequencies in Negroid Type 1 diabetic patients and control subjects

DQβ Pattern	Patients $(n=39)$	Control subjects $(n=77)$	RR	p_c
I	6 (15.4)	25 (32.5)	0.40	NS
III	14 (35.9)	10 (13.0)	3.7	<0.04
IV	15 (38.5)	17 (22.1)	2.2	NS
V	6 (15.4)	28 (36.4)	0.34	NS
VIa	4 (10.3)	33 (42.9)	0.17	<0.005
VIb	3 (7.7)	10 (13.0)	0.62	NS
VIIb	5 (12.8)	12 (15.6)	0.84	NS
VIIc	19 (48.7)	8 (10.4)	7.8	$<10^{-4}$

This table shows the numbers (percentages) of patients and control subjects with each DQβ RFLP. n=total number of subjects in each group. Six patients and eleven control subjects are presumptive homozygotes.
RR = relative risk; p_c = corrected significance level; NS = non-significant

Table 5. DNA restriction fragments characteristic of each DRβ RFLP

DRβ pattern	TaqI/DRβ fragments
I	6.0+4.5
II	12+2.0+1.5
III	12+7.0+4.0
IV	16+6.0+5.5+2.5
V	10+6.5+4.0
VI	10+7.0+4.0
VII	16+6.0/7.0+4.0+2.5

The table shows the size (in kilobases) of the DNA fragments characteristic of each DRβ RFLP. DRβVII may include either a 6.0 or 7.0 kb fragment

Table 6. Associations between DR antigens and DQβ RFLPs

Association	++	+−	−+	−−	p
DR1:DQβI	9	0	11	44	$<10^{-4}$
DR2:DQβVIa	18	4	13	29	$<10^{-4}$
DR3:DQβIII	8	11	1	44	0.001
DR3:DQβIV	11	8	3	42	$<10^{-4}$
DR4:DQβIV	1	0	13	50	NS
DR5:DQβV	14	8	9	33	<0.001
DR5:DQβVIa	12	10	19	23	NS
DRw6:DQβVIa+b	14	4	19	24	<0.02
DR7:DQβVIIb+c	7	1	8	48	<0.0005
DRw8:DQβV	6	0	17	38	<0.005
DRw9:DQβVIIc	3	0	4	54	<0.002

The figures shown represent the numbers of control subjects in each category of DR-DQβ association. p=significance level of the association

Table 7. Associations between DQβ, DRβ and DQα RFLPs

Antigen	DRβ	DQβ	DQα
DR2	II	-VIa	-6.2 kb
DR3	III	-III	-4.6 kb
	VI	-III	-4.6 kb
	V	-IV	-6.2 kb
DR4	IV	-IV	-5.5 kb
	IV	-V	-5.5 kb
DR5	V	-V	-4.6 kb
	V	-VIa	-6.2 kb
DR7	VII	-VIIb	-5.5 kb
	VII	-VIIc	-5.5 kb
DRw9	VII	-VIIc	-5.5 kb

The table shows the most commonly associated DRβ-DQβ-DQα RFLP combination(s) for selected DR antigens

patients (38.5% vs 22.1% of control subjects). DQβVIa was markedly reduced in patients vs control subjects (10.3% vs 42.9%; RR=0.17 (0.06–0.49); p_c<0.005).

RFLPs defined with DRβ and DQα probes were the same as those seen in Caucasoid subjects [10, 24, 25]. DRβ RFLPs defined with TaqI were classified into seven patterns which are listed in Table 5. These patterns correlate closely with serologically-defined DR-types [10, 24, 25]. DQα probing with TaqI defined fragments of size 2.6, 4.6, 5.5, 6.2 and 6.8 kb which are known to represent alleles of the DQα gene [26].

Relationships between DR antigens and DQ and DR RFLPs

There were close associations (due to linkage disequilibrium) between DR antigens and DQα and DQβ RFLPs. Table 6 shows the DR antigen-DQβ RFLP relationships (defined in control subjects), and Table 7 shows, for selected DR antigens, the most commonly associated DRβ/DQβ/DQα RFLP combinations. Many of the associations were similar to those previously found in Caucasoid subjects [10, 24, 25], but the following differences were seen in Negroid subjects: (1) the novel RFLP, DQβVIIc, was found in association

with DRw9 and a subset of DR7. (2) DR3 was in linkage disequilibrium with DQβIII (as in Caucasoid subjects), but also showed an association with DQβIV not seen in Caucasoid subjects. (3) DQβVIa was strongly associated with DR2 (as in white Caucasoid subjects), but there was also a weaker (non-significant) association with DR5.

Of eight DR7-positive control subjects tested, six had DQβVIIb, one had DQβVIIc and one had DQβIV. Among eleven DR7-positive diabetic patients, seven had DQβVIIc and five had DQβVIIb (one patient was heterozygous for the two RFLPs). DR7 was thus increased in frequency in patients only in combination with DQβVIIc (RR=9.8 (2.0–49); p<0.01). DR7 in combination with DQβVIIb was neutral (RR=1.4 (0.44–4.5); p=NS). DR7-DQβIV was not seen in any patient. DRw9 was closely associated with DQβVIIc, in both patients and control subjects (RR for DRw9-DQβVIIc=5.5 (1.6–19); p<0.01). DR7-associated DQα and DRβ RFLPs were as described for DR7 haplotypes in Caucasoid subjects [10, 24, 25] (see Table 7).

In control subjects, DR3 was associated with either DQβIII or DQβIV. DRβ and DQα RFLPs also differed between these two subsets of DR3 (see Table 7). Eight of 19 (42%) DR3-positive control subjects tested had DQβIII, and 11/19 (58%) had DQβIV. DQβIII was

present in 14/15 (93%) DR3-positive patients, whereas DR3-DQβIV was absent from the patient group (one DR3-positive patient had neither of these two DQβ patterns). Thus, only the DR3-DQβIII combination was increased in frequency in diabetic patients (RR = 3.8 (1.5–9.8); $p < 0.01$); for DR3-DQβIV, RR was 0.06 (0.01–0.48) ($p < 0.01$). The difference in proportions of the two DQβ patterns between DR3-positive patients and DR3-positive control subjects was statistically significant ($p < 0.001$).

Eleven of 15 (73%) DR4-positive patients had DQβIV, but the remaining four had DQβV. The latter patients had the following DR antigens and DQβ RFLPs - (1) DR3/4 (DQβIII/V); (2) DR3/4 (DQβIII/V); (3) DR4/w6 (DQβV/VIb); (4) DR4/w6 (DQβV/I). The single DR4-positive control subject tested had DQβIV.

DQβVIa was significantly associated with DR2, but was also found in 12/22 DR5-positive control subjects. In six of these subjects, the presence of DQβVIa could be accounted for by the second DR antigen (DR2 or DRw6), but in the remaining six subjects, this was not the case. DQβVIa was absent from the three DR5-positive patients.

Discussion

The Afro-Caribbean population of the United Kingdom is of recent origin, being principally derived from a wave of migration which occurred during the 1950s and 1960s. Our selection criteria excluded patients and control individuals with post-migration Caucasoid ancestry and our subjects are, therefore, genetically representative of the indigenous Negroid population of the Caribbean region. Current knowledge of HLA associations with Type 1 diabetes in Negroid subjects has relied heavily on studies from the United States, but the American Negroid population has a substantial proportion (approximately 20%) of Caucasoid genetic admixture [27]. In the Negroid population of Jamaica, Caucasoid admixture has been estimated at 10% [27], and thus, the subjects of the present study are genetically closer to the West African Negroid peoples from whom both American and Caribbean Negroid subjects are descended.

Our analysis indicated that four DR antigens are increased in frequency in Negroid patients with Type 1 diabetes - DR3, 4, 7 and w9. In all other races studied, DR7 is reduced in frequency in patients [28], and, thus, this antigen is a race-specific marker. Ethiopian Negroid patients do not appear to show this association [23], possibly due to their distinct Negroid-Caucasoid ancestry. DRw9 shows only a weak predisposing effect in Caucasoid patients [12], but is increased in Japanese diabetic patients [11]. The DR4 association has been noted in previous studies of Negroid subjects [17–20, 23]. In Caucasoid subjects, the relative risk for DR3 is

three to four [29], but our analysis indicated a value of approximately two for Negroid subjects. There was significant variation in relative risk estimates for DR3 in Negroid subjects, which may be due to Caucasoid genetic admixture in some of the populations studied. Both DR2 and DR5 were significantly reduced in frequency in Negroid patients with Type 1 diabetes. In Caucasoid subjects, the negative association of DR2 is well-recognised, but DR5 does not consistently confer a marked protective effect.

To investigate further these racial differences in DR antigen associations, we studied class II HLA RFLPs with DNA probes corresponding to the DQα, DQβ and DRβ chain genes.

DRw9, and a subset of DR7, were associated with a DQβ pattern (DQβVIIc) which has not been previously identified in other races [10, 24, 25]. This pattern was associated with DQα and DRβ RFLPs which are also characteristic of Caucasoid DR7 and DRw9 haplotypes [10, 24, 25]. The frequency of DQβVIIc was markedly increased among Negroid diabetic patients compared with control subjects. As in Caucasoid subjects [10], DR7-DQβVIIb was not increased in Type 1 diabetes. These results suggest that the positive associations of DR7 and DRw9 with Type 1 diabetes in Negroid subjects correlate with variation in the DQ subregion on DR7 and DRw9 haplotypes in this race. DNA sequencing of the class II genes on the diabetes-associated DR7 haplotype is now required to confirm the results of RFLP analysis.

The other DQβ patterns seen in Negroid subjects are also found in Caucasoid subjects [10, 24, 25]. Their linkage disequilibrium relationships with DR antigens were also similar to those seen in the latter groups, with certain important exceptions. In Caucasoids, one DQ RFLP pattern, DQβIII-DQα4.6 kb, is found in the great majority of DR3-positive subjects (patients and controls). This pattern corresponds to the DR3-DQw2 haplotype. In Negroids, DR3 in control subjects was associated with two patterns - DQβIII-DQα4.6 kb, and DQβIV-DQα6.2 kb. This split has recently been recognised by others [30]. The latter pattern corresponds to the DR3-DQ"blank" haplotype, present in approximately 50% of DR3-positive American Negroid subjects [30]. DNA sequence analysis has shown that the DR3-DQ"blank" and DR3-DQw2 DQα chains are very similar, but the DR3-DQ"blank" DQβ chain is similar to that previously described for a DR4-DQ"blank" haplotype [30].

In the present study, only the DR3-DQw2 pattern was increased in frequency in Negroid patients, whereas the DR3-DQ"blank" pattern was absent from this group. The negative association of DR3-DQ"blank" with Type 1 diabetes explains the lower relative risk for DR3 in Negroid subjects compared with Caucasoid subjects, and is consistent with the view that the DR3-related component of genetic susceptibility to Type 1 diabetes is DQ-related. No firm conclusion can be

reached regarding the relative roles of DR and DQ in DR3-related susceptibility, however, because DR3-DQw2 and DR3-DQ"blank" have distinct DRβ RFLPs, implying that their DRβ chains are also different.

DQβVIa is associated with DR2 in Caucasoid subjects and with DRw6 in North Indian subjects but has a protective effect against Type 1 diabetes in both groups [10]. In Negroid subjects, DQβVIa was strongly associated with DR2, but there was also evidence for an association with DR5. Although the latter association was not statistically significant, it is known that DR5 haplotypes frequently carry DQw1 (of which DQβVIa is a subset) in Negroid subjects [31]. As in white Caucasoid and North Indian subjects, DQβVIa in Negroid subjects was markedly reduced in frequency in patients with Type 1 diabetes. Thus, a single DQβ pattern with varying DR antigen associations in different races confers a consistent protective effect against Type 1 diabetes, supporting the view that a component of susceptibility to the condition is DQ-encoded.

Two DQ-defined subsets of DR4 - DQw3.1 and DQw3.2 - have been described using serology and RFLP analysis; DQw3.1 correlates with a positive reaction with TA10 antisera, whereas DQw3.2 is TA10-negative [32]. In our terminology, DQβIV is characteristic of DQw3.2, and DQβV corresponds to DQw3.1. Current evidence indicates that DQw3.2 is present in approximately 90% of DR4-positive Caucasoid diabetic patients, whereas DQw3.1 is reduced in frequency in patients [9]. This has been interpreted as evidence that DR4 susceptibility is DQ-encoded. If, however, the negative association of DR4-DQw3.1 with Type 1 diabetes is a general phenomenon, then the low frequency of DR4 in the Afro-Caribbean Negroid control population should lead to a very low frequency of DR4-DQβV in patients of this race. In the present study, however, four of fifteen DR4-positive patients had DQβV rather than DQβIV. DQ may not, therefore, be the only locus influencing susceptibility to Type 1 diabetes on DR4 haplotypes. This view is supported by a recent study of DR4-DQw3 subtypes (defined by TA10 serology) in Caucasoid patients, which found that the specific positive association of DR4-DQw3.2 with Type 1 diabetes may only apply to DR3/4 heterozygous subjects; among DR4-positive, DR3-negative patients the frequencies of DQw3.1 and 3.2 were similar to those found in DR4-positive control subjects [33].

In conclusion, trans-racial genetic analysis indicates that the DQ region has a primary role in determining predisposition to Type 1 diabetes. This is supported by two lines of evidence from this study. First, the increased frequencies of DR7 and DRw9 in Type 1 diabetes in Negroid subjects correlated with the association of these antigens with a novel DQβ RFLP which was markedly increased in frequency among patients. Second, a DQβ RFLP in linkage disequilibrium with different DR antigens in three races, confers a consistent protective effect against Type 1 diabetes. Furthermore, our results are compatible with a model in which the DR3 association is mediated by HLA-DQ. Studies in Caucasoid subjects support a role for DQ in DR4-related susceptibility also, but the possibility of an additional non-DQ component of DR4 predisposition cannot be excluded.

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