HLA Class II Alpha Chain Gene Polymorphisms in Patients with Insulin-dependent Diabetes Mellitus, Dermatitis Herpetiformis, and Celiac Disease

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Abstract

We have investigated DNA polymorphism of the class IIα chain genes in HLA typed patients with insulin dependent diabetes mellitus (IDDM; $n = 79$), celiac disease (CD; $n = 46$), dermatitis herpetiformis (DH; $n = 53$), and controls ($n = 86$). Preferential allelic associations of HLA genes and gene products have thus been constructed for susceptibility to these diseases. DRα and DQα gene polymorphisms indicated heterogeneity of HLA DR3, DRw6, and DR7, and HLA DR2 and DRw6, respectively. In DR7 positive CD patients a 3.8-kilobase (kb) DRα fragment, which correlated with DQw3, was found in only 11% of patients compared with 45% of corresponding controls ($P < 0.05$). An increased frequency of a DXα genotype UU in all three diseases was found (IDDM 59%, DH 45%, CD 48%, compared to 21% in controls, $P < 0.001$), which is not explained solely by the increased frequencies of DR3-DXαU. We therefore conclude part of the genetic susceptibility for these three conditions is encoded by genes within the DQ-DX subregion.

Introduction

Genes determining susceptibility or resistance to a variety of diseases have been localized to the HLA region on chromosome 6. Individual serologically defined HLA antigens were initially shown to be increased in frequency in groups of patients when compared to healthy controls from the same population group. In insulin dependent diabetes mellitus (IDDM),[1] dermatitis herpetiformis (DH), and celiac disease (CD), statistically significant associations are found with HLA-DR3; in addition the frequency of DR4 is increased in IDDM (1–3), DR2 in DH (4), and DR7 in CD (5, 6). Since a proportion of patients with these conditions do not have the relevant antigens, the likelihood is that the genes encoding them are not those conferring the disease susceptibility, but are markers for other closely linked genes. Even after more detailed examination of other major histocompatibility complex (MHC) gene products, such as the class II HLA DQ series (7), the lymphocyte activating HLA-Dw determinants (8) and the complement components C2, C4 and Bf (9), none appear to be invariably present, although some combinations of several closely linked gene products show clearer associations than individual antigens. For example the associations of the complement component C4AQ0 with DR3, or C4AB3 with DR4 are more significant in IDDM patients compared to controls than each antigen taken individually. Additionally, some evidence has been presented that non-MHC genes may be involved in these diseases. In IDDM associations have been described with the class 1 allele adjacent to the insulin gene located on chromosome 11 (10, 11) and the β-chain of the T cell receptor on chromosome 7 (12). In celiac disease an association has been described with heavy chain immunoglobulin (Gm) allotypes (13).

Analysis of the MHC has thus been mainly confined to detection of serologically defined antigenic determinants. More recent analysis at the genomic level has defined heterogeneity within serological specificities. Thus the search for the disease susceptibility genes within the HLA-class II region has been extended by detection and comparison of restriction fragment length polymorphisms (RFLP) in patients and healthy control populations. The HLA-D region consists of three main adjacent subregions (Fig. 1). The HLA DR locus consists of three β-chain genes and an α-chain gene. The DQ region consists of a single α- and β-chain gene at both the DQ and DX loci. The DP subregion consists of two α-chain genes and two β-chain genes. Marked differences in frequency of various RFLPs within the HLA D region have been described in HLA serologically matched patients and controls. Owerbach et al. 1983 (14) found a decreased prevalence of a Bam HI 3.7 kb DQβ related fragment in IDDM (2% in IDDM subjects compared with 27% controls). Subsequently, Festenstein et al. (15) and Cohen-Haguenauer et al. (16) found an increase in certain DQβ RFLPs (designated as omega and DQR4, respectively) in IDDM subjects who possess DR4.

As the class II molecule consists of both an α- and β-chain we felt it would be equally important to study α-chain polymorphism in relation to autoimmune disease. We were especially interested in studying the DXα locus at the genomic level as no expression has been detected in the cell lines so far studied despite an apparently normal gene structure based on nucleotide sequencing (17). We have previously demonstrated an increased frequency of a Taq 1 DXα gene related fragment sized 2.1 kb (U allele) in IDDM patients compared with healthy controls using a genomic DQα probe (18). The DQα probe also identifies the DXα gene because of close nucleotide homology between these two loci (17). This study (*a*) provides additional information on DR associations in individuals with the UU genotype in

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1. *Abbreviations used in this paper:* CD, celiac disease; DH, dermatitis herpetiformis; IDDM, insulin-dependent (type 1) diabetes mellitus; MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism.

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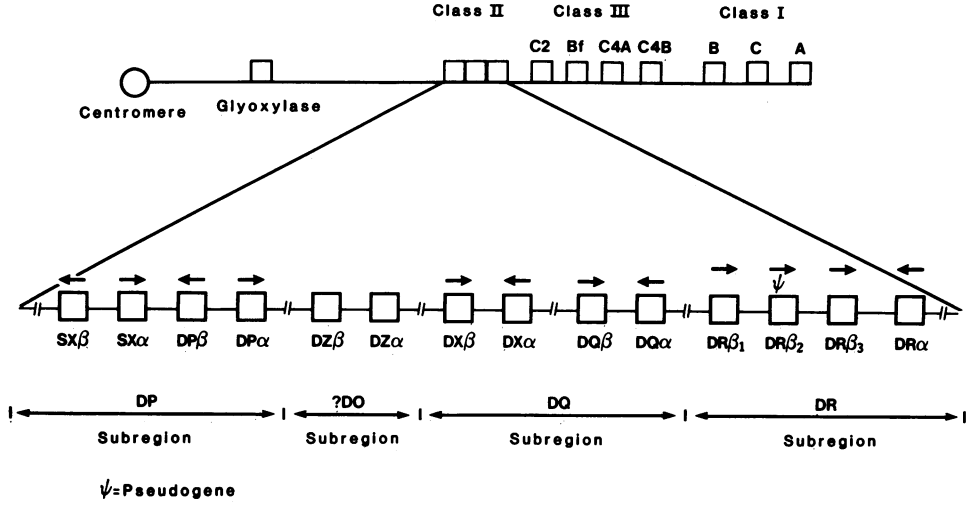

Figure 1. Map of Class II MHC genes on the short arm of chromosome 6 (taken from reference 30). The exact orientation of individual genes with respect to each other is not exactly known, although the direction of transcription is indicated by the arrows. The location of the DO subregion is not known and may be telomeric to the DR subregion rather than between the DP and DQ subregions as shown in this diagram.

IDDM; (*b*) compares the distribution of DXα and DQα RFLPs in patients with IDDM, CD and DH and healthy controls; (*c*) analyses the distribution of DRα RFLPs in the four population groups; (*d*) defines preferential allelic associations in the four population groups.

Methods

Subjects. Unrelated British Caucasian healthy individuals with no known personal or family history of diabetes mellitus, celiac disease, or dermatitis herpetiformis served as controls for RFLP studies. A reference control panel (n = 161) (19) was used for comparison of DR and DQw antigen frequencies with the patient groups. Some of the unrelated British Caucasian IDDM subjects were those previously described (10). Unrelated British Caucasian patients with celiac disease and dermatitis herpetiformis were serially selected from outpatient clinics and most of them were described in a previous study (4). 86 healthy controls, 79 patients with IDDM, 53 with DH, and 46 with CD were analyzed.

DNA analysis. DNA was extracted by a modification of the method of Kunkel from leukocytes obtained from whole blood samples anticoagulated with EDTA and previously stored at − 20°C (20). After digestion with the appropriate restriction enzyme according to manufacturers specifications (Boehringer Mannheim, Cambridge, England; Anglian Biotechnology Ltd., Colchester, England) DNA fragments were separated by electrophoresis on a 0.85% agarose gel and then transferred to a nylon membrane (Gene Screen Plus; New England Nuclear Research Products, Boston, MA) using an alkaline solvent (21). Hybridization with nick translated ^{32}P-labeled gene probes was performed according to a standard protocol (New England Nuclear Research Products). All filters were washed down to a high stringency as recommended by the manufacturers and exposed to Kodak XAR5 film in the presence of two high speed intensifying screens for 36 h at − 70°C. Hybridization bands were sized by comparison with an internal control (GAH genomic DNA) and Hind III digested lambda phage (Bethesda Research Laboratories, Paisley, Scotland) present on each filter. GAH genomic DNA allowed standardization of sizes of alleles between autoradiograms and also acted as an internal control for adequacy of DNA digestion. The genomic DQα (22) and the cDNA DRα (23) probes used were kind gifts of J. Trowsdale and W. Bodmer (London, England) and P. A. Peterson (Uppsala, Sweden), respectively.

HLA typing. Tissue typing for HLA-A, -B, -C (24), -DR, and -DQ antigens (25) was performed according to previously described techniques. All subjects were typed for HLA-A, -B, -C, and -DR antigens; 54 subjects with IDDM and 12 with CD were not typed for DQ antigens. It was also not always possible to identify the DQw2 antigen in individuals who were both DR3 and DR7 as appropriate absorbed antisera were not available.

Statistical methods. Serological and RFLP data were analyzed for differences in allelic or genotype frequencies by chi-square tests using 2 × 2, 3 × 2, or 5 × 2 contingency tables.

Results

HLA-DR frequencies

The HLA-DR frequencies (Table I) in the RFLP control panel and HLA-DR reference panel were similar. As expected the frequency of DR3 is highly significantly raised in all three patient groups ($P < 0.001$) compared with the HLA-DR reference panel. The frequency of DR4 ($P < 0.001$) and the heterozygote DR3/DR4 ($P < 0.001$) were also significantly increased in IDDM compared to the controls. Similarly DR7 is increased in the CD panel when compared to all three other panels ($P < 0.001$). No

Table I. Distribution of DR Frequencies in Different Groups

DR antigen	HLA reference control panel (n = 161)	RFLP controls (n = 86)	IDDM (n = 79)	DH (n = 53)	CD (n = 46)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
DR1	16	19	15	11	0*
DR2	32	27	6‡	26	13*
DR3	32	34	63‡	80‡	89‡
DR4	37	34	71‡	28	6‡
DR5	14	14	4	4	2
DRw6	19	16	9	9	9
DR7	27	30	9	22	54‡
DR3/2	6	7	2	26‡	0
DR3/4	9	9	37‡	11	2
DR3/7	8	7	0*	15	48‡
DR3/X	2	1	4	0	11
DR4/X	4	2	6	6	2
DR7/X	2	2	0	0	2

X, no other antigen detected implying homozygosity.
P values were calculated from 2 × 2 contingency tables by chi-square analysis for comparison of patients with the HLA reference control panel.
* $P < 0.05$; ‡ $P < 0.001$.

significant difference was seen in frequency of DR2 in controls and patients with DH in this study although the DR3/DR2 heterozygotes were significantly increased in patients compared with controls (26 vs 6%; $P < 0.001$).

HLA-DQ frequencies

The frequency of DQw2 was increased (in association with the increase of DR3) in all three disease groups compared to the control group ($P < 0.001$; data not shown). The frequencies of DQw1 and DQw3 were examined only in individuals who were DR3 (Table II). Compared to the reference control panel DQw1 was decreased in IDDM patients ($P < 0.01$) and increased in DH patients ($P < 0.05$). The frequency of DQw3 was markedly decreased ($P < 0.001$) in patients with CD and DH. By subtracting from 100 the DQw1 and DQw3 percentages in individuals who are DR3 DQw2, the frequency of DQw2 with a second DR antigen (predominantly DR7) was determined. This revealed that DQw2 occurred with a second DR antigen in 10% of controls compared with 17% of IDDM patients (P = NS), 28% of DH patients ($P < 0.05$) and 62% of CD patients ($P < 0.001$).

When the DQ association in DR7 individuals is analyzed, 9/24 RFLP controls are DQw3 associated compared to 0/8 IDDM, 2/25 CD, and 0/11 DH patients ($P < 0.05$ for all comparisons).

DQα polymorphism

Using the DQα genomic probe five DQα Taq I fragments sized 6.8, 6.2, 5.5, 4.6, and 2.6 kb were detected (Fig. 2) (Table III). In all subjects studied the 5.5-kb fragment is associated with DR4 and DR7, and 4.6 kb with DR3 and DR5 (irrespective of their different DQw associations). The 2.6-kb fragment was present in 22/23 DR1 individuals. One DR2, and two DRw6 individuals both without DR1 also had the 2.6-kb band. In all others, DR2 and DRw6 associated with either the 6.8 or the 6.2-kb fragments. Thus 18/21 (86%) DR2 controls and 10/14 (71%) DR2 DH patients (P = NS) have the 6.2-kb fragment as do 2/8 controls, 6/6 DRw6 IDDM ($P < 0.05$), 4/4 DRw6 DH, and 1/6 DRw6 CD patients.

DXα polymorphism

The DQα probe also hybridizes with two Taq I allelic DXα fragments sized 2.1 kb (U for upper) and 1.9 kb (L for lower) (Fig. 1) because of close nucleotide homology between the two loci (17). The frequencies of homozygotes of the U and L alleles and heterozygotes for both alleles, according to DR phenotypes are given for the different groups in Table IV.

Based on the incidence of individuals homozygous for U or L, and their DR type, in the control subjects the U allele tends to coinherit with DR2, DR3, DRw6, and the L allele with DR1, DR4, DR5, and DR7 (Table IV).

DXα in IDDM

In IDDM patients the frequency of UU is considerably increased and that of LL considerably decreased when compared with the control distribution (Table III) ($P < 0.001$). This reflects the DR3-U association obtained in controls as well as the high correlation of DR4 with the U allele in IDDM in contrast to the L allele in controls: of the 24 DR4 patients who are not DR3, 12 (50%) are UU and only 5 (21%) are LL; of the corresponding 30 controls, 3 (10%) are UU and 16 (53%) are LL ($P < 0.01$). By the same token, not withstanding the strong association of the U allele with all DR3 individuals, the distribution of the U and L alleles in DR3/4 IDDM patients is significantly different ($P < 0.05$) from that of DR3/4 controls (see Table IV).

DXα in DH

In DH an increased frequency of UU (45%) is found with a corresponding decrease of the LL genotype (8%) compared with 21 and 34%, respectively, in controls ($P < 0.001$) (Table III), which is partly explained by the increased prevalence of U associated DR3 in the patient group. There is no clearcut association between the U allele and other DR antigens.

DXα in CD

In CD patients 48% have the UU genotype and only 15% the LL genotype compared to 21 and 34%, respectively, in controls ($P < 0.001$) (Table III). This is partly due to the increase in the U associated DR3 as well as the nonsignificant increase in the U-DR7 association when compared to the controls (Table IV).

DRα polymorphism

Using a DRα cDNA probe with restriction enzyme Bgl II, three different fragments, 4.5 kb, 4.2 kb, and 3.8 kb, were identified (Fig. 3). A fourth band, 0.7 kb, always appeared together with the 3.8-kb band. No more than two of the three large fragments were found in each individual. Individuals who possess DR2, DR4, and DR5 always had the 4.5-kb fragment and DR1 individuals always had the 3.8-kb fragment. In contrast, individuals possessing DR3, DRw6, or DR7 were heterogeneous with respect to the DRα RFLP (Table V).

DR3 polymorphism. 90 percent of all DR3 individuals had the 4.2-kb fragment and the 4.5-kb fragment was present in the remainder (Table V). There was no significant difference in their distribution in the individual population groups.

DRw6 polymorphism. DRw6 (both DRw13 and DRw14) associated with either the 4.5- or 4.2-kb fragment.

DR7 polymorphism. Individuals with DR7 possessed either the 3.8 or 4.5-kb fragment. The 3.8-kb fragment was present in 6/14 (43%) controls compared with 2/17 (12%) patients with CD ($P < 0.05$), 0/7 with DH and 0/4 with IDDM. Although it was not possible to determine the DQ typings in some subjects,

Table II. Frequency Distribution of the Second DQ Antigen in Individuals Who Are Positive for DR3 and DQw2

DQ antigen specificity	Reference panel	RFLP controls	IDDM	DH	CD
DQw1	17/51 (31)*	13/37 (35)	7/48‡ (15)	23/43§ (53)	12/42 (29)
DQw3	30/51 (59)	17/37 (46)	33/48 (69)	7/43‖ (16)	4/42‖ (10)
DQw2 (calculated)	5/41 (10)	7/37 (19)	8/48 (17)	13/43 (30)	26/42‖ (62)

P values were calculated from 2 × 2 contingency tables by chi-square analysis for comparison of patients with HLA reference control panel.
* Percentages in parentheses. ‡ $P < 0.05$; § $P < 0.01$; ‖ $P < 0.001$.

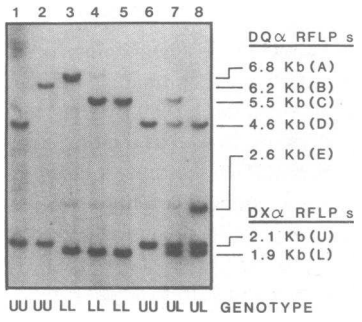

Figure 2. Autoradiogram of DQα and DXα restriction fragment length polymorphisms. DNA from 8 individuals (labeled 1–8) was digested with Taq 1 and studied by Southern blot hybridization methods using a genomic DQα gene probe. This probe also detects DXα genomic sequences because of the close nucleotide homology between the DQα and DXα genes (17).

DQw3 always occurred in those patients with the 3.8-kb fragment and not in those with the 4.5-kb fragment.

Preferential allelic associations

Based on the serologically defined class I and class II antigens, and RFLPs obtained with the Taq 1 restriction enzyme and the DQα probe and with Bgl II restriction enzyme and the DRα probe, the following HLA haplotype constructs could be derived (Table VI):

(a) (A1-B8) = DR3 = DQw2 = DQα-4.6 kb = DXα-2.1 kb = DRα-4.2 kb occurs in all four population groups and its increased frequency in DH, CD, and IDDM is concomitant with the increased association previously found with DR3 in these three conditions.

(b) (A3-B7) = DR2 = DQw1 = DQα-6.2 kb = DXα-2.1 kb = DRα-4.5 kb occurs frequently in the controls and patients with DH. The DQα 6.8-kb fragment occurs instead of the 6.2-kb fragment in ~ 25% of both groups.

(c) DR4 = DQw3 = DQα-5.5 kb = **DXα-1.9 kb** = DRα-4.5 kb was unequivocally assigned in 17/22 DR4 control individuals and 4/34 IDDM patients, whereas DR4 = DQw3 = DQα-5.5 kb = **DXα-2.1 kb** = DRα-4.5 kb was assigned to 5/22 controls and 30/34 IDDM patients.

(d) DRw6 = DQw1 = **DQα-6.8 kb** = DXα-2.1 kb = **DRα-4.5 kb** was found in 6/7 controls whereas DRw6 = DQw1 = **DQα-6.2 kb** = DXα-2.1 kb was found in 6/7 IDDM patients (the rogue fragment was DQα 2.6 kb) and 4/5 tested for **DRα** had the **4.2-kb** band. The five IDDM patients that were DR4/w6 had the same profiles DR4 = DQw3 = DQα-5.5 kb = DXα-2.1 kb = DRα-4.5 kb DRw6 = DQw1 = DQα-6.2 kb = DXα-2.1 kb. Four had the DRα-4.2-kb fragment as well.

(e) (B57) = DR7 = **DQw3** = DQα-5.5 kb = **DXα-1.9 kb** = **DRα-3.8 kb** profile was found in 6/14 controls and only 2/29 of all patients, whereas DR7 = **DQw2** = DQα-5.5 kb = **DXα-1.9/2.1 kb** = **DRα-4.5 kb** was found in all the other DR7 patients and controls.

Discussion

Results from this study suggest that some disease associated susceptibility genes previously attributed to the DR region are associated with genes within or centromeric to the DQ and DX MHC region.

The frequencies of the relevant serologically defined HLA-DR antigens in our RFLP control group are similar to those of the DR reference control group. The increased frequencies of DR3 and DR4 in IDDM, DR3, and DR7 in CD, and DR3 in DH are similar to other studies. In DH no difference was found in DR2 frequency compared with controls but the DR3/DR2 heterozygote and DQw1 were significantly increased. In a larger group of DH patients ($n = 70$) both DQw1 ($P < 0.05$) and DR2 ($P < 0.05$) were increased (unpublished observations). These data are consistent with the current view that in all three diseases at least one disease susceptibility gene is associated with one HLA haplotype and another with the second haplotype specifically DR3 and DR4 in IDDM, DR3 and DQw1/DR2 in DH, and DR3 and DR7 in CD. It is interesting to note that in CD the DQw2 antigen is possibly more important for susceptibility DR3 and/or DR7, confirming the findings of Tosi et al. (26). Thus in DH and CD the serological data is consistent with the view that at least for one haplotype the susceptibility gene resides closer to DQ than DR. The increase of DQw3 in IDDM patients (Table III) can be correlated with the increase of the DR4 antigen with which it is in linkage disequilibrium. It is not possible to allocate the DR4 susceptibility gene in IDDM to either the DR or DQ region using standard serological criteria alone.

RFLPs have been identified with DRα and DQα probes in the four different population groups of similar ethnic origin. DRα polymorphisms have been previously reported both at the protein (27) and the genomic level (28). The increased frequency of the DRα 4.2-kb fragment found in all three diseases reflected the increase of DR3. Heterogeneity of DR3 has also been defined by HLA-B serology. It was therefore of interest that the DRα RFLP did not preferentially associate with B8 which is DR3 associated in this British Caucasian population. Only three individuals were B18-DR3 of which two were DRα-4.2 and one

Table III. DXα, DQα, and DRα Polymorphisms in Controls, and Patients with IDDM, DH, and CD

		DXα			DQα						DRα		
Group		UU	LL	UL	6.8	6.2	5.5	4.6	2.6		4.5	4.2	3.8
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
Con	$n = 86$	21	34	45	13	27	66	42	26	$n = 53$	91	42	30
IDDM	$n = 79$	59	8	32*	3	13	81	68	18‡	$n = 50$	86	56	20 (NS)
DH	$n = 54$	45	8	47*	7	30	46	83	13‡	$n = 36$	83	78	8‡
CD	$n = 46$	48	15	37‡	8	9	59	93	2*	$n = 31$	81	84	6‡

P values were calculated from 3 × 2 and 5 × 2 contingency tables by chi-square analysis for comparison of patients with controls. * $P < 0.001$; ‡ $P < 0.01$.

Table IV. Distribution of DXα 2.1 kb (U) and 1.9 (L) kb Polymorphisms

DR antigen	Controls ($n = 86$)			IDDM ($n = 79$)			DH ($n = 53$)			CD ($n = 46$)		
	UU	LL	UL	UU	LL	UL	UU	LL	UL	UU	LL	UL
DR1	0	8	8	2	3	7	2	0	2	—	—	—
DR2	10	3	11	2	0	3	12	0	2	3	0	2
DR3	9	2	17	34	1	16*	22	0	20	14	4	11
DR4	2	16	17	36	6	15‡	3	2	10	0	0	1
DR5	0	4	3	0	0	3	0	1	0	0	0	0
DR6	5	0	2	7	0	0	2	0	3	5	0	1
DR7	3	10	12	2	1	5	1	2	8	6	6	11
DR3/2	4	—	2	1	—	1	12	—	2	3	—	3
DR3/4	2	1	5	24	1	7§	1	—	5			
DR3/7	2	—	4	1	—	5	1	2	8	5	3	11
DR3/X	—	—	1	3	—	—	4	—	—	4	—	—
DR4/X	—	1	1	4	1	—	2	—	1	—	—	—

X, no other antigen detected implying homozygosity. *P* values were calculated from 3 × 2 contingency tables by chi-square analysis for comparison of patients with controls. * $P < 0.01$; ‡ $P < 0.001$; § $P < 0.05$.

DRα4.5. We are unable to confirm the recently reported findings of Stetler et al. (28) who found 16% of 55 controls compared with 50% of 12 IDDM probands positive for the DRα 4.2-kb allele. They also studied 6 IDDM families, compared frequencies of the 4.2-kb allele in the probands and controls and demonstrated an increased relative risk associated with the 4.2 DRα allele compared to DR3 (11.8 vs. 5.5, respectively). They therefore concluded that the DRα polymorphism is a closer marker of IDDM than DR3 alone. This apparent discrepancy with our data might reflect either the increased numbers of IDDM subjects studied in our patient group (50 in our study versus 12 in Stetler's) or that Caucasian subjects in the United States and Britain have varying HLA-D region associations.

The DRα 3.8-kb fragment of the DR7 polymorphism was less frequent in patients with CD and DH compared with controls. This fragment is associated with the DR7-DQw3 haplotype which is also reduced, in favor of the DR7-DQw2 haplotype. The RFLP data based on the DRα polymorphism thus confirms the serological data aligning the disease susceptibility gene of this haplotype to the DQ region in CD patients.

Five different DQα related hybridization bands were detected as previously reported (18) not all of which correspond to DR-DQw serological associations. The increased frequency of the 4.6-kb fragment in all three patient groups is a reflection of its DR3 association determined from controls. The DQw heterogeneity found in DR2 individuals is similar in all four groups. In DRw6 individuals, the 6.8-kb fragment occurs more frequently in the control and the CD patient groups, whereas the 6.2-kb fragment occurs at a higher frequency in the IDDM and DH patient groups (although the numbers involved are too few to establish whether the differences are meaningful).

We have previously observed that the 2.1-kb fragment (U) of the DXα di-allelic Taq 1 polymorphism correlated with DR3 in population studies of control and IDDM patients and in family studies (18). We now find that the frequency of the UU genotype

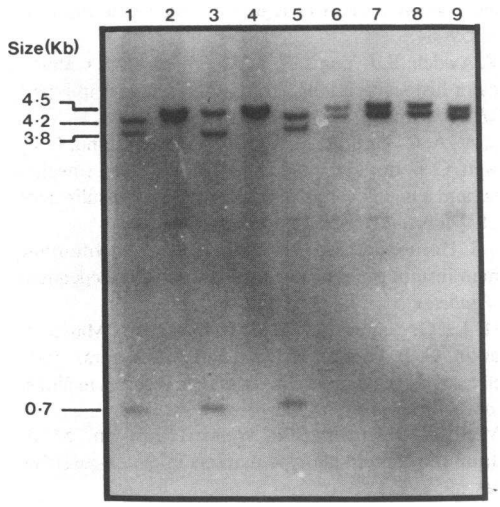

Figure 3. Autoradiogram of DRα restriction fragment length polymorphism. DNA from nine individuals (labeled 1–9) was digested with Bgl II and studied by Southern blot hybridization methods using a cDNA DRα probe.

Table V. RFLPs Associated with the DRα Probe

DR	RFLP	Control	IDDM	DH	CD
	kb				
DR3*	4.5	3 (13%)	4 (15%)	1 (3%)	3 (11%)
	4.2	20 (87%)	22 (85%)	28 (97%)	25 (90%)
DR7	4.5	8 (57%)	3	7	15‡ (88%)
	3.8	6 (43%)	0	0	2‡ (11%)

* Excludes DR3/DRw6 individuals in whom it was not possible to assign the 4.5/4.2 fragments. *P* values were calculated from 2 × 2 contingency tables by chi squares for comparison of patients with controls. ‡ $P < 0.05$.

Table VI. Preferential Allelic Associations and Disease Susceptibility

DXα	DQα	DQw	DR	DRα	B	At risk of disease association
2.1	6.2	1	2	4.5	7	None
2.1	6.8	1	2	4.5	—	None
2.1	4.6	2	3	4.2	8	IDDM, CD, DH
1.9	4.6	2	3	4.5	8	None
2.1	5.5	3	4	4.5	62	IDDM
1.9	5.5	3	4	4.5	44	RA(15)*
2.1	6.2	1	w6	4.5	—	IDDM
2.1	6.8	1	w6	4.5	—	CD
1.9	5.5	2	7	4.5	—	CD
1.9	5.5	3	7	3.8	57	None

* RA, rheumatoid arthritis.

in DH and CD as well as IDDM is significantly raised compared with controls (CD 56%, DH 50%, IDDM 57% vs. controls 21%; $P < 0.001$ for all comparisons).

We have previously suggested that no single gene product encoded by the MHC gene is responsible for the pathogenesis of IDDM but that several genes in linkage disequilibrium with each other can be implicated (15). We therefore correlated all the common DR antigens with either the U or L allele by studying individuals homozygous at the DXα locus. In all subjects the U allele was associated with DR3 and DRα 4.2 kb but failed to differentiate patients from controls. Thus the exact location within the HLA-D region of the DR3 related disease susceptibility gene cannot be determined from our investigations. The increased frequency of the UU genotype in IDDM can now be explained by an increased association of DR4 with the U allele in contrast to controls, as well as a consequence of the increased frequency of U-DR3 in this condition.

It has been suggested that the DQ and DX are neighboring loci on chromosome 6 (17). In addition to the IDDM DR4-DXαU association, we have also described an IDDM associated DQβ gene polymorphism that identifies a subset of DR4 (15). In a preliminary study we have examined the frequency of DXα genotypes in those subjects with the high risk DQβ polymorphism (designated omega [15] and characterized by Taq 1 fragments sized 2.0, 2.3, and 2.5 kb). Of 48 subjects who possessed the omega pattern, 39.5% were UU, 39.5% UL and 21% LL. As at least one-fifth of DR4 subjects who possess omega do not possess DXαU, this implies that there is either a recombinational hot spot between DQ and DX loci or that these loci are independent with respect to susceptibility to IDDM. The formation of MHC mixed isotypic dimers has been recently observed in the murine model by Germain and Quill (29) and has been hypothesised as a mechanism of disease susceptibility. One intriguing possibility therefore to explain this data is that in IDDM DXα and DQβ genes (or genes in linkage disequilibrium with one or both of them) code for products which form a dimer molecule of etiological significance in this disease.

The increased frequency of the UU genotype in CD and DH is more difficult to account for in terms of DR antigen associations (Table IV). Thus a possibility is that the second non-DR3 related U allele is the primary genetic determinant rather than DR2 or DR7 in DH and CD, respectively.

The serological and RFLP data point to the DR4, DR2, and DR7 related genes involved in the immunopathogenesis of IDDM, DH and CD, respectively, being located in the HLA-DQ (which includes the DX genes) rather than the DR MHC sub-region.

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