Oligonucleotide probes for HLA-DQA and DQB genes define susceptibility to Type 1 (insulin-dependent) diabetes mellitus

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Summary. We have typed 27 Caucasoid families for DNA restriction fragment length polymorphisms and specific sequences using HLA class II specific cDNA, genomic and oligonucleotide probes. DNA haplotypes were identified by restriction fragment length polymorphism analysis that correlated with previously serologically-defined extended major histocompatibility haplotypes. These DNA haplotypes sort into positive, neutral or negative associations with Type 1 (insulin-dependent) diabetes mellitus. The DNA susceptibility haplotypes are even more simply and specifically defined by oligonucleotide probes for sequences of DQA and DQB genes. Our oligonucleotide probes define variabilities in nucleotide sequences coding for amino acid residues

26, 37 and 38 in the DQB-chain. Probes defining DQA sequences are also important for defining susceptibility since certain DQA genes appear to modify DQB susceptibility by conferring resistance. Thus, major histocompatibility conferred susceptibility to diabetes cannot be adequately explained by an amino acid change at a single position in the DQB-chain. These probes allow the direct identification of major histocompatibility susceptibility genes in Type 1 diabetes without the necessity of determining full haplotypes.

Key words: Type 1 (insulin-dependent) diabetes, HLA, families, haplotypes, oligonucleotide probes.

Type 1 (insulin-dependent) diabetes mellitus, is characterised by selective destruction of pancreatic B cells, low or absent insulin secretion, and an absolute requirement for exogenous insulin. The mechanism(s) determining the loss of pancreatic B cells are not known; however, a number of immunologic abnormalities are associated with its clinical onset [1, 2]. Studies in the last decade have shown that a significant portion of the genetic susceptibility to Type 1 diabetes is provided by class II genes near the HLA-D(R) sub-region on chromosome 6. Population studies showed that 90–95% of Type 1 patients have HLA-DR3 or DR4, or both, and in family studies, sibling pairs affected with Type 1 diabetes tend to share both HLA haplotypes [3, 4]. Furthermore, specific extended haplotypes of the major histocompatibility complex (MHC) composed of HLA-A, B, C, DR and the complement proteins properdin factor B (Bf), C2, C4A, and C4B alleles have been defined that predispose or protect against the development of Type 1 diabetes [5]. Restriction fragment length polymorphism (RFLP) studies have shown that HLA-DQ region genes, in linkage disequilibrium with the DR genes, are also strongly associated with Type 1 diabetes [6–14]. Additionally, a recent study by Todd et al. [15], reports that amino acid residue 57 of the DQ β -chain specifies the autoimmune response in Type 1 diabetes.

We have used HLA class II specific cDNA, genomic and oligonucleotide probes to define specific DNA haplotypes that describe the susceptibility to Type 1 diabetes in a group of families previously typed for serologically-defined MHC extended haplotypes [5]. The DNA haplotypes thus identified can be independently sorted into positive, neutral or negative influences on the susceptibility to Type 1 diabetes.

Subjects and methods

Study population

We analysed 27 Caucasoid families previously typed for extended MHC haplotypes [5]. Twenty-three families had a single diabetic proband, while four had 2 diabetic patients each. Among the four multiplex families, 2 HLA-DR3/4 diabetic subjects, MHC identical to their diabetic probands, were not available for DNA analysis. In the remaining two multiplex families, the diabetic siblings shared either 1 or both MHC haplotypes respectively. Because the majority of our families contained a single diabetic subject, the distribution of haplotypes in the non-diabetic siblings in this study is essentially random, showing the expected 1:2:1 ratios (22.1%:48.5%:29.4%) of sharing 2, 1 or 0 haplotypes, respectively, with the diabetic probands. In addition, the patient population under study is a homogeneous, young Type 1 group of diabetic patients enrolled and followed in the Diabetes Control and Vascular Disease Study for the past 12 years [5, 16, 17]. All diabetic subjects had onset of diabetes before 15 years

Fig. 1. Dot blot analysis on amplified DNA using oligonucleotide probes. Amplified genomic DNA from individuals 1–8 were hybridised with 32 P-19 nucleotide probes $\alpha 1$, $\alpha 7$, $\alpha 3$, $\beta 3$, $\beta 4B$ and $\alpha 4$. Lane C was hybridised with a genomic probe containing DQB exon 2 sequences and is a positive control lane. The DNA haplotypes of the individuals are: 1, 3A/4B; 2, 3A/4B; 3, 3A/2A; 4, 4A/7A; 5, 4B/7C; 6, 3A/8A; 7, 3B/5A; and 8, 4A/10. Hybridisation with probes $\beta 1$ and $\beta 4A$ are not shown

of age, with the mean age of onset being 8 years. As of this writing, the non-diabetic siblings are all greater than 20 years of age except for 4 siblings ages 12–18. Because of the young age of onset of diabetes in our patients and the current age of the non-diabetic siblings, few new cases of similar age of onset or phenotype are possible.

Southern and dot blot analysis

Ten μ g DNA from each individual in the study was digested with the restriction endonuclease *TaqI* and the restriction endonuclease products analysed by electrophoresis on 1% agarose gels followed by Southern transfer to Zetabind nylon membranes [18]. 32 P-labelled genomic probes DRB [19–21] and DQB [18, 20, 21] and cDNA probe DQA [22] were used to detect homologous class II sequences on the filters. The DRB and DQB genomic probes were used instead of commonly used cDNA probes, because these genomic probes more specifically detect DRB and DQB (DXB) sequences respectively. The DQB probe is derived from a 2kb region that is downstream from exon 5 on the DQB gene [18]. The DRB probe is derived from exon 4 and adjacent intervening sequences of a DRB gene [19]. Hybridisation and washing of filters were as previously described [18].

For dot blot analysis using oligonucleotide probes, genomic DNA (1 μ g) was treated to 30 rounds of amplification essentially as described by Saiki et al. [23] except that the reaction volume was 50 μ l and contained primers for both DQA (5'-GGA TCC ATT GGT GGC AGC GGT AGA, 5'-GAA TTC TAT GTG GAC CTG GAGA) and DQB (5'-TGC TAC TTC ACC AAC GGG AC, 5'-GGT AGT TGT GTC TGC ACA CC). After denaturation at 95°C, samples were snap frozen in dry-ice ethanol, spun 1 s in the microfuge and equilibrated at 45°C for 2 min. Polymerisation reactions were run at 45°C for 2 min using 2 units of random prim-

ing grade DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA). Enzyme was added during each round of amplification. After 30 rounds of amplification, samples were stored at -20°C. DNA from 2 members of each family were amplified. One-tenth of the amplification mixture was denatured and applied to Zetabind membranes (AMF Corp, Meriden, CT, USA) using an apparatus and procedure provided by Schleicher and Schuell (Keene, NA, USA). Filters were baked for 2 h at 80°C, pretreated in 0.1 $\times$ SSC, 0.5% SDS for 1 h at 65°C and prehybridised for 3 or more hours. Prehybridisation and hybridisation buffer consisted of 900 mmol/l NaCl, 6 mmol/l EDTA, 90 mmol/l Tris pH 7.4, and 5 $\times$ Denhardt's. 19-mer oligonucleotides were labelled by standard procedures [24] using 32 P- γ -ATP to specific activities of approximately 1×10^9 cpm/ μ g. Approximately 1 ng probe was added per ml hybridisation solution and the filters were incubated at 45°C for 2 or more hours. Filters were washed, 3 times in 2 $\times$ SSC, 0.1% SDS at room temperature for 15 min each wash. A final wash was for 30 min in 6 $\times$ SSC, 0.1% SDS at 45–65°C ($\alpha 7$, 45°C; $\alpha 1$, $\alpha 3$, $\alpha 4$, $\beta 4B$, 55°C; $\beta 3$, $\beta 4A$, 60°C; $\beta 1$, 65°C). Filters were exposed to Kodak XAR-5 film for 8–36 h. The sequences of DQA oligonucleotide probes $\alpha 1$, $\alpha 3$, $\alpha 4$ and $\alpha 7$ were previously reported [23]. The sequences of the DQB probes are $\beta 1$ (5'-CGT GCG GGG TGT GAC CAGA), $\beta 3$ (5'-GAG AAG AGA TCG TGC GCTT), $\beta 4A$ (5'-GAG AGG AGT ACG CAC GCTT) and $\beta 4B$ (5'-CGT GCG TTA TGT GAC CAGA). The $\beta 1$ and $\beta 4B$ probes detect DQB sequences which encode amino acid residues 24–29 while $\beta 3$ and $\beta 4A$ detect DQB sequences which encodes amino acid residues residues 35–39.

Statistical analysis

The difference in frequency between control and test samples was estimated by the X^2 test. The level of significance was accepted to be $p < 0.05$.

Results

Ten μ g of genomic DNA from the parents ($n=54$), diabetic subjects ($n=29$) and non-diabetic siblings ($n=68$) were digested with the restriction endonuclease *TaqI*, and the restriction endonuclease products were analysed by Southern blot hybridisation using 32 P-labelled probes specific for DRB, DQB and DQA gene sequences (see Subjects and Methods). In addition, specific DQA and DQB sequences which encode amino-terminal hypervariable regions of these genes, were characterised using oligonucleotide probes (Fig. 1).

Twenty-one different haplotypes were deduced from the patterns of restriction fragment length polymorphisms (RFLPs) and oligonucleotide probe-detected sequences in the families (Table 1). For example, four DNA haplotypes were associated with HLA-DR4. All contained identical DRB polymorphisms but varied with respect to DQA and/or DQB RFLP or oligonucleotide probe detected polymorphisms.

Previously, serologically defined extended haplotypes composed of HLA class I (A, B, C), class II (DR) and class III (BF, C2, C4A and C4B) alleles were examined in these families [5]. For example, the pedigree shown in Figure 2 has two diabetic siblings, both of whom are heterozygous with haplotypes [HLA-A25,

Table 1. DNA haplotypes defined by restriction fragment length polymorphism and oligonucleotide probes

Haplotype	DR	DQA RFLP	(Oligo)	DQB RFLP	(Oligo)	DR RFLP ^a	Number of haplotypes ^b
1	1	2.6	($\alpha 1$)	2.3	($\beta 1$)	6.7,4.4	(11)
2A	2	6.5	($\alpha 1$)	2.3	($\beta -$) ^c	14,1.9,1.6	(6)
2B	2	6.5	($\alpha 1$)	2.3	($\beta -$)	1.9	(3)
2C	2	2.6	($\alpha 1$)	2.3	($\beta 1$)	4.4	(1)
2D	2	6.5	($\alpha 1$)	2.3	($\beta 4B$)	1.4,1.9,1.6	(1)
3A	3	4.6	($\alpha 3$)	5.0	($\beta 3$)	12,7.0,4.0	(20)
3B	3	4.6	($\alpha 3$)	5.0	($\beta 3$)	14,7.0,4.0	(8)
3C/6A	3/w6	6.5	($\alpha 1$)	2.3	($\beta -$)	12,7.0,4.0	(6)
4A	4	5.5	($\alpha 4$)	2.0	($\beta 4A$)	16,6.5,5.5,3.0	(21)
4B	4	5.5	($\alpha 4$)	5.0	($\beta 4A, \beta 4B$)	16,6.5,5.6,3.0	(6)
4C	4	6.5	($\alpha 1$)	2.3	($\beta -$)	16,6.5,5.5,3.0	(1)
4D	4	5.5	($\alpha 4$)	5.0	($\beta 3$)	16,6.5,5.5,3.0	(1)
5A	5	4.6	($\alpha 3$)	5.0	($\beta 4A, \beta 4B$)	14,6.7,4.0	(10)
5B	5	4.6	($\alpha 3$)	7.0	($\beta 3$)	14,6.7,4.0	(1)
6B	w6	2.6	($\alpha 1$)	2.3	($\beta -$)	14,7.0,4.0	(1)
7A	7	5.5	($\alpha 7$)	7.0	($\beta 3$)	16,7.2,2.9	(2)
7B	7	5.5	($\alpha 7$)	7.0	($\beta 3$)	16,2.9	(3)
7C	7	5.5	($\alpha 7$)	2.6	($\beta 4A$)	16,2.9	(1)
8A	w8	6.5	($\alpha 3$)	2.0	($\beta -$)	10	(3)
8B	w8	6.5	($\alpha 1$)	2.3	($\beta 1$)	10	(1)
10	w10	2.6	($\alpha 1$)	2.3	($\beta 1$)	4.4	(1)

^a Taq 1 restriction fragment length polymorphisms, in kilobases; ^b Haplotypes found in the parents ($54 \times 2 = 108$); ^c ($\beta -$) no hybridisation with $\beta 1, \beta 3, \beta 4A$ or $\beta 4B$

Fig. 2. Pedigree of a family showing serologically and DNA defined extended haplotypes. 2A, 3A, 4A and 4C represent DNA haplotypes (see Table 1). Squares ($\square$) = males. Circles ($\circ$) = females. Type 1 subjects are shaded. ND = Not determined

B18, DR4, SC31] and [HLA-A3, B8, DR3, SC01]. SC31 and SC01 are class III alleles and are given in the order of BF, C2, C4A and C4B [5]. These two DR4 and DR3 associated serologically defined haplotypes type as DNA haplotypes 4A and 3A, respectively

(Fig. 2, Table 1). Neither the DR3 SC02-3C nor the DR2 SC31-2A haplotypes were present in Type 1 subjects in this family (Fig. 2) or any other Type 1 subjects in other families in this study.

Table 2 shows DNA haplotypes 3A, 3B, 4A, 4B

Table 2. DNA haplotypes defined serologically

DNA haplotype 3A	DNA haplotype 3B	DNA haplotype 4A	DNA haplotype 4B	DNA haplotype 2A
B8 DR3 SC01 ^a (15) ^c	B18 DR3 F1C30 ^a (5)	B15 DR4 SC33 ^a (3)	B44 DR4 SC30 ^b (3)	B7 DR2 SC31 ^b (3)
B8 DR3 SC31 (2)	B14 DR3 F1C30 (1)	B7 DR4 SC31 (4)	B8 DR4 SC01 (1)	B37 DR2 SC31 (1)
B38 DR3 SC01 (1)	B49 DR3 F1C30 (1)	B35 DR4 SC31 (1)	B7 DR4 ND (1)	B51 DR2 SC31 (1)
B8 DR3 FC01 (1)	B35 DR3 SC31 (1)	B18 DR4 SC31 (1)	B60 DR4 ND (1)	B18 DR2 SC42 (1)
B37 DR3 ND (1)		B44 DR4 SC31 (1)		
		B51 DR4 SC31 (1)		
		B60 DR4 SC31 (1)		
		B39 DR4 SC30 (1)		
		B15 DR4 SB42 (1)		
		BX DR4 ND (7)		

ND: Not Determined; BX = B7, 27, 35, 44, 60 or 62; ^aSerologically defined haplotypes associated with Type 1 diabetes [5]; ^bSerologically defined haplotypes protective for Type 1 diabetes [5]; ^c(*n*) = number of parental haplotypes

Table 3. DNA haplotypes frequencies in Type 1 diabetic subjects and non-diabetic siblings

DNA haplotype	Oligonucleotide sequences	Diabetic subjects haplotypes # (%)	Non-diabetic siblings haplotypes # (%)	Statistics χ^2 , 1df (<i>p</i>)
4A	$\alpha 4/\beta 4A$	19 (32.8)	23 (16.9)	6.02 ($p < 0.05$)
4A,4B,4C,4D	$\alpha 1, \alpha 4/\beta 3, \beta 4A, \beta 4B$	21 (36.2)	34 (25.0)	2.51 (NS) ^a
4B,2D,5A	$\alpha 1, \alpha 3, \alpha 4/\beta 4A, \beta 4B$	1 (1.7)	20 (14.7)	7.10 ($p < 0.01$)
2A,2B,3C	$\alpha 1/\beta (-)$	2 (3.4)	28 (20.6)	9.14 ($p < 0.005$)
4C,6A,6B				
3A,3B,5B	$\alpha 3/\beta 3$	22 (37.9)	35 (25.7)	2.96 (NS) ^a
1,2C,8B,10	$\alpha 1/\beta 1$	9 (15.5)	13 (9.6)	1.44 (NS) ^a
7A,7B,7C	$\alpha 7/\beta 3, \beta 4A$	2 (3.4)	14 (10.3)	3.50 (NS) ^a

^a NS = non-significant ($p > 0.05$)

and 2A and their association with serologically-defined extended MHC haplotypes. DNA haplotypes 3A, 3B, 4B and 2A are predominantly found with serologically-defined haplotypes [B8, DR3, SC01], [B18, DR3, F1C30], [B44, DR4, SC30] and [B7, DR2, SC31] respectively (Table 2). The two DR3 haplotypes are positively associated with Type 1 susceptibility, while the [B44, DR4, SC30] and [B7, DR2, SC31] haplotypes are negatively associated with Type 1 susceptibility [5]. DNA haplotype 4A was found with a number of different serologically-defined haplotypes including [B15, DR4, SC33] (Table 2), which was previously found to be positively associated with Type 1 susceptibility [5].

The HLA associations were further analysed by comparing the frequencies of DNA haplotypes defined by RFLP and oligonucleotide probe analysis, in the diabetic subjects and their non-diabetic siblings (Table 3). 4A DNA haplotypes are 32.8% of all haplotypes of the diabetic subjects but only 16.9% of the haplotypes in the non-diabetic siblings ($p < 0.05$, Table 3). By comparison, the frequency of all serologically defined HLA-DR4 haplotypes (represented by DNA haplotypes 4A+4B+4C+4D), is not statistically different in the Type 1 subjects and the non-diabetic siblings (Table 3). The 4B and 4C DNA haplotypes were not present in the Type 1 subjects.

The 4B as well as 2D and 5A DQB genes encode amino acid sequences containing a unique tyrosine at position 26 [15] and these are detected with the $\beta 4B$

oligonucleotide probe (Fig.1). The haplotypes detected with this probe are found in 14.7% of the non-diabetic siblings but only 1.7% of the diabetic subjects ($p < 0.01$, Table 3). Similarly, DNA haplotypes 2A, 2B, 6A, 6B, 3C and 4C are detected with the $\alpha 1$ probe but none of our β probes. These oligonucleotide probes ($\beta 1$, $\beta 3$, $\beta 4A$ or $\beta 4B$) detect hypervariable sequences of the DQB gene encoding, in part residues 26 or 37 and 38. The frequency of DNA haplotypes containing the $\alpha 1/\beta (-)$ hybridisation phenotype are negatively associated with Type 1 susceptibility ($p < 0.005$, Table 3).

The comparison of haplotype frequencies in the diabetic subjects and the non-diabetic siblings does not directly measure the combined effect of both parental haplotypes on Type 1 susceptibility. For example, in haploidentical siblings sharing the DNA haplotype 4A, the non-shared haplotype would likely be critical in defining Type 1 susceptibility. In Table 4, we examine the contribution of both parental haplotypes and find that 62.1% of our diabetic subjects ($n=18$) have DQA and DQB genes that are found on DNA haplotypes 3A, 3B, 5A, 4A, 4D and 8A and are detected with oligonucleotide probes $\alpha 4$, $\beta 4A$ and/or $\alpha 3$, $\beta 3$ only. In contrast only 17.6% of the non-diabetic siblings ($n=12$) have these combinations of sequences ($p < 0.0001$). We therefore conclude that the genes associated with these DQA and DQB sequences are positively associated with Type 1 diabetes.

Table 4. Combination of parental DNA haplotypes in Type 1 diabetic Subjects and non-diabetic siblings

	DNA haplotypes A/A ^a # (%)	DNA haplotypes A/B ^b # (%)	DNA haplotypes C/A,B,C ^c # (%)
Type 1 diabetic subjects	18 (62.1)	6 (20.7)	5 (17.2)
Non-diabetic siblings	12 (17.6)	7 (10.3)	49 (72.1)
Haploidentical siblings ^d	7 (21.2)	1 (3.0)	25 (75.8)

^a Frequency of individuals having haplotypes defined by group A only; ^b Frequency of individuals having haplotypes defined by groups A and B; ^c Frequency of individuals having haplotypes defined by groups C and A, B or C; ^d Haploidentical siblings share 1 haplotype with their diabetic siblings.

Group A DNA Haplotypes: 3A,3B,5B,4A,4D,8A

Group B DNA Haplotypes: 1,2C,8B,10

Group C DNA Haplotypes: 2A,2B,2D,3C,4B,4C

5A,6A,6B,7A,7B,7C

[α3, β3, α4, or β4A]

[β1]

[α7, β4B or α1]

positive Type 1 susceptibility

neutral Type 1 susceptibility

negative Type 1 susceptibility

Statistics: χ^2 ldf A/A + A/B versus C/A,B or C.

Type 1 vs non-diabetic siblings $\chi^2 = 24.86$ ($p < 0.0001$)

Type 1 vs haploidentical siblings $\chi^2 = 21.16$ ($p < 0.0001$)

Additionally, 20.7% of the diabetic subjects have a single positively associated DNA haplotype and another composed of a DNA haplotype detected with the β1 oligonucleotide probe (1, 2C, 8B or 10) (Table 4). HLA-DR1 and w8 have previously been shown to be neutral with respect to diabetes susceptibility [25] and we therefore suggest that the β1 probe is detecting DNA haplotypes neutrally associated with Type 1 diabetes. In total, 82.8% of the diabetic subjects have DNA haplotypes we indicate as positively/positively or positively/neutrally associated with Type 1 versus only 27.7% of the non-diabetic siblings ($p < 0.0001$, Table 4). Furthermore, in the 33 non-diabetic siblings haploidentical with their diabetic proband, only 24.2% have DNA haplotypes we define as positive/positive or positive/neutral (Table 4).

In contrast, 72.1% of the non-diabetic siblings and 75.8% of the haploidentical non-diabetic siblings, but only 17.2% of the Type 1 subjects have DNA haplotypes 2A, 2B, 2D, 3C, 4B, 4C, 5A, 6A, 6B, 7A, 7B and 7C which are detected with oligonucleotide probes α1, α7 or β4B ($p < 0.0001$, Table 4). Furthermore, 22.1% of non-diabetic siblings and none of the Type 1 subjects have two of these haplotypes. Thus these DNA haplotypes appear to be negatively associated with Type 1 susceptibility.

The interaction of specific DQA and DQB genes seems to be critical in the determination of Type 1 susceptibility. For example, DNA haplotypes containing DQB genes encoding the β3 specificity in combination with DQA genes encoding α3 or α4 (Table 1, 3A, 3B, 4D and 5B) are associated with Type 1 diabetes (Table 4). However, the DNA haplotypes 7A and 7B containing DQA and DQB genes having the β3 and α7 specificities (Table 1) are negatively associated with Type 1 susceptibility (Table 4). The DQA gene containing the unique α7 specificity, thus appears to be acting in a dominant fashion over DQB genes having β3. Similarly, DQA and DQB genes present on DNA haplotypes 4A and 4B, share oligonucleotide speci-

ficities α4 and β4A, but differ with respect to sequences detected with the β4B probe (Table 1). The β4B detected specificity, thus appears to be acting in a dominant fashion, and thereby provide the negative association of the 4B DNA haplotype and Type 1 susceptibility.

Discussion

These family studies demonstrate the importance of both DQA and DQB genes for susceptibility to Type 1 diabetes mellitus. Our data show that amino acid residues in addition to residue 57 of the DQβ-chain [15] are probably important for diabetes susceptibility. For example, our β4B and β1 probes recognise sequences that code for ²⁶Tyr and ²⁶Gly and are negatively and neutrally associated with diabetes susceptibility respectively. In addition, the β3 probe which specifies amino acid residues that include ³⁷Ile and ³⁸Val detect sequences conferring positive diabetes susceptibility. In a recent model of class II molecular structure, the β-chain residues 26, 37 and 38 are located on the floor of the antigen binding site, while residue 57 is located at the cleft of the site [26]. The 57 versus 26, 37 and 38 polymorphic residues may therefore control interaction with antigen by completely different mechanisms. Clearly, no single amino acid residue in the β-chain can per se determine diabetes susceptibility. While other nucleotide substitutions in the DQB gene may yet be shown to affect diabetes susceptibility, the probes we have defined are sufficient to characterise the majority of Type 1 diabetic patients (~95%).

Our data also suggest that the DQA genes play an active role in diabetes susceptibility. The MHC haplotypes containing HLA-DR7 were previously shown to be negatively associated with diabetes in numerous studies. However, HLA-DR7 DNA haplotypes in this study are associated with two different types of DQB

sequences (Table 1), both of which are independently associated with positive diabetes susceptibility (Table 4). In the instances where these positive DQB susceptibility sequences are present with the unique DQA sequence found on DR7 haplotypes, there is a negative association with diabetes susceptibility. This suggests that the DQA gene is acting in a dominant fashion over the positive susceptibility conferred by the DQB genes.

The specific interaction of DQA and DQB genes thus seems to be of critical importance in Type 1 diabetes. The well documented excess of HLA-DR3/4 heterozygotes [3, 4], may be a result of transallelic complementation of the DQA and DQB loci. For example, DQ α molecules encoded by DQA genes detected by the α 4 probe are associated with DQ β molecules encoded by DQB genes detected by the β 3 probe. Normally the products of these genes are encoded on separate haplotypes. However, on DNA haplotype 4D (Table 1), a chance genetic recombination seems to have placed both the α 4- and β 3-detected specificities on the same chromosome in a diabetic patient.

Nineteen non-diabetic siblings have DQA and DQB genes on both haplotypes that are strongly associated with Type 1 susceptibility, but do not have the disease. Because of the young age of onset of diabetes in our patients and the current age of the non-diabetic siblings, few new cases with a similar age of onset or phenotype are possible. Therefore, absence of diabetes in our 19 non-diabetic siblings with the high risk DQA and DQB genes cannot be explained solely by the susceptibility haplotypes in the MHC region. Studies in identical twins, with a broader age delimitation of Type 1 diagnoses, indicate that the concordance rate (genetic contribution) is approximately 50% [27], although in those twin pairs having a younger age of onset, the concordance rate of diabetes is much higher [28]. Taken together, these findings suggest the necessity of non-MHC genes being involved in Type 1 diabetes mellitus. Since these MHC susceptibility haplotypes (and presumably the same DQA and DQB genes) are involved in some other autoimmune diseases [29, 30], the additional genes must serve to target the specific organs.

This knowledge of the DQA and DQB genes predisposing the population to Type 1 diabetes makes it possible to identify subjects at risk (15–20% of the Caucasoid population), without the need to do family haplotype studies. Furthermore, identification of these MHC susceptibility genes will facilitate the search for the other genes and/or environmental factors that interact with the MHC components to precipitate and perpetuate the disease.

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