Implication of Specific DQB1 Alleles in Genetic Susceptibility and Resistance by Identification of IDDM Siblings With Novel HLA-DQB1 Allele and Unusual DR2 and DR1 Haplotypes

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Genetic susceptibility to insulin-dependent diabetes mellitus (IDDM) is associated with the HLA-DR3 and DR4 haplotypes. The HLA-DR2 haplotype is negatively associated with IDDM, an association that has been interpreted as dominant protection. Here, we describe the molecular analysis of the HLA class II genes in an unusual family with three HLA-DR1/2 siblings, all of whom have IDDM. With polymerase chain reaction amplification and sequence analysis to characterize the class II alleles, we identified a novel DQB1 allele on the DR1 haplotype and an unusual DQB1 allele on the DR2 haplotype. However, the DRB1 alleles on these DR1 and DR2 haplotypes are the conventional alleles (*0101 and *1501, respectively). These results suggest that it is the conventional DQB1 allele (*0602) not the DRB1 allele (*1501) on the protective DR2 haplotype that confers protection in the general population and, furthermore, that these unusual DQB1 alleles may confer susceptibility to IDDM in this family. The unusual DQB1 allele on this DR2 haplotype encodes Asp at position 57, indicating that it is the allele DQB1*0602 and not simply the presence of this residue that is responsible for the protective effect. *Diabetes* 40:478–81, 1991

Insulin-dependent diabetes mellitus (IDDM) is a chronic autoimmune disease in which the regulation of glucose metabolism is dysfunctional due to the immunological destruction of the pancreatic islet β-cells, which produce insulin (1). IDDM and many other autoimmune diseases have been associated with serologically defined alleles of the HLA class II antigens (2,3). In population studies, the serological types HLA-DR3 and DR4 are positively associated and DR2 negatively associated with IDDM (2,3). In addition, the analysis of patterns of HLA haplotype sharing among affected sibling pairs (4) and disease and HLA linkage in families (2,3) has implicated loci in the HLA region on chromosome 6 in IDDM susceptibility. To date, the unusual DR2 haplotypes identified in rare $DR2^+$ IDDM patients that have been sequenced differ at both the HLA-DRB1 and DQB1 loci from the common DR2 haplotypes that confer resistance to diabetes (5–7). Thus, the specific HLA class II genetic locus conferring resistance to diabetes could not be ascertained.

We describe a highly unusual family in which two HLA-identical siblings (DR1/2) had IDDM (8) and a third (also DR1/2) later developed IDDM (Fig. 1). In this family, the three siblings who shared the maternal DR1 and the paternal DR2 haplotype all have IDDM and islet cell antibodies (ICAs). This striking pattern suggests that these two haplotypes may predispose to IDDM. To identify which genes may be candidates for susceptibility alleles in this family, we determined the sequence of the polymorphic second exon of the DQB1, DRB1, and DRB5 loci on these two predisposing haplotypes.

These studies reveal, on the DR2 haplotype, the conventional DR2, DRB1, and DRB5 alleles but an unusual DQB1 allele, DQB1*0402, usually found only on other haplotypes. On the DR1 haplotype, the conventional DR1 DRB1 allele (*0101) was found coupled with a novel DQB1 allele. These results indicate that DRB1 alleles of these haplotypes were not associated with diabetes resistance or susceptibility and suggest that, in this family, it is the unusual DQB1 alleles on these IDDM-associated haplotypes that confer the increased susceptibility to IDDM.

RESEARCH DESIGN AND METHODS

The family studied has three HLA-identical siblings with IDDM and three unaffected siblings. The three diabetic children developed diabetes at ages 11 (1974), 17 (1983), and 29 (1988) yr. When studied in 1985, neither of the two diabetic siblings had detectable C-peptide secretion (basal and post-Sustacal stimulation), and on initial evaluation of this

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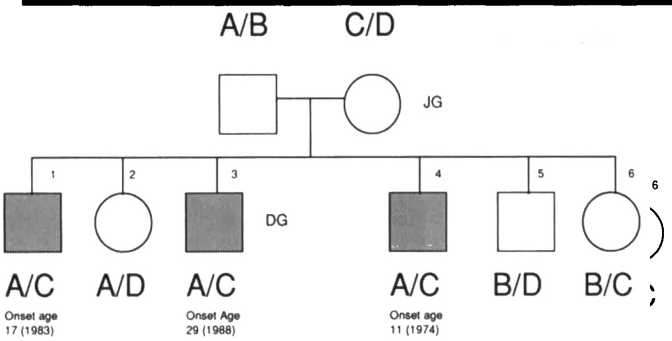

FIG. 1. Pedigree of reference family (serological typing recorded in ref. 8)

	Haplotypes	
Serology	**DNA typing**	
A: A3, B7, DR2	**DQA1*0401, DQB1*0402**	**DRB1*1501**
B: A11, Bw35, DR4	**DQA1*0301, DQB1*0301**	**DRB1*0402**
C: A26, Bw49, DR1	**DQA1*0102, DQB1*05 variant (new)**	**DRB1*0101**
D: A25, B18, DR2	**DQA1*0102, DQB1*0602**	**DRB1*1501**

Ages of onset for all diabetic siblings and initials of diabetic patient (DG) and nondiabetic mother (JG) are shown in pedigree. DQB1 allele on haplotype C is new allele.

family, a strongly ICA$^+$ sibling was identified. This sibling was sequentially evaluated from 1983 until 1988, when he also developed overt diabetes. During the prodromal phase of diabetes, he was continuously cytoplasmic ICA$^+$ and insulin-autoantibody negative and had markedly decreased first-phase insulin secretion. After development of overt diabetes, he has remained ICA$^+$ and, in response to insulin therapy, has developed insulin antibodies. At the time of initial screening, the mother in the family was also found to be and has continued to be cytoplasmic ICA$^+$ and insulin-autoantibody negative. Her first-phase insulin secretion, HbA_{1c}, and glucose on oral glucose and intravenous glucose tolerance testing have remained normal.

Samples of genomic DNA for the diabetic proband and his parents were obtained. Polymerase chain reaction (PCR) amplification (9–11) and oligonucleotide typing for the HLA-DQA1 locus were performed as described previously (12,13). Similarly, DQB1 amplification was carried out with the primers

DB130: 5′ [*Bam*HI]agggatccCCGCAGAGGATTTCGTGTACC 3′
DB131: 5′ [*Pst* I]tcctgcagGGCGACGACGCTCACCTCCCC 3′

and for the DRB loci with primers

GH46: 5′ [*Bam*HI]ccggatccTTCGTGTCCCCACAGCACG 3′
GH50: 5′ [internal *Pst* I]CTCCCCAACCCCGTAGTTGTGTctgca 3′

(see ref. 12). Lowercase letters include sequences that generate a restriction enzyme site in the double-stranded PCR product. One hundred–microliter reactions contained standard PCR salts, 1 mM deoxynucleotides, 1 μg genomic DNA, and 2.5 U *Taq* polymerase (Perkin-Elmer/Cetus, Amplitaq, Norwalk, CT). PCR was performed on a Perkin-Elmer/Cetus thermal cycler with 35 cycles of 95°C denaturation (1 min), 55°C annealing (30 s), and 72°C extension (45 s). PCR product was subsequently digested with *Bam*HI and *Pst* I restriction endonucleases (Boehringer-Mannheim, Mannheim, Germany), purified by extraction with phenol-chloroform and Centricon 30 (Amicon) dialysis, and ligated into M13mp18 with T4 DNA ligase (BRL, Bethesda, MD). *Escherichia coli* DG98 was transformed with these recombinants, and the resulting plaques were probed with nick-translated cDNA probes for the appropriate locus. Positive plaques were used to infect a plate of DG98, and confluent plates were extracted the next morning by gentle shaking with Luria broth (5 ml). Template DNA was prepared for each clone of interest. Multiple PCR reactions, clonings, and sequencing reactions were done to confirm the new sequence and to ensure that a potential misincorporation had not occurred or that misprimed shuffling capable of generating an artifactual hybrid sequence was not involved (11). DNA sequencing was carried out with the dideoxy-chain–termination process (14). DQB1 DNA sequences were confirmed by oligonucleotide typing (D. Bugawan, unpublished observations).

RESULTS

DNA samples from patient D.G. (DR1/2; haplotypes c and a; see Fig. 1) and the mother (DR1/2; haplotypes c and d) were analyzed by PCR amplification of the DQB1 and the DRB loci; the amplified DNA was characterized by M13 cloning, chain-termination sequencing (14), and dot-blot hybridization with sequence-specific oligonucleotide probes (12,13). The allelic sequences are shown in Fig. 2 and their assignment to the haplotypes segregating in this family in Fig. 1. The DR2 haplotype in patient D.G. contained a conventional DRB1 allele (Dw2 or *1501) common to most white DR2 haplotypes (15,16). This haplotype typically contains

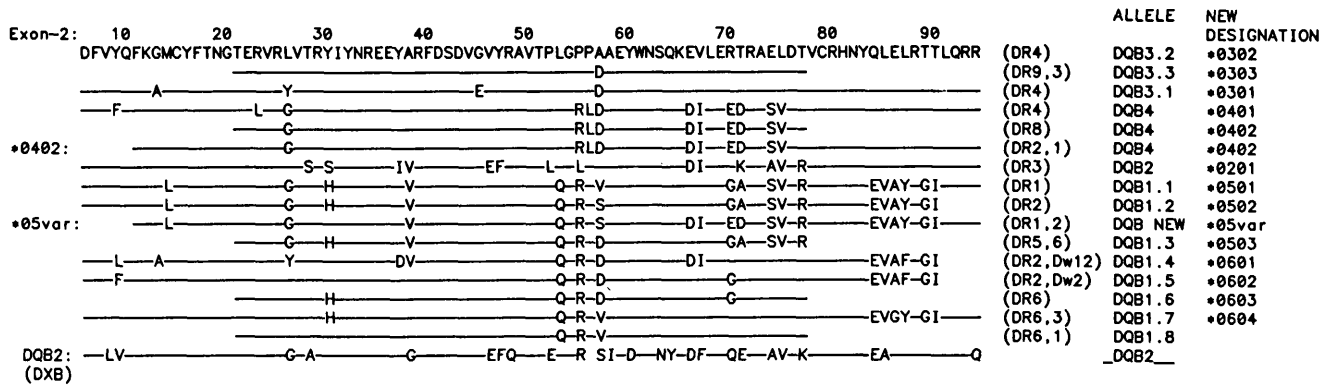

FIG. 2. Alignment of protein sequences of HLA-DQB1 alleles. Sequences were translated into standard 1-letter amino acid code and aligned to DQB1*0302 allele. Allele designations are our local nomenclature (e.g., 3.2) used in previous publications, whereas those on *right* are official World Health Organization HLA nomenclature (e.g., 0302). *Asterisks on left*, *0402 allele found on unusual DR2 haplotype and new DQB1 allele, *05 variant, found on DR1 haplotype in this family.

the DQA1 allele 1.2 or *0102 and the DQB1 allele 1.5 or *0602 as does the maternal DR2 haplotype (haplotype d). However, in this family, the paternal DR2 haplotype (haplotype a) contained the DQA1 allele 4.2 or *0401 and the DQB1 allele 4.2 or *0402. This combination of DQA1 and DQB1 alleles is usually found only on DR8 haplotypes in whites and is rare (<4%). The DRB1 allele of the DR1 haplotype also contains the conventional DR1 sequence *0101; DR1 haplotypes typically contain the DQA1 allele *0101 and the DQB1 allele *0501. However, this DR1 haplotype contained the DQA1 allele *0102 and a novel previously unreported DQB1 allele termed provisionally *DQB1*05 variant*, which appears to be a recombinant between the *0502 and the *0402 DQB1 alleles (Fig. 2).

TABLE 1
DQB1 alleles on 6 DR2 haplotypes in 4 unrelated DR2+ insulin-dependent diabetes mellitus patients

Haplotypes (*n*)	Allele	Codon 57 amino acid	DR type
2	DQB1*0602	Asp	DR2/DR-
1	DQB1*0502	Ser	DR2/DR3
2	DQB1*0603	Asp	DR2/DR-
1	DQB1*0402	Asp	DR2/DR1

DISCUSSION

In this family, all three siblings who inherited the unusual maternal DR1 (haplotype c) and unusual paternal DR2 (haplotype a) haplotypes have IDDM. This observation suggests that the combination of these two haplotypes is responsible for the high penetrance of diabetes seen in this family. This interpretation is consistent with the notion based on family-segregation analysis that the highest IDDM susceptibility is associated with the presence of two predisposing haplotypes (2–4). Note that the mother (55 yr of age), who contains the unusual DR1 haplotype along with the conventional DR2 haplotype, has ICAs but does not have IDDM and has maintained normal first-phase insulin secretion on prospective evaluation.

The DR2,Dw2 haplotype is strongly negatively associated with IDDM and therefore has been considered protective (2,3), whereas the DR1 haplotype is weakly associated with IDDM (17). Given the strong linkage disequilibrium between the DQ and DR regions of HLA class II haplotypes, it has been difficult to attribute these haplotype disease associations to specific class II alleles. The analysis of highly predisposing haplotypes with an unusual combination of DQB1 and DRB1 alleles, like those observed in this family, allows the tentative identification of predisposing alleles. On the DR2 haplotype, the DQB1*0402 allele is implicated in susceptibility because the DRB1 allele (*1501) is the same as on the protective DR2,Dw2 haplotype. Thus, the conventional DQB1 allele *0602 on DR2,Dw2 haplotypes is probably responsible for the protection associated with this haplotype. The DQB1 allele found on this unusual DR2 haplotype is identical to the DQB1*0402 allele found on white DR8 haplotypes. DR8 is weakly associated with IDDM (17). A similar DQB1 allele, *0401, is also found on Japanese DR4 haplotypes, which are positively associated with IDDM (18), but in this population, it is coupled with the DQA1*0301 allele.

Studies of class II sequence polymorphism and IDDM have revealed a general correlation with the charge of the amino acid residue at position 57 of the DQ β-chain (5–7,19). One study reported that the presence of Asp at position 57 was per se protective (19). Because the DQB1*0402 allele found on this DR2 haplotype has Asp at position 57, the observations reported here are clearly inconsistent with the notion that Asp-57 is protective. In fact, of the six DR2 haplotypes found in the four DR2+ IDDM patients we studied, five contained Asp-57 (7; Table 1). However, only two had the DQB1*0602 allele, expected in >90% of white DR2 haplotypes. Thus, the entire allele DQB1*0602 may confer resistance rather than a single residue at position 57 of the DQ β-chain.

The new DQB1 allele found on the unusual DR1 haplotype may also be responsible for conferring IDDM susceptibility, because the DRB1 allele (*0101) observed in the patient is the one typically found on control DR1 haplotypes. This novel DQB1 allele has Ser at position 57 like the rare DQB1 allele (*0502) we previously found in a DR2+ IDDM patient (6,7). The new allele appears to be a complex recombinant of the *0502 and *0402 alleles; this sequence may have been created by a gene conversion inserting a DQB1*0402 segment into the DQB1*0502 allelic framework, with the sites of a putative recombination somewhere between codons 58 and 74. According to the structural model for HLA class II molecules, a peptide-binding groove is formed by a β-pleated sheet and two α-helices (20). Thus, in this new allele, a segment encoding part of the α-helix is common to *0402, whereas the rest of the DQ β-chain is similar to the DQB1*0502 allele. The presence of these two unusual haplotypes in all three IDDM siblings indicates that they may both confer susceptibility. Although the contribution of unknown loci on one or both of these haplotypes remains a formal possibility, the sequence analysis of DRB1 and DQB1 alleles strongly suggests that the DQB1 locus on both of these predisposing haplotypes confers genetic susceptibility to IDDM.

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REFERENCES

1. Eisenbarth GS: Type I diabetes mellitus, a chronic autoimmune disease. *N Engl J Med* 314:1360–68, 1986
2. Svejgaard A, Platz P, Ryder LP: HLA and disease 1982: a survey. *Immunol Rev* 70:193–218, 1983
3. Tiwari JL, Terasaki PI: *HLA and Disease Associations*. New York, Springer-Verlag, 1985
4. Thompson G, Bodmer WF: The genetic analysis of HLA and disease association. In *HLA and Disease*. Dausset J, Svejgaard A, Eds. Copenhagen, Munksgaard, 1977, p. 84–93
5. Todd JA, Bell JI, McDevitt HO: HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
6. Horn GT, Bugawan TL, Long C, Erlich HA: Allelic sequence variation of the HLA-DQ loci: relationship to serology and insulin-dependent diabetes susceptibility. *Proc Natl Acad Sci USA* 85:6012–16, 1988
7. Erlich HA, Bugawan TL, Scharf S, Nepom GT, Tait B, Griffith RL: HLA-DQβ sequence polymorphism and genetic susceptibility to IDDM. *Diabetes* 39:96–103, 1990
8. Eisenbarth GS, Srikanta S, Fleischnick E, Ganda OP, Jackson RA, Brink SJ, Soeldner JS, Yunis EJ, Alper C: Progressive autoimmune beta cell insufficiency: occurrence in the absence of high risk HLA alleles DR3,

DR4. *Diabetes Care* 8:477–80, 1985
9. Mullis K, Faloona F: Specific synthesis of DNA in vitro via a polymerase catalysed chain reaction. *Methods Enzymol* 155:355–70, 1987
10. Saiki RK, Scharf S, Faloona F, Mullis K, Horn G, Erlich HA, Arnheim N: Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–54, 1985
11. Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi RH, Horn GT, Mullis KB, Erlich HA: Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–91, 1988
12. Erlich HA, Bugawan TL: HLA class II gene polymorphism: DNA typing, evolution, and relationship to disease susceptibility. In *PCR Technology: Principles and Applications for DNA Amplification*. Erlich HA, Ed. New York, Stockton, 1989, p. 193–208
13. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA: Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele-specific oligonucleotide probes. *Nature* (Lond) 324:163–66, 1986
14. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463–67, 1977
15. WHO Nomenclature Committee: Nomenclature for factors of the HLA system, 1989. *Immunogenetics* 31:131–40, 1990
16. Marsh SGE, Bodmer JG: HLA-DR and -DQ epitopes and monoclonal antibody specificity. *Immunol Today* 10:305–12, 1989
17. Thomson G: HLA disease associations: models for insulin dependent diabetes mellitus and the study of complex human genetic disorders. *Annu Rev Genet* 22:31–50, 1988
18. Aparicio JMR, Wakisaka A, Takada A, Matsuura N, Aizawa M: HLA-DQ system and insulin-dependent diabetes mellitus in Japanese: does it contribute to the development of IDDM as it does in Caucasians? *Immunogenetics* 28:240–46, 1988
19. Morel PJ, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the HLA-DQβ chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA* 85:8111–15, 1988
20. Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* (Lond) 332:845–50, 1988