A gene in the HLA class I region contributes to susceptibility to IDDM in the Finnish population

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Summary In Finland the haplotype A2, Cw1, B56, DR4, DQ8 is the third most common haplotype in insulin-dependent diabetic (IDDM) patients and has the highest haplotype-specific absolute risk for IDDM. Cw1, B56, DR4, DQ8 haplotypes containing HLA-A alleles other than A2 are infrequent in the population and are not associated with IDDM. Comparison of the A2 and non-A2 haplotypes at the DNA level showed that they were identical at HLA-B, -DR, and -DQ loci. Evidence that class I alleles confer susceptibility to IDDM was obtained from the two HLA-C, -B, -DR and -DQ haplotypes most frequently found in IDDM patients in Finland. A24, A3 and A2 on the Cw3, B62, DR4, DQ8 haplotype, and A28, A2 and A1 on the Cw7, B8, DR3, DQ2 were all found to be associated with IDDM. In Finland these seven haplotypes, including A2, Cw1, B56, DR4, DQ8, account for 33 % of diabetic haplotypes and 10.3 % of non-diabetic haplotypes ($p < 0.00001$). The contribution of the class I region to IDDM susceptibility was also apparent in those IDDM patients lacking the disease-predisposing class II alleles. Significantly more non-DR3/non-DR4 IDDM patients (47 of 55) possessed two of the IDDM-associated HLA-A alleles compared to non-DR3/non-DR4 control subjects (40 of 58; $p = 0.038$). Moreover, IDDM patients confirmed by oligotyping as unable to form a 'diabetes-susceptibility' DQ heterodimer, tended to possess two diabetes-associated HLA-A alleles (12 of 13) compared to control subjects (12 of 20; $p = 0.056$). [Diabetologia (1994) 37: 937–944]

Key words Genetics, haplotype, HLA-A, HLA-DQ, HLA-DR, tumour necrosis factor, diabetes mellitus.

Insulin-dependent diabetes mellitus (IDDM) is caused by an autoimmune destruction of the insulin-producing beta cells of the pancreas, the aetiology of which remains uncertain. Although a strong association exists between IDDM and markers in the HLA-DQ region of the major histocompatibility complex, HLA associations with IDDM were first described for the class I alleles HLA-B8, B18 and B15 (of which B62 is the major subtype) [1]. The higher relative risks of IDDM associated with DR3 compared to B8, and DR4 compared to B62, have been used as evidence to support the hypothesis that the primary susceptibility to IDDM exists in the class II region [2]. Highly poly-

Table 1. Asociations of HLA-A alleles with IDDM on Cw1, B56, DR4, DQ8 haplotypes

Haplotype	Diabetic haplotypes ($n = 1492$ (%))	Non-diabetic haplotypes ($n = 1254$ (%))	p value. Diabetic vs non-diabetic haplotypes	Corrected p value[a]
A2, Cw1, B56, DR4, DQ8	82 (5.5)	14 (1.1)	$p < 0.0001$	$p < 0.0004$
A1, Cw1, B56, DR4, DQ8	3 (0.2)	1 (0.08)	NS	NS
A3, Cw1, B56, DR4, DQ8	3 (0.2)	1 (0.08)	NS	NS
A11, Cw1, B56, DR4, DQ8	1 (0.1)	0 (0)	NS	NS
	89 (6)	16 (1.3)		

p value generated by Fisher's exact test.
[a] p values were corrected for the number of haplotypes compared ($n = 4$)

morphic markers are not ideal for population studies because individual allelic frequencies are so low that comparisons become meaningless. In this sense, HLA-DR serological and DNA HLA-DQ markers are ideal for association studies, whereas the class I HLA-B antigens are less useful, unless very large numbers of individuals are studied, because they are so polymorphic. However, because of linkage disequilibrium, each DR and DQ marker will be tightly associated with a number of class I antigens and these haplotypes are likely to be of similar discriminative value in association studies, as are individual alleles defined by the DQ locus. Indeed, Raum et al. [3] found associations of IDDM with four extended haplotypes: B8, SC01, GL02, DR3; B18, F1C30, DR3; B15, SC33, DR4; and B38, SC21, DR4. These associations were stronger than for DR3 or DR4 alone.

Finland has the highest incidence of IDDM in the world (35 of 100,000 per year in children aged 0–14 years) [4]. In the nationwide DiMe study 37 haplotypes, based on HLA-A, -C, -B, -DR and -DQ typing, have been found to be present in 84% (634 of 757) of the diabetic probands. The newly-described A2, Cw1, B56, DR4, DQ8 haplotype (abbreviated to A2, B56, DR4) which is present in 5.5% of diabetic haplotypes and 1.1% of non-diabetic haplotypes ($p < 0.0001$), has the highest absolute risk for IDDM (218 of 100,000 per year) compared to all other haplotypes [4] and is specific for the Finnish population, not being found in eight other European populations (Swedish [5], Norwegian [6], English [7], French [8, 9], Dutch [10], Estonian [11], Hungarian (E. Tuomilehto-Wolf, personal communication) and (former) East German [12] nor in the fourth and fifth Genetic Analysis Workshops [13–15]. In the Finnish population the Cw1, B56, DR4, DQ8 haplotype is a conserved haplotype and it is only associated with four HLA-A alleles; A2, A1, A3 and A11. Only the HLA-A2 associated haplotype is significantly increased in diabetes (Table 1) suggesting that on this haplotype at least the MHC class I region contributes to genetic susceptibility to IDDM.

HLA-DR3 and -DR4 containing haplotypes are strongly associated with IDDM in most ethnic groups, however, nearly 7% of IDDM patients in Finland are non-DR3/non-DR4. Khalil et al. [16] proposed that aspartic acid at position 57 of the DQβ chain and amino acids other than arginine at position 52 of the DQα chain protect against IDDM, whereas arginine at position 52 of the DQα chain and amino acids other than aspartic acid at position 57 of the DQβ chain lead to susceptibility to IDDM. A 'diabetes-susceptibility' DQ heterodimer is composed of a susceptible DQα chain and susceptible DQβ chain. In the Finnish population there is a wide variation in IDDM predisposition in those individuals who can form a disease-associated DQ heterodimer according to which HLA class I alleles are present on the haplotype, ranging from the same as the background population (35 of 100,000 cases per year) to 218 of 100,000 cases per year [4]. Furthermore, 2.5% of Finnish IDDM patients do not possess 'susceptibility' alleles at both DQA1 and DQB1 and are therefore incapable of forming the 'diabetes-susceptibility' DQ heterodimer. If genes in the class I MHC are important to disease predisposition, then patients who are non-DR3/non-DR4 or who are unable to form a 'diabetes-susceptibility' DQ heterodimer should demonstrate these associations.

The objectives of this study were to 1) characterize the A2, B56, DR4 haplotype at the DNA level and to compare this haplotype with non-A2, B56, DR4 haplotypes, 2) identify the HLA-A alleles that are most frequently found in haplotypes associated with IDDM and 3) compare these with the HLA-A alleles found in non-DR3/non-DR4 IDDM patients and IDDM patients who do not possess 'susceptibility' alleles at both DQA1 and DQB1.

Subjects and methods

Subjects. All individuals were taken from the "Childhood Diabetes in Finland" (DiMe) study [4, 17, 18] a population-based prospective family study carried out in Finland between September 1986 and April 1989. A group of 757 newly-diagnosed diabetic children aged 14 years or younger and 112 control children matched with the early-onset diabetic patients (6 years and under, $n = 122$) were HLA-A, -C, -B, -DR genotyped by serology together with their parents. The four haplotypes found in a family were divided into 'diabetic' and 'non-diabetic'. 'Diabetic' haplotypes were both haplotypes found

in the proband, 'non-diabetic' haplotypes were those parental haplotypes found neither in the proband nor in diabetic parents nor diabetic siblings.

Sequencing of MHC class I genes. PolyA$^+$ RNA was isolated by oligo (dT) cellulose (Invitrogen, San Diego, Calif., USA) batch adsorption from a Finnish A2, B56, DR4 Epstein-Barr virus transformed B-cell line. cDNA was synthesised [19] and the α1 to α3 regions (bases 97–808) of class I HLA genes were amplified from the cDNA by PCR with the primers 5'-CGAGAAGCTTGCTCCCACTCCATGAGGTATTTC-3' and 5'-CCGGAGGATCCAGAAGGCACCACCACAG-3'. The PCR conditions were 50 pmol of each primer, 50 mmol/l KCl, 20 mmol/l Tris-Cl pH 8.3, 1.5 mmol/l MgCl$_2$, 0.1 g/l BSA, 125 μmol/l dNTPs, 10 % v/v dimethyl sulphoxide, 2 units Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA) for 30 cycles of 94 °C for 60 s, 50 °C for 90 s, 72 °C for 120 s. The amplification products were desalted using a Centricon concentrator (Amicon, Beverly, Mass., USA) and digested with *Hind* III and *Bam* HI restriction endonucleases, sites of which were incorporated into the PCR primers. Digested PCR products were extracted with phenol, excluded from Sephadex G50 (Pharmacia, Milton Keynes, UK) and then purified by electrophoresis in low gelling temperature agarose (FMC Bioproducts, Rockland, Md., USA). Purified digested products were ligated into bacteriophage M13mp18 and M13mp19 vectors which had been digested with *Hind* III and *Bam* HI restriction endonucleases. The ligated product was used to transform competent *Escherichia coli* JM109 [20]. Stocks of M13 clones were prepared [20] and dot blotted onto a Hyband-N+ nylon membrane (Amersham International, Amersham, Bucks., UK) following the manufacturer's recommendations. HLA-A clones were found to be in excess of the other class I clones. M13mp18 clones containing HLA-C and -B were identified using the oligonucleotide 5'-CCCAAAGACACACGTGAC-3' and M13mp19 clones containing HLA-C and -B were identified using the oligonucleotides 5'-TGCCTGGCGCTTGTACTTCTG-3' and 5'-TTCTA TCTCCTGCTGGTCT-3', respectively. Oligonucleotides were labelled with [α-^{32}P] dCTP (3000 Ci/mmol) (Amersham International) using terminal deoxynucleotide transferase (Gibco BRL, Uxbridge, UK) following the supplier's recommendations and the membrane was hybridized with the oligonucleotides [20]. Using a Sequenase kit (USB, Cleveland, Ohio, USA) six M13 clones of HLA-A and -B in each orientation were sequenced and three clones of HLA-C genes were fully sequenced.

Typing of MHC class II loci. MHC class II genes were PCR amplified from proteinase K digested blood lysates [21]. Using protocols from the Eleventh International Histocompatibility Workshop [22] DRB1*04 and DQB1 alleles were PCR amplified, the product dot blotted onto Hybond-N+ nylon membranes and hybridized [20] with DRB1*04 and DQB1 SSOs which had been labelled with [α-^{32}P] dCTP using terminal deoxynucleotide transferase. DRB1*04 SSOs identified DRB1*0401-*0411 inclusive. Any inconclusive typings at DQB1 were further analysed by PCR-RFLP [23]. DQA1 alleles were PCR amplified and typed either using an AmpliType kit (Perkin Elmer Cetus) or by PCR-RFLP [24].

TNFα microsatellite analysis and complement typing. The TNFα microsatellite was PCR amplified with 5 pmol of the primers IR2 5'-GCCTCTACTATTTCATCCAGCCA-CAG-3' and IR4 5'-CCTCTCTCCCCTGCAACACACA-3' [25] in a 10 μl reaction containing 50 mmol/l KCl, 10 mmol/l Tris-HCl pH 9.0, 1.5 mmol/l MgCl$_2$, 1 % v/v Triton X-100,

0.5 μmol/l dNTPs, 1 μCi [α-^{32}P] (3000 Ci/mmol) dCTP and 1 unit of Taq DNA polymerase. Amplification was for 15 cycles of 94 °C for 60 s, 64 °C for 90 s. Amplified product was electrophoresed on an 8 % w/v acrylamide/bis-acrylamide (19 : 1) gel containing 48 % w/v urea, 89 mmol/l Tris-borate, 2 mmol/l EDTA, the gel was then dried and autoradiographed. Complement Bf and C4 types were determined using immunofixation agarose gel electrophoresis [26, 27].

Statistical analysis

Statistical comparisons were by Chi square analysis employing a Fisher's exact two tailed test where appropriate. Corrected p values were calculated by multiplying by the number of comparisons made; $p < 0.05$ is taken as statistically significant.

Results

Characterization of the A2, B56, DR4 haplotype. A2 and non-A2, B56, DR4 haplotypes were compared at the DNA level. The class I region was studied by sequencing, the class III region using the TNFα microsatellite and the class II region by oligonucleotide typing. HLA-A, -C and -B genes were sequenced on the A2, B56, DR4 haplotype. HLA-A and -C alleles on the A2, B56, DR4 haplotype were identical to A*0201 and Cw*0101 [28]. HLA-B was identical to B*5601 which had been previously sequenced on a non-A2, B56 haplotype (A1,3; Cw1; B8,56) [29].

TNFα, a microsatellite located 3.5 kb telomeric to TNFβ, is highly polymorphic with fourteen alleles reported and a polymorphism information content (PIC) value of 0.86 [30]. Haplotypes with known associated TNFα alleles [30] were included as controls. Allele TNFα6 (sized 107 bp) was shown to segregate with the A2, B56, DR4 haplotype in five families. Allele TNFα6 was present in all 15 individuals tested with the A2, B56, DR4 haplotype (seven diabetic and eight non-diabetic) and all nine individuals tested with non-A2, B56, DR4 haplotypes (seven diabetic and two non-diabetic). The A2, B56, DR4 subject, from whom the class I sequence data had been derived, also possessed a TNFα6 allele. The complement typing of the A2, B56, DR4 haplotype was BFS, C4A3, C4B0.

DRB1*04 alleles in 16 individuals with the A2, B56, DR4 haplotype (nine diabetic and seven non-diabetic individuals) and nine individuals with the non-A2, B56, DR4 haplotype (seven diabetic and two non-diabetic individuals) were oligotyped and all possessed DRB1*0401.

DQA1 and DQB1 alleles in 14 individuals with the A2, B56, DR4 haplotype (seven diabetic and seven non-diabetic individuals) and nine individuals with the non-A2, B56, DR4 haplotype (seven diabetic and two non-diabetic individuals) were typed and all possessed DQA1*03, DQB1*0302.

Table 2. Associations of HLA-A alleles with IDDM on Cw3, B62, DR4, DQ8 haplotypes

Haplotype	Diabetic haplotypes ($n = 1492$ (%))	Non-diabetic haplotypes ($n = 1254$ (%))	p value. Diabetic vs non-diabetic haplotypes	Corrected p value[a]
A28, Cw3, B62, DR4, DQ8	4 (0.3)	0 (0)	NS	NS
A24, Cw3, B62, DR4, DQ8	17 (1.1)	3 (0.2)	0.006	< 0.036
A2, Cw3, B62, DR4, DQ8	138 (9.2)	19 (1.5)	< 0.0001	< 0.0006
A1, Cw3, B62, DR4, DQ8	3 (0.2)	2 (0.2)	NS	NS
A3, Cw3, B62, DR4, DQ8	41 (2.7)	9 (0.7)	< 0.0001	< 0.0006
A11, Cw3, B62, DR4, DQ8	2 (0.1)	0 (0)	NS	NS
	205 (13.7)	33 (2.6)		

p value generated by Fisher's exact test.
[a] p values were corrected for the number of haplotypes compared ($n = 6$)

Table 3. Associations of HLA-A alleles with IDDM on Cw7, B8, DR3, DQ2 haplotypes

Haplotypes	Diabetic haplotypes ($n = 1492$ (%))	Non-diabetic haplotypes ($n = 1254$ (%))	p value. Diabetic vs non-diabetic haplotypes	Corrected p value[a]
A28, Cw7, B8, DR3, DQ2	29 (1.9)	6 (0.5)	0.0005	0.003
A24, Cw7, B8, DR3, DQ2	3 (0.2)	0 (0)	NS	NS
A2, Cw7, B8, DR3, DQ2	50 (3.4)	19 (1.5)	0.0021	< 0.0126
A1, Cw7, B8, DR3, DQ2	135 (9.0)	59 (4.7)	< 0.0001	< 0.0006
A3, Cw7, B8, DR3, DQ2	11 (0.7)	4 (0.3)	NS	NS
A11, Cw7, B8, DR3, DQ2	4 (0.3)	2 (0.2)	NS	NS
	232 (15.5)	90 (7.2)		

p value generated by Fisher's exact test.
[a] p values were corrected for the number of haplotypes compared ($n = 6$)

Association of HLA-A alleles on C, B, DR, DQ haplotypes with IDDM. Amongst haplotypes containing B56, DR4 only A2, B56, DR4 is associated with IDDM (Table 1). This haplotype is the third most abundant haplotype found in IDDM patients in Finland and yet it only accounts for 5.5 % of diabetic haplotypes compared to 1.1 % of non-diabetic haplotypes. To test if associations between HLA-A alleles and IDDM were a broader phenomenon, C, B, DR, DQ haplotypes based on the two most abundant diabetic haplotypes i.e., A2, Cw3, B62, DR4, DQ8 and A1, Cw7, B8, DR3, DQ2, were similarly analysed. A24, A3 and A2 on the Cw3, B62, DR4, DQ8 haplotype (Table 2) and A28, A2 and A1 on the Cw7, B8, DR3, DQ2 haplotype (Table 3) were associated with IDDM. These seven haplotypes, including the A2, B56, DR4 haplotype, associated with diabetes in the above analyses account for 33 % of diabetic haplotypes and 10.3 % of non-diabetic haplotypes (492 of 1492 diabetic haplotypes vs 129 of 1254 non-diabetic haplotypes; $p < 0.00001$), suggesting that certain HLA-A alleles (A28, A24, A2, A3 and A1) on these haplotypes are associated with an important factor in susceptibility to IDDM.

HLA class I in IDDM patients lacking HLA class II diabetes predisposing alleles. If the HLA class I is involved in disease predisposition it is most likely to be observed in those IDDM patients lacking the class II susceptibility alleles. HLA-DR3 and -DR4 are strongly associated with IDDM although it is the HLA-DQ regions in linkage disequilibrium with these HLA-DR antigens, DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302, respectively, that are believed to confer the susceptibility [31]. Comparison of HLA-A alleles in non-DR3/non-DR4 probands and control subjects revealed that 47 of 55 probands compared to 40 of 58 control subjects ($p = 0.038$) possessed two copies of the IDDM-associated HLA-A alleles.

Amongst the non-DR3/non-DR4 IDDM patients 19 were identified, using the HLA associations listed in Table 4, who were unable to form a 'diabetes-susceptibility' DQ heterodimer. Of these patients, from whom genomic DNA was available, 13 were typed at DQA1 and DQB1 to confirm that they did not possess 'susceptibility' alleles at both loci (Table 5); nine did not possess a 'susceptible' allele at DQA1, three did not possess a 'susceptible' allele at DQB1 and one did not possess a 'susceptible' allele at either locus. Twelve of the 13 IDDM patients compared to 12 of 20 control subjects who were unable to form a 'diabetes-susceptibility' heterodimer ($p = 0.056$) possessed two alleles of either HLA-A28, A24, A3 or A2, all of which are associated with diabetes on at least one of the three haplotypes most frequently

Table 4. Protective (DQA1 non-Arg 52, DQB1 Asp 57), (P) and susceptible (DQA1 Arg 52, DQB1 non-Asp 57), (S) DQA1 and DQB1 alleles associated with DRB1 alleles

DRB1	DQA1	DQB1
DR2 (15)	P 0102	P 0602
DR6 (13)	P 0103	P 0603
DR6 (14)	P 0101	P 05031
DR7	P 0201	P 0303
DR1	P 0101	S 0501
DR2 (16)	P 0102	S 0502
DR6 (13)	P 0102	S 0604/0605
DR7	P 0201	S 0201
DR10	P 0101	S 0501
DR4[a]	S 03011	P 0301
DR5 (11)	S 05012	P 0301
DR5 (12)	S 0501	P 0301
DR8	S 0401	P 0402
DR9	S 0302	P 0303
DR3	S 05011	S 0201
DR4	S 03011	S 0302

[a] HLA-B44 and -B51, DR4 haplotypes can be either DQB1*0301 or *0302

Associations were generated by predicting DQA1 and DQB1 alleles from the *Taq* I, *Pst* I and *Bam* HI restriction fragment length polymorphism in DQA1 and DQB1 genes determined in 68 Finnish haplotypes [4]. The predictions are also based on the known linkage disequilibrium between specific alleles of the HLA-A, -C, -B, -DR and -DQ loci [22]

found in IDDM. Repeating this comparison but including the IDDM patients that have not been typed at DQ, then 17 of 18 are unable to form a 'diabetes-susceptibility' DQ heterodimer ($p = 0.02$; Fisher's exact two-tailed test). Therefore, in those IDDM patients who do not possess the HLA-DQ alleles known to predispose to IDDM, certain HLA-A alleles are conferring susceptibility.

Discussion

Studies of the immune response in the pancreatic islets at IDDM onset implicate the HLA class I in pathogenesis. In human islets CD8 lymphocytes, which are MHC class I restricted, rather than CD4 lymphocytes, which are class II restricted, predominate at the time of disease onset [32] and when pancreatic grafts are rejected [33, 34]. In the nonobese diabetic mouse CD8 lymphocytes are involved in the selective destruction of beta cells [35, 36] which could be prevented with anti-class I antibodies and anti-cytotoxic T-cell antibodies [37] and, furthermore, anti-class I antibody could prevent diabetes [38]. Hyperexpression of MHC class I molecules has been observed on all cell types in human pancreatic islets at onset of IDDM [32, 39] and rejection of xenogenic pancreatic islets can be prevented by antibodies directed against class I molecules [40]. However, peripheral blood lymphocytes from pre-diabetic (normoglycaemic) [41] and diabetic individuals [42] have

reduced class I MHC expression. This phenomenon has been observed in other autoimmune diseases and may be a primary underlying defect leading to the failure of self-tolerance to develop.

A recent nationwide survey in Finland revealed a haplotype that had not been previously associated with IDDM. A2, B56, DR4 was the third most abundant haplotype in IDDM patients and carried the highest haplotype-specific absolute risk for IDDM compared to all other haplotypes. We have characterized this haplotype and compared it with non-A2, B56, DR4 haplotypes which are not found in increased frequency in diabetes. HLA-A, -B and -C sequences were identical to those found in Caucasians. The HLA-A allele A*0201 accounts for 89 % of A2 allotypes found in Caucasians [43]. The published B*5601 sequence was derived from a non-A2, Cw1, B56 haplotype [29] and is identical to the sequence found on the A2, B56, DR4 haplotype. Since Cw*0101 was only sequenced on an A2, B56, DR4 haplotype and not on a non-A2, Cw1, B56 haplotype we cannot exclude a difference between A2 and non-A2 haplotypes existing at this locus. However, we feel that these two haplotypes are unlikely to differ at HLA-C because they are identical at the more polymorphic loci of HLA-B and -DR, and HLA-C has limited sequence polymorphism. Thus, there are no novel HLA-A, HLA-C or HLA-B sequences on the A2, B56, DR4 haplotype. All individuals with the B56, DR4 haplotype possessed the class II haplotype DRB1*0401, DQA1*03, DQB1*0302. A2 and non-A2 haplotypes from diabetic and non-diabetic individuals were typed at the class II loci using SSOs and PCR-RFLP methodologies. These have the limitation of only being able to identify characterized alleles. However, exon 2 of DQB1, which is arguably the single most important locus for conferring susceptibility to IDDM, had already been sequenced on the A2, B56, DR4 haplotype [4] and was identical to DQB1*0302. All individuals were DRB1*0401, DQA1*03, DQB1*0302. The DR4 subtyping on this highly diabetogenic haplotype is consistent with the report that the DRB1*0401 is the subtype most strongly associated with IDDM [44].

The MHC class III region was typed with a highly polymorphic microsatellite close to TNFβ because complotyping was not available on the non-A2, B56, DR4 haplotype. A2 and non-A2 haplotypes possessed the same microsatellite allele. Thus, there are no differences between the A2 and non-A2 haplotypes centromeric of HLA-B.

Having demonstrated that HLA-A alleles are markers of susceptibility to IDDM on one haplotype, we wanted to confirm that this locus would also act as a susceptibility marker on other haplotypes. A2, B56, DR4 is the third most abundant haplotype in IDDM and therefore, the two most abundant C, B, DR, DQ haplotypes were selected for analysis.

Table 5. HLA-A, -DR and -DQ alleles in IDDM patients predicted and confirmed, or [ ] predicted only, as unable to form a 'diabetes-susceptibility' DQ heterodimer

Paternal haplotype				Maternal haplotype			
HLA-A	-DR	-DQA1	-DQB1	HLA-A	-DR	-DQA1	-DQB1
2	6 (13)	P 0103	P 0603	3	1	P 0101	P 0503
28	1	P 0101	S 0501	2	2 (16)	P 0102	P 0503
3	5 (11)	S 0501	P 0301	2	6 (13)	P 0103	P 0603
3	1	P 0101	S 0501	3	6 (13)	P 0102	S 0604
24	6 (13)	P 0102	S 0604	3	1	P 0101	S 0501
2	1	P 0101	S 0501	24	1	P 0101	S 0501
2	1	P 0101	S 0501	11	1	P 0101	S 0501
24	6 (13)	P 0102	S 0604	3	1	P 0101	S 0501
2	7	P 0201	S 0201	2	1	P 0101	S 0501
2	7	P 0201	S 0201	3	1	P 0101	S 0501
3	1	P 0101	S 0501	24	1	P 0101	S 0501
2	1	[P] [0101]	[S] [0501]	28	7	[P] [0201]	[S] [0201]
24	6 (13)	[P] [0102]	[S] [0604]	2	7	[P] [0201]	[S] [0201]
2	7	[P] [0201]	[S] [0201]	3	1	[P] [0101]	[S] [0501]
2	8	S 0401	P 0402	24	8	S 0401	P 0402
2	4	S 0301	P 0301	2	9	S 03	P 0303
24	9	[S] [03]	[P] [0303]	28	8	[S] [0401]	[P] [0402]
3	5	[S] [0501]	[P] [0301]	28	8	[S] [0401]	[P] [0402]

P, Protective; S, susceptible (refer to Table 4 for definition)

Cw3, B62, DR4, DQ8 and Cw7, B8, DR3, DQ2 haplotypes are heterogeneous at the HLA-A locus allowing comparison between HLA-A alleles and disease susceptibility. Comparison of HLA-A alleles on a predetermined C, B, DR, DQ haplotype takes into account the known, and unknown, contribution these loci may make to disease susceptibility and assumes there is no variation at other MHC loci that influences disease susceptibility. It is especially important to control for the class II region which is well known to predispose to IDDM. Haplotypes containing certain HLA-A alleles were associated with diabetes and this was interpreted as conferring increased susceptibility to the disease. These IDDM-associated haplotypes are present in a third of Finnish diabetic patients.

An alternative approach to controlling for the class II genes is to examine HLA-A alleles in those patients that do not possess disease predisposing class II genes. Non-DR3/non-DR4 diabetic patients were more likely to possess two of the HLA-A alleles that increased susceptibility to diabetes on the three most abundant diabetic haplotypes. A similar tendency was noted in diabetic patients that could not form a 'diabetes-susceptible' DQ heterodimer.

Other studies have implicated the HLA-A region in diabetes. In Japanese IDDM patients HLA-A24 is associated with the acute onset of disease [45] and the complete loss of beta-cell function [46]. Also, there is an inverse association of HLA-A2 with age of disease onset in Pima Indians with NIDDM which is dose-related [47], and A2 was increased in South African blacks with NIDDM [48]. In all of these studies genes in linkage disequilibrium with HLA-A2 could in fact be contributing to the disease suscep-

tibility. In Finland, HLA haplotypes which have a moderate association with IDDM are also strongly associated with mild glucose intolerance in elderly men [49]. However, haplotypes strongly associated with IDDM were rarely found in these individuals. This suggests a graded genetic susceptibility to diabetes depending on allelic variation of several genes on the MHC haplotype.

We have demonstrated that on the haplotypes most frequently found in IDDM which also contain the class II IDDM-associated alleles, the HLA-A alleles A28, A24, A3, A2 and A1 modulate the disease susceptibility. Furthermore, these HLA-A alleles may explain the existence of the disease in non-DR3/non-DR4 patients and patients who cannot form the 'diabetes-susceptibility' DQ heterodimer. We propose that a gene or genes in the class I region modulates the risk of IDDM in association with the class II 'diabetes-susceptibility' DQ heterodimers.

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