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**REVIEW ARTICLE**

# Genetic susceptibility factors of Type 1 diabetes in Asians

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## Summary

Type 1 diabetes is a multifactorial disease in which the insulin producing $\beta$-cells of the pancreas are destroyed by the immune system, a process determined by the activity of major histocompatibility complex (MHC)-restricted T lymphocytes. Progress has been made in elucidating genetic factors involved in Type 1 diabetes in Caucasians, with less data available from Asia. For Asians, the human MHC locus (HLA region), especially the class II region, is the major susceptibility interval. The role of *IDDM2*, the insulin locus, has been questioned in Asia. In contrast to Caucasians, Asian populations have a very low incidence of Type 1 diabetes (0.4–1.1 cases/year/100 000 individuals). This low incidence rate in the Asian population may be related to the population frequency distribution of susceptible Type 1 diabetes genes, especially of HLA. The overall risk for Type 1 diabetes from HLA DR and DQ is determined by polymorphic residues (alleles) and particular combinations of alleles (haplotypes and genotypes) in a given individual. In Asians, it is very common that a protective DR4 allele is associated with susceptible DQ alleles while neutral/protective DQ alleles are associated with the susceptible DR4 alleles. Our analyses indicate that the counterbalancing between susceptible *DRB1* and protective *DQB1*, and vice versa, is a factor that may contribute to the low incidence of diabetes in Asians. We find that identical HLA DRB1-DQB1 haplotypes of Asians and Caucasians have similar transmission to diabetic children and similar associations with diabetes. Moreover, the association with diabetes and transmission to a diabetic offspring of DR4 haplotypes varies depending on the haplotype borne on the homologous chromosome. This might contribute not only to the synergistic effect of DR3/4, but also to the susceptibility influence of DQB1*0401 haplotypes confined to DR4/X. High-risk DR4 subtypes were predominant in DR4/X, whereas protective DR4 subtypes were observed mainly in the DR3/4 genotype. Since in Asians DQB1*0401 is in linkage disequilibrium (LD) with DRB1*0405, we find more DRB1*0405-DQB1*0401 haplotypes in patients with DR4/X than in patients with DR3/4, suggesting that the contribution of the DRB1 locus may be greater in DR4/X than in DR3/4 genotypes. Several genome scans suggested additional susceptibility intervals and provided supporting evidence for several previously reported linkages. Other studies focused on the confirmation of linkage using multipoint sib-pair analyses with densely spaced markers and multiethnic collection of families. Although significant and consistent linkage evidence was reported for the susceptibility intervals *IDDM12* (on 2q33) even in Asia, evidence for most other intervals varies in different data sets. LD mapping has become an increasingly important tool for both confirmation and fine-mapping of susceptibility intervals, as well as identification of etiological mutations. The examination of large and ethnically varied data sets including those of Asia has allowed identification of haplotypes that differ only at a single codon in a single locus. As more data become available, the study of pairs of haplotypes which differ at a single polymorphic site, but have

different effects on disease susceptibility, should allow more precise definition of the polymorphisms involved in the disease process. 

**Keywords** HLA; genetics; Type 1 diabetes; epidemiology

## Introduction

Type 1 diabetes is one of the first disorders with a complex genetic basis that researchers have begun to unravel [1,2]. More than 20 years ago, the HLA region was found to contain a major locus that influences predisposition to Type 1 diabetes [3,4], and a decade ago a locus with a smaller effect was identified in the insulin gene region [5]. With the advent of numerous microsatellite markers suitable for genome screening, more than 20 loci that influence susceptibility to Type 1 diabetes have been reported (Table 1) [6–14]. Some of the new loci appear to predispose individuals to Type 1 diabetes independently of HLA and may be important factors in families with Type 1 diabetes who lack strong HLA susceptibility [13]. Other loci may interact with HLA to cause susceptibility, and specific combinations may be diabetogenic [2,14]. Although isolating the actual predisposing genes in Type 1 diabetes is more difficult than isolating those involved in single-locus genetic disorders, the fact that the genes can be identified with the use of a reasonable number of families is very encouraging for future research on other genetically complex disorders.

Mapping complex disease genes is, however, a challenging undertaking because a large number of genes may be involved and each gene only accounts for a small percentage of the total familial aggregation [15,16]. For example, each of the recently confirmed Type 1 diabetes genes may explain only 5–10% of the total genetic contribution to Type 1 diabetes. To demonstrate the effect of such genes on a disease, many hundreds of families are required. This is further complicated by heterogeneities in different populations and/or ethnic groups. Moreover, if an etiological factor has either an extremely high or low frequency (close to monomorphic) in one population, its susceptibility effect cannot be detected by conventional genetic studies [16]. This possibility can be illustrated by the insulin (*INS*) gene polymorphisms in Asia. *INS* polymorphisms have been associated with Type 1 diabetes in many Caucasian populations [5], but not in Asian populations because the frequency of the susceptible *INS* allele is $\gg$95% in Asian controls and close to 100% in Asian Type 1 diabetes (Y. Park and J. X. She, unpublished data), although some investigators reported that they found a significant association when they subtyped the class I allele [17,18].

The incidence rate of Type 1 diabetes occurring below the age of 15 years varies considerably across the world [19]. In Caucasian populations, including those in Northern Europe, Type 1 diabetes incidence rates are high with rates in excess of 20 cases/year/100 000 individuals. In contrast, countries in Asia, including Korea, have extremely low Type 1 diabetes incidence rates, less than 1 case/year/100 000 individuals. This low incidence rate in the Asian population may be related to the population frequency distribution of susceptible Type 1 diabetes genes, including HLA alleles. It is apparent that population frequencies of HLA alleles and haplotypes vary dramatically between ethnic groups. Because of this, some highly 'susceptible' alleles that are relatively uncommon in a population may be mistakenly considered neutral in population association studies [20]. The haplotype distribution and its transmission pattern, either in patients with Type 1 diabetes, or in the general population in Asia, have not been well described.

Some points to be considered in investigating genetic susceptibility factors in Asia include the following. First, since the incidence is very rare, Asian data sets are usually small. We should consider that the small Asian data set in contrast to the large Caucasian data set might lead to the possibility of overstating the conclusions. Moreover, because of the extensive polymorphism of the human candidate alleles, the data sets contain many different but relatively infrequent haplotypes [20–25]. Second, since there are very few cases of multiplex families and because it is very difficult to obtain family-based samples, case-control studies are more prevalent rather than affected sib-pair approaches or family-based association studies. Case-control studies are very sensitive to ascertainment of patient and control subjects as well as sample sizes. Such study designs are not very powerful in detecting small differences between patient and control populations unless a very large data set is studied.

**Table 1. Proposed MLSs for suggested Type 1 diabetes susceptibility intervals in various studies**

| | | Studies | | | |
|---|---|---|---|---|---|
| Locus | Region | UK | US/UK | Others | References |
| IDDM1 | 6p21 | 34 | 33 | – | [7,8] |
| IDDM2 | 11p15 | 2.8 | 0.6 | – | [7,8] |
| IDDM3 | 15q26 | 0.0 | 0.0 | 2.5 | [7,8,9] |
| IDDM4 | 11q13 | 0.5 | 0.4 | 1.5 | [7,8,10] |
| IDDM5 | 6q25 | 1.0 | 1.5 | 3.0 | [7,8,10] |
| IDDM6 | 18q | 1.2 | 0.0 | 2.8 | [7,8,10] |
| IDDM7 | 2q33 | 1.0 | 0.7 | – | [7,8,10] |
| IDDM8 | 6q27 | 1.9 | 1.1 | 3.4 | [7,8,10,11] |
| IDDM9 | 3q | 1.1 | 0.2 | – | [7,8,11] |
| IDDM10 | 10p13-q11 | 4.7 | 0.4 | – | [7,8,11] |
| IDDM11 | 14q24-q31 | 0.0 | 0.3 | 4.0 | [7,8,12] |
| IDDM12 | 2q33 | 0.0 | 0.9 | – | [7,8,10] |
| IDDM13 | 2q33 | 0.0 | 0.4 | 3.3 | [7,8,10,13] |
| IDDM15 | 6q21 | – | 2.3 | 4.2 | [7,11] |
| D14S70-D14S276 | 14q12-q21 | 2.0 | | – | [8] |
| D16S515-D16S520 | 16q22-q24 | 3.4 | – | – | [8] |
| D19S247-D19S226 | 19p13 | 1.7 | – | – | [8] |
| D19S225 | 19q13 | 1.6 | – | – | [8] |
| D1S1644-AGT | 1q | – | 2.8 | – | [7] |
| IDDM17 | 10q25 | – | – | – | [14] |

## Type 1 diabetes is a genetic disease even in Asians

Type 1 diabetes is much less frequent in Asia than in countries with a predominantly Caucasian population. To investigate whether the genetic determinants influence the development of Type 1 diabetes in this low incidence countries, Ikegami *et al*. [25] studied siblings of Type 1 diabetes probands with age-at-onset under 20 years. The ratio of the risk for siblings of Type 1 diabetes patients and the population prevalence ($\lambda s$), often used to assess the degree of familial clustering of a disease is more than 200, a much higher value than that in Caucasian populations. Familial clustering of a disease does not necessarily indicate a genetic component, since the disease may be caused by sharing of the same environment among family members. If a genetic factor is responsible for the high $\lambda s$ value for Type 1 diabetes with low population prevalence in Asians, then susceptibility genes whose frequencies are very low in the general population may be segregating in Type 1 diabetes families.

## HLA association in Asians

The HLA class II alleles on chromosome 6p21 are the most highly related to diabetes susceptibility [1–4]. Even in Asians, both the DR and DQ alleles are genes that have the highest association with Type 1 diabetes [20–26]. Table 2 shows the distribution of HLA DR alleles in Korean patients with Type 1 diabetes and control subjects [21]. In the Type 1 diabetes cases, HLA DR3 and DR9 were increased. DR4 as a group was not significantly increased in diabetic patients compared to controls. Among the DR4 subtypes, DRB1*0401 and DRB1*0405 had increased frequencies in patients. Two DR4 subtypes (0403 and 0406) had lower frequencies in patients. As expected, DR15 confers strong protection. DR12 was also strongly protective. When the HLA DQB1 alleles were identified in the Type 1 diabetes patients, the DQB1*0201 allele had significantly higher frequencies in patients, while three DQB1 alleles (0301, 0601 and 0602) had significantly lower frequencies in patients compared to controls. Five haplotypes (DRB1*03-DQB1*0201, DRB1*0401-DQB1*0302, DRB1*0405-DQB1*0302, DRB1*0407-DQB1*0302 and DRB1*0901-DQB1*0303) had significantly increased frequencies in diabetic patients. Four other haplotypes (DRB1*15-DQB1*0601, DRB1*15-DQB1*0602, DRB1*08-DQB1*0601 and DRB1*10-DQB1*05) had significantly lower frequencies in patients.

**Table 2. Distribution of HLA-DR phenotypes among Korean Type 1 diabetes patients and controls**

| HLA-DR specificity | Type 1 diabetes patients (%) ($n=146$) | Control subjects (%) ($n=140$) | $P_C$ value[a] |
|---|---|---|---|
| DR1 | 21 (14.4) | 15 (10.7) | NS |
| DR2 | 9 (6.2) | 35 (25.0) | $<0.001$ |
| DR3 | 33 (22.6) | 6 (4.3) | $<0.001$ |
| DR4 | 81 (55.5) | 54 (38.6) | NS |
| DR5 | 12 (8.2) | 31 (22.1) | $<0.01$ |
| DR6 | 23 (15.8) | 34 (24.3) | NS |
| DR7 | 19 (13.0) | 17 (12.1) | NS |
| DR8 | 20 (13.7) | 23 (16.4) | NS |
| DR9 | 53 (36.3) | 34 (24.3) | NS |
| DR10 | 1 (0.7) | 8 (5.7) | NS |

[a]$P_C$ were obtained by multiplying the $P_{NC}$ values by ten DR allele groups. NS, Not significant.

## Genotypic vs haplotypic interaction

It has been proposed that the contribution of the DQ molecule to overall disease susceptibility may be genotype dependent [2,22,23]. While haplotype analyses are very informative in revealing the effects of DR and DQ alleles, genotypes of DR/DQ are most useful in predicting the risk of Type 1 diabetes for each individual [22,24,25]. In addition to DQB1*0302, DQB1*0401 in Asians has been known to be positively associated with disease [21–26]. The role of the DQB1*0401 molecule in Type 1 diabetes risk is not clear. It may be slightly predisposing or neutral. We analyzed the association of DQB1*0302, DQB1*0301 and DQB1*0401 haplotypes in Asian patients with Type 1 diabetes [26]. Although the prevalence of the DR4-DQB1*0302 haplotype did not differ in patients vs controls, the HLA DR3/4-DQB1*0302 genotype had an increased risk indicating a synergistic effect (Figure 1). When DR3/4 diabetic patients are compared to all other DR4 positive patients, a significant association of DQB1*0302 with the DR3/4 genotype was also found. In contrast, the distribution of DQB1*0401 haplotypes in DR4/X (X: other than 3, 4) is different from that of DR3/4 and DR4/4. To be noted is that a significant association of DQB1*0401 with DR4/X (X: other than 1, 3, 4) was found. The frequency of transmission of the DQB1*0401 haplotypes to diabetic offspring with DR3 was decreased compared to those without DR3. High-risk DR4 subtypes were predominant in DR4/X, whereas protective DR4 subtypes were observed mainly in the DR3/4 genotype. The association with diabetes and transmission to a diabetic offspring of DR4 haplotypes varies depending on the haplotype borne on the homologous chromosome. This might contribute not only to the synergistic effect of DR3/4, but also to the susceptibility influence of DQB1*0401 haplotypes confined to DR4/X. Moreover, since in Asians DQB1*0401 is in linkage disequilibrium (LD) with DRB1*0405, we find more DRB1*0405-DQB1*0401 haplotypes in patients with DR4/X than in patients with DR3/4, suggesting that the contribution of the DRB1 locus may be greater in DR4/X than in DR3/4 genotypes. The specific molecular mechanism underlying this difference is unknown. The two major hypotheses are the effect of DQ transcomplementation [4,22,24] and presentation of peptides of one allele by the alternative molecule [27]. Though we do not understand the effect, in the prediction of diabetes risk, genotypic associations will be important.

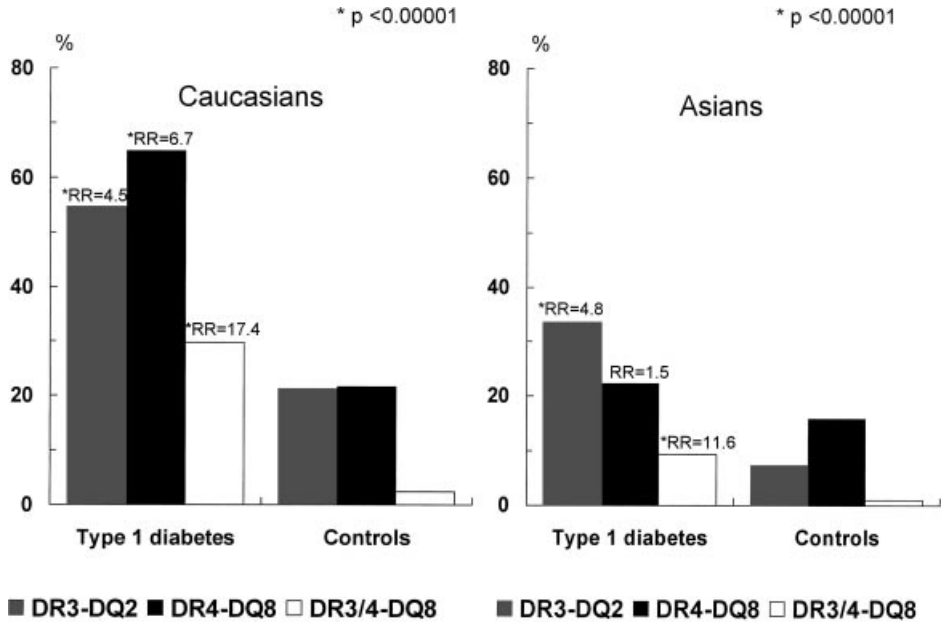


**Figure 1. Documentation of DR4-DQ8/DR3-DQ2 heterozygotic (synergistic) effect in Caucasian (Barbara Davis Center) and Asian patients with Type 1 diabetes mellitus**

## Common transmission of HLA haplotypes in Asians compared to Caucasians

It has been suggested that HLA alleles of Asian patients associated with diabetes differ from those of Caucasians [23]. We analyzed the common susceptibility and transmission pattern of a series of HLA DRB1-DQB1 haplotypes to Korean and Caucasian patients with Type 1 diabetes [20]. Although the haplotype frequencies in the two populations are quite different, when identical haplotypes are compared, their odds ratio (OR) is nearly the same. We also analyzed the transmission of a series of these haplotypes from non-diabetic parents to their children with Type 1 diabetes and compared transmission with that in Caucasians. For all parental haplotypes which were identical at DRB1 and DQB1, the transmission to diabetic offspring was similar for Koreans and Caucasians families ($r=0.8$, $p<10^{-4}$) (Figure 2).

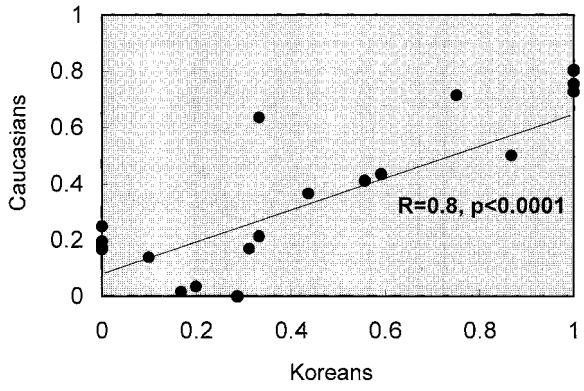


**Figure 2. Correlation of transmission frequencies of HLA DRB1-DQB1 haplotypes**

Allowing for ethnic differences in allelic associations due to different frequencies of DRB1 and DQB1 haplotypes, these data show, not only by case-control comparison but also by the transmission analyses of the haplotypes, that the susceptibility effects of DRB1-DQB1 haplotypes are consistent in Koreans and Caucasians. Thus, the influence of class II susceptibility and resistance alleles appears to transcend ethnic and geographic diversity of Type 1 diabetes incidence.

## Class I, III, and novel class II gene association

It is known that more than one genetic locus even within the HLA region is important for disease risk [28,29]. The highest risk genotype for Type 1 diabetes consists of individuals heterozygous for DR3 and DR4 associated haplotypes. This HLA DR3-DQB1*0201/DR4-DQB1*0302 genotype can only explain some 20% or 12% of the total genetic contribution in Caucasians, assuming a 30% or 50% concordance rate in monozygotic twins, respectively [30,31]. The population frequency of this genotype is still 10–20 times higher than the prevalence of Type 1 diabetes associated with this genotype. DRB1 subtyping might influence the risk of Type 1 diabetes in this high-risk DQ population, though it is assumed to account for only 10% of additional familial aggregation [28]. This implies that additional alleles of protective or risk determining genes (HLA or non-HLA) and/or environmental factors influence susceptibility to the disease.

The HLA complex encompasses 3.5 Mb of DNA from the centromeric HLA-DPB2 locus to the telomeric HLA-F locus on chromosome 6p21. Since there are extensive LD

between these regions, analyses of haplotypes are likely to be more informative than analyses of individual alleles. However, given the strong LD observed for alleles at the class I, class III and class II loci, it has proven difficult to identify which allele on a disease-associated haplotype may be responsible for the genetic predisposition. One valuable approach has been to examine the pattern of HLA haplotype disease association in different ethnic groups with differing allele frequencies and LD patterns.

Recent investigations have evaluated novel genes in the HLA class II region [i.e. the transporter associated with antigen processing (TAP) and the large multifunctional protease (LMP) genes], which are involved in the presentation of peptides by HLA molecules to T cells [32,33]. There have been other reports investigating the role of class III region genes [i.e. heat shock protein (HSP) and tumor necrosis factor (TNF) genes] that are involved in cell protection and immunomodulation [28,34]. However, the results of these studies have been inconsistent even in Asians [35–37], possibly reflecting variations in study methodology, sample size, ethnicity, and other factors.

One Japanese group reported that HLA class I molecule, A as well as B, are associated with early age-of-onset Type 1 diabetes [38]. We found a similar significant deviation of transmission of B in our family-based association study of Type 1 diabetes, age-of-onset less than 15 years (Y. Park and J. Noble, unpublished observation). Furthermore, a novel family of the class I genes termed MIC (MHC class I chain-related genes), MICA, has been recently identified near the HLA B gene on the short arm of human chromosome 6. The predicted amino acid sequence of the MICA chain suggests that it folds similarly to typical class I chains and may have the capacity to bind peptides or other short ligands. Therefore, MICA is predicted to have a specialized function in antigen presentation or T cell recognition [39,40]. The polymorphism of the MICA gene and its location in the HLA region warrant studies aimed at identifying an association with the risk for various autoimmune diseases [41,42]. Lie and co-workers have implicated another MHC region associated risk with microsatellite D6S2223 near class I region [29]. We have also analyzed D6S2223 microsatellite markers in 102 general population DQ8/DQ2 children in addition to 58 similarly studied (DQ8/DQ2 heterozygous patients) families from the Caucasian Human Biological Data Interchange (HBDI) repository [42]. Our analysis suggests the presence of an additional Type 1 diabetes gene approximately 5.5 Mb telomeric of the class II region near D6S2223. When we analyzed the LMP7, D6s273, D6S2223 polymorphisms in our genetically distinct Koreans, they were not associated with Type 1 diabetes, but MICA and TNF $\alpha$ microsatellites were associated with diabetes. This deviated distribution was not changed even after controlling for HLA haplotype [43]. Although the genes at these loci appear to contribute to Type 1 diabetes susceptibility, their roles are likely to be minor compared with those for the HLA class II genes.

## Non-HLA markers

Three proposed intervals, *IDDM7*, *IDDM12*, and *IDDM13*, have been identified on chromosome 2q31-35 [7,8,10,13,44]. These three intervals span approximately 23 cM. One of the 'confirmed' Type 1 diabetes susceptibility loci is *IDDM12* located on chromosome 2q33 [45]. Transmission disequilibrium test (TDT) revealed highly significant deviation of transmission for alleles at the (AT)n microsatellite marker and the A/G polymorphism within the *CTLA4* gene in the data sets with Mediterranean origins (Italian, Spanish, French and Mexican Americans) ($p < 10^{-7}$) [44]. In contrast to a negative result in Japanese [46] and Chinese [45], a positive result was also observed in a small Korean [45] and another Chinese data set [47]. More precise definition of the *IDDM12* interval led to a locus within a BAC clone of 200 kb using a multiethnic collection of families [45]. In contrast to a positive report of Japanese population [48], the association of D2S137 within *IDDM13* region with Type 1 diabetes was not confirmed in Korean and Chinese populations [49]. The transcription factor NeuroD/Beta2 has been mapped to chromosome 2q33 in human, and chromosome 2 in mouse [50]. Although a recent case-control study in the Japanese population has shown association of this marker with Type 1 diabetes ($p = 0.006$) [51], we could not confirm this finding in Korean and Chinese populations [52]. These differences may be because the one Asian population differs from other Asian populations indicating genetic heterogeneity even in Asians.

Since the early 1990s, several whole-genome approaches to the mapping of Type 1 diabetes susceptibility genes have been reported [6–8]. These studies together revealed over 20 genomic intervals with variable degree of linkage evidence (Table 1). Recently Concannon *et al*. reported the results of a genome screen for linkage with Type 1 diabetes and analyzed the data by multipoint linkage methods [7]. An initial panel of 212 affected sib-pairs (ASPs) were genotyped for 438 markers spanning all autosomes, and an additional 467 ASPs were used for follow-up genotyping. Other than the well-established linkage with the HLA region at 6p21.3, they found only one region, located on 1q. Due to a weak effect of the disease genes, genetic heterogeneity or random variation, these differences are an obstacle for the confirmation and fine-mapping of susceptibility intervals and identification of etiological mutations [15,16]. These problems will lead to a delay in the completion of genetic mapping, because a chromosomal region may cosegregate with a disease in some families but not in others. However, irrespective of the many obstacles that can hamper genetic dissection of Type 1 diabetes, some approaches have been successful in narrowing down the susceptibility interval. Using a large consanguineous Bedouin Arab family (18 affected relatives in three generations,), Fain and co-workers [14] found a locus causing Type 1 diabetes in the long arm of chromosome

10 (10q25). D10S554 and D10S592 and the closest flanking markers are contained in a 1240 kb yeast artificial chromosome, indicating that the target region is sufficiently small to proceed with positional cloning. If oligogenicity rather than polygenicity apply to human Type 1 diabetes, studies of large multiplex families from genetically and culturally homogeneous populations are likely to improve the prospects of identifying susceptibility genes by genetic linkage studies followed by positional cloning.

Since the incidence of Type 1 diabetes in Asia is very low, it is not easy to find multiplex cases of Type 1 diabetes, or to get a large data set of incidence cases. Moreover, because of cultural differences, it is very difficult to get any family-based samples. Therefore, it is not easy to find a study trying to dissect genes determining Type 1 diabetes susceptibility applying whole-genome approaches in Asia. However, population genetic theory and data suggest that there will be greater genetic and allelic homogeneity in a more genetically isolated population such as Korea and Japan in Asia than in a large, mixed population [15,16]. Focusing on a highly restricted population may also offer advantages for eventual positional cloning, because one may be able to exploit LD for fine-structure genetic mapping.

## Approaches for evaluating functions of the genes determining susceptibility of Type 1 diabetes

Complete molecular understanding of the function of the genetic susceptibility factors may permit the design of rational and effective means of prevention. Prevention could then replace insulin therapy, which is effective but associated with long-term renal, vascular, and retinal complications. Tisch and McDevitt reviewed the molecular understanding of the pathogenesis of this autoimmune disease and described the role of the major histocompatibility complex (MHC) [53]. Ikegami *et al*. identified a new susceptibility locus to Type 1 diabetes on MHC by ancestral haplotype cogenic mapping [54]. This is a novel strategy based on the prediction that recombination had occurred many times during historical meiosis even in a small interval with strong LD. The HLA region, also known as the human MHC, is a cluster of over 150 genes contained in about 3.5 Mb of DNA on the short arm of chromosome 6. The products of many of these genes play a central role in the immune response and in susceptibility to Type 1 diabetes. Functional studies indicate that the susceptible and protective HLA class II molecules, HLA DR and DQ, bind and present non-overlapping peptides [55]. In fact, the overall risk for Type 1 diabetes from the HLA DR and DQ molecules is determined by combinations of polymorphic residues (alleles) and combinations of alleles (haplotypes and genotypes) in a given individual [22,31]. One explanation for this might be that multiple peptides are involved in the process. Consistent with this notion, studies of peptide binding to HLA demonstrate that different HLA alleles bind different peptide epitopes from a single candidate diabetes antigen, such as GAD or insulin [56–58]. Recent research focuses on possible mechanisms of the susceptibility effect of HLA. A study of antigen presentation using human APCs and murine GAD65 specific T cell hybridomas and development of TCR transgenic mice specific for an immunodominant DR*0405 restricted T cell epitope of GAD65, peptide 341–355. They found that DR*0405 and DR*0403 transgenic mice identify both a set of 'communal' (shared) T cell epitopes, which are also shared with DR*0401 transgenic mice and a set of 'private' CD4+ T cell epitopes from recombinant human GAD65 [58]. They found two 'private' immunodominant CD4+ T cell epitopes for DR*0405 and the one single immunodominant 'private' DR*0403 epitope of human GAD65. These TCR transgenic mice were used for studies of positive selection in the thymus, when the TCR transgenic mice were on a DR*0405 only vs a heterozygous DR*0405/*0403 background. Furthermore, they will establish a hierarchy of human antigen presenting cells restricted with DQB1*0302 only and DQB1*0401 only, which are positively associated with Type 1 diabetes in Asia vs those restricted with heterozygous DQB1*0302/DQB1*0401 background, which is neutral in Asia (G. Sonderstrup, personal communication). Recently, Wen and co-workers have demonstrated that mice with islet expression of the co-stimulatory molecule B7-1 and transgenic for human DQB1*0302 develop diabetes, while mice with DQB1*0601, which is quite common in Asians, do not [59]. This provides direct evidence for the diabetogenicity of DQB1*0302.

Several genes other than HLA might have a functional role in susceptibility to Type 1 diabetes. These include the insulin gene (*INS*) on the short arm of chromosome 11 [5], the interleukin-1 gene cluster at 2q12-21 [60], the T cell receptor $\alpha$- and $\beta$-chains on chromosomes 12 and 7, respectively [61], and the immunoglobin heavy chain allotypes on chromosome 14 [62]. Several studies have confirmed the association between diabetes and markers for the *INS* region at 11p15.5, now known as *IDDM2* [5,17,18]. The marker showing the strongest association with disease is a tandemly repeated 14 bp oligonucleotide, about 365 bp from the transcriptional initiation site at the 5′ end of the gene. The number of repeat units varies from individual to individual. The frequency of the shortest (class I) alleles are increased in patients with diabetes compared to controls, while the frequencies of the longest (class III) alleles are in decreased frequency in patients compared to controls. Although it seems much less in Asia, estimates of the contribution of *INS* region haplotype sharing to the 15-fold increased risk in sibs (total genetic risk) are in the range 1.25–1.6 ($\lambda_{(INS,S)}$) [63]. With the exception of *INS* [17,18], there is either no evidence, or there is conflicting evidence that any of these genetic regions contribute to diabetes, especially in Asia.

Of note, variable nucleotide tandem repeat (VNTR)

alleles correlate with differential *INS* mRNA expression in the thymus, where a small subset of dendritic cells produce insulin and the protective class III VNTRs are associated with higher *INS* mRNA expression [64]. This finding provides a plausible explanation for the dominant protective effect of class III VNTRs, and suggests that diabetes susceptibility and resistance associated with *IDDM2* may derive from the VNTR influence on INS transcription in the thymus. Higher levels of insulin in the thymus may promote negative selection of insulin specific T lymphocytes which play a critical role in the pathogenesis of Type 1 diabetes. Moreover, ICA512/IA-2, one of the genes found to be expressed in thymus, is an autoantigen in Type 1 diabetes and therefore could be a good candidate for a Type 1 diabetes susceptibility marker [65]. An alternatively spliced variant of the ICA512/IA-2 mRNA transcript has been recently identified, and translates into a protein lacking the transmembrane and juxta-membrane domains encoded by exon 13 [66]. Because some of the epitopes recognized by autoantibodies in patients with Type 1 diabetes overlap with these domains, we investigated whether splicing of the ICA512/IA-2 gene could be differentially regulated in lymphoid organs compared to pancreas as this may affect immune responsiveness to ICA512/IA-2. Thymus and spleen exclusively expressed the alternatively spliced transcript and always lacked the full-length transcript expressed instead in pancreas [67]. Therefore, differential tissue splicing of the ICA512/IA-2 gene could generate cryptic epitopes unknown to the immune system and predispose to autoimmunity once such cryptic epitopes are expressed in pancreatic ß-cells and exposed to the immune system.

Other studies have focused on a region about 10 cm distal to 2q31 at 2q33 (*IDDM12*), the location of candidate genes cytotoxic T lymphocyte antigen 4 (CTLA4) and CD28 [44–47]. CTLA4 is of special interest due to reports of an association between CTLA4 and Graves' disease [68] in addition to the role of the gene in T cell apoptosis and proliferation [69]. More recent studies have confirmed the association between CTLA4 and Graves' disease [70] and have additionally reported that the same CTLA4 variants are in increased frequency in Graves' disease as in Type 1 diabetes [71]. These results suggest that CTLA4 or a nearby locus may predispose to organ-specific autoimmunity, thereby contributing to the association between Type 1 diabetes and other autoimmune diseases.

It is likely that identification of non-MHC genes will facilitate understanding the pathogenesis of Type 1 diabetes, but to date with only loci identified, each with a relatively small effect upon familial clustering, current knowledge of non-MHC diabetes susceptibility loci do not contribute to the diagnosis or prediction of Type 1 diabetes. However, both studies in humans clarifying the difference between Caucasians and Asians and studies in animal models applying various transgenic mice models and congenic approach, in addition to *in vitro* experiments, will help us to understand the genetic components of Type 1 diabetes further.

## Conclusions

Type 1 diabetes is much less frequent in Asia than in countries with a predominantly Caucasian population. These low incidence/prevalence rates in Asians in contrast to Caucasians suggest that the former may be genetically protected against Type 1 diabetes compared with the latter. In general, in Asians, it is very common that the protective DR4 is associated with the susceptible DQ alleles while the neutral/protective DQ alleles is associated with the susceptible DR4 alleles. This counterbalancing influence between susceptible *DRB1* and protective *DQB1*, and vice versa, might be an important factor responsible for the low incidence of diabetes in Asians. As we indicated before, however, the risk of developing Type 1 diabetes in first-degree relatives of diabetic patients is much increased in Asia. Caucasian immunogenetic markers for Type 1 diabetes confer disease susceptibility among Asians, but different HLA susceptibility alleles and a lower strength of the associations are found because of the different genetic make-up of the population, or the different LD patterns compared with other racial groups [20,21]. Seropositivity to islet cell antigens appeared to be associated with the similar but different HLA genotype as in Caucasians [72]. Different genetic/environmental interactions might be involved in the etiology of Type 1 diabetes in Asia compared with Caucasians, or there may be a difference in persistence of autoantibodies in those with different genetic backgrounds.

Several studies have clearly demonstrated that both DQ and DR influence Type 1 diabetes susceptibility. It has now become evident that there are both susceptible and protective alleles at DRB1, DQA1 and DQB1 loci. Specific DQ and DR alleles are non-randomly associated with each other on what are termed extended haplotypes, the typing of which provide the best risk determinants of Type 1 diabetes. According to our data [20], the influence of a given haplotype on Type 1 diabetes susceptibility is consistent in Caucasian and Asian populations. Although the effects of these alleles are the same across different countries, ethnic differences in the prevalence of specific alleles in patients vary with population allele frequencies. Because of this, some highly 'diabetogenic' DR/DQ alleles which are relatively uncommon in a population may be mistakenly considered neutral in population association studies.

The study of the genetic susceptibility factors in Asia is also helpful in understanding the role of individual amino acids. The overall risk for Type 1 diabetes from the DR and DQ molecules is determined by combinations of polymorphic positions (alleles) and combinations of alleles (haplotypes and genotypes) in a given individual. The contribution of individual alleles at the HLA DRB1 and DQB1 loci to Type 1 diabetes susceptibility can be

unclear because of the strong LD between the two loci. Because those HLA haplotypes associated with disease frequently contain multiple sequence differences, the role of individual polymorphic amino acid residues is difficult to assess. However, identification of pairs of haplotypes in which polymorphisms encoding a single amino acid change in a single polypeptide chain result in dramatic differences in disease susceptibility (i.e. DRB1*1302-DQB1*0604 vs DRB1*1302-DQB1*0609 and DRB1*0403-DQB1*0302 vs DRB1*0407-DQB1*0302) is useful, although it is difficult and requires very large data sets from varied ethnic origins, because of the extreme polymorphism of the HLA loci, and because individual haplotypes tend to be confined to one or a few ethnic groups. As more data become available, the study of pairs of haplotypes which differ at a single polymorphic site, but which have different effects on disease susceptibility, should allow more precise definition of the amino acids involved in the disease process. Identification of the exact polymorphisms that can trigger Type 1 diabetes or protect from it will facilitate the development of biological tools with which to study the disease process.

Other novel regions could potentially harbor disease loci, but confirmation and fine-mapping can not be pursued effectively using conventional linkage analysis. Instead, more powerful LD-based and haplotype mapping approaches will be required. In particular, markers reported to be linked to Type 1 diabetes in one population are strong candidates for LD mapping by association studies in other populations. A large number of genes showed heterogeneities in different populations and/or ethnic groups. In order to confirm the genetic interval, it is necessary to identify markers in LD with it by demonstrating allelic association of the markers with Type 1 diabetes. Association of markers with Type 1 diabetes in genetically distinct populations, such as Korean, Japanese and Caucasians, should be a useful approach for fine-mapping Type 1 diabetes gene because different haplotypes segregate in different ethnic groups.

As we mentioned earlier, there are very few reports investigating susceptibility factors other than *IDDM1* and *IDDM2* region in Asians. Family-based linkage and association studies are considered essential for confirming putative associations because case-control studies are prone to population stratification. Since the incidence of Type 1 diabetes is very rare in Asia, it is not easy to find the multiplex cases of Type 1 diabetes, or to get a large data-set of incidence cases. Because of cultural differences, it is very difficult to obtain family-based samples. Because of these various reasons, we could not easily find a study trying to dissect genes determining Type 1 diabetes susceptibility using whole-genome approaches. The Human Biological Data Interchange (HBDI) is a repository for cell lines derived from families with Type 1 diabetes in Caucasians [31]. The HBDI collection was established in part for the purpose of mapping Type 1 diabetes-associated, non-HLA genes by linkage analysis. Most of the HBDI families are nuclear families with unaffected parents and at least two affected siblings. Some of these HBDI samples were used in the mapping of recently reported Type 1 diabetes genetic loci. To have a collaborative DNA collection like HBDI will help to investigate and dissect the genetic susceptibility factors in Asia and we think it might be a feasible option for Asia. Since the Japanese, Korean and Chinese populations are very homogenous, collaborative approaches using the pooled DNA repositories will overcome the problems of the small sample size of the data-set and scarcity of Type 1 diabetes.

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# References

1. Eisenbarth GS. Type 1 diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 1986; **314**: 1360–1368.
2. Thomson G. HLA disease associations: models for insulin dependent diabetes mellitus and the study of complex human genetic disorders. *Annu Rev Genet* 1988; **22**: 31–50.
3. Wolf E, Spencer KM, Cudworth AG. The genetic susceptibility to Type 1 (insulin-dependent) diabetes: analysis of HLA-DR association. *Diabetologia* 1983; **24**: 224–230.
4. Todd JA, Bell JI, McDevitt HO. HLA DQ gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 1985; **329**: 599–604.
5. Bell GI, Horita S, Karam JH. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 1984; **33**: 176–183.
6. Davies JL, Kawaguchi Y, Bennett ST, *et al*. A genome-wide search for human Type 1 diabetes susceptibility genes. *Nature* 1994; **371**: 130–136.
7. Concannon P, Gogolin-Ewens KJ, Hinds DA, *et al*. A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. *Nat Genet* 1998; **19**: 292–296.
8. Mein CA, Esposito L, Dunn MG, *et al*. A search for Type 1 diabetes susceptibility genes in families from the United Kingdom. *Nat Genet* 1998; **19**: 297–300.
9. Field LL, Tobias R, Magnus T. A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. *Nat Genet* 1994; **8**: 189–194.
10. Luo DF, Buzzetti R, Rotter JI, *et al*. Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5 and IDDM8. *Hum Mol Genet* 1996; **5**: 693–698.
11. Delepine M, Pociot F, Habita C, *et al*. Evidence of a non-MHC susceptibility locus in Type 1 diabetes linked to HLA on chromosome 6. *Am J Hum Genet* 1997; **60**: 174–187.
12. Field LL, Tobias R, Thomson G, Plon S. Susceptibility to insulin-dependent diabetes mellitus maps to a locus (IDDM11) on human chromosome 14q24.3–q31. *Genomics* 1996; **33**: 1–8.
13. Morahan G, Huang D, Tait BD, Colman PG, Harrison LC. Markers on distal chromosome 2q linked to insulin-dependent diabetes mellitus. *Science* 1996; **272**: 1811–1813.
14. Verge CF, Vardi P, Babu S, *et al*. Evidence for oligogenic inheritance of Type 1 diabetes in a large Bedouin Arab family. *J Clin Invest* 1998; **102**: 1569–1575.
15. Lernmark A, Ott J. Sometimes it's hot, sometimes it's not. *Nat Genet* 1998; **19**: 213–214.
16. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; **11**: 241–247.
17. Awata T, Kurihara S, Kikuchi C, *et al*. Evidence for association between the class I subset of the insulin gene minisatellite

 

(IDDM2 locus) and IDDM in the Japanese population. *Diabetes* 1997; **46**: 1637–1642.
18. Kawaguchi Y, Ikegami H, Shen GQ, *et al*. Insulin gene region contributes to genetic susceptibility to, but may not to low incidence of, insulin-dependent diabetes mellitus in Japanese. *Biochem Biophys Res Commun* 1997; **233**: 283–287.
19. Karvonen M, Tuomilehto J, Libman I, LaPorte R. A review of the recent epidemiological data on the worldwide incidence of Type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. *Diabetologia* 1993; **36**: 883–892.
20. Park Y, She JX, Wang C-Y, *et al*. Common susceptibility and transmission pattern of HLA DRB1-DQB1 haplotypes to Korean and Caucasian patients with type 1 diabetes. *J Clin Endocrinol Metab* 2000; **85**: 4538–4542.
21. Park YS, Wang CY, Ko KW, *et al*. Combinations of HLA DR and DQ molecules determine the susceptibility to insulin-dependent diabetes mellitus in Koreans. *Hum Immunol* 1998; **59**: 794–801.
22. She J-X. Susceptibility to Type 1 diabetes: HLA-DQ and DR revisited. *Immunol Today* 1996; **17**: 323–329.
23. Penny NA, Mijovic CH, Cavan DA, *et al*. An investigation of the association between HLA-DQ heterodimers and Type 1 (insulin-dependent) diabetes mellitus in five racial groups. *Hum Immunol* 1993; **38**: 179–183.
24. Chuang L-M, Lin BJ, Wu H-P, Tai T-Y, Tsai W-Y. Transcomplementation of HLA DQA1-DQB1 in DR3/DR4 and DR3/DR9 heterozygotes and IDDM in Taiwanese families. *Diabetes Care* 1995; **18**: 1483–1486.
25. Ikegami H, Ogihara T. Genetics of insulin-dependent diabetes mellitus. *Endocr J* 1996; **3**: 605–613.
26. Park YS, She JX, Noble J, Erlich H. Transracial evidence for the influence of the homologous DR-DQ haplotype on transmission of DR4 haplotypes to diabetic children. *Diabetes* 1999; **48** (Suppl. 2): A188.
27. Chapoval SP, Neeno T, Krco CJ, Marietta EV, Harders J, David CS. HLA-DQ6 and HLA-DQ8 transgenic mice respond to ragweed allergens and recognize a distinct set of epitopes on short and giant ragweed group 5 antigens. *J Immunol* 1998; **161**: 2032–2037.
28. Moghaddam PH, de Knijf P, Roep BO, *et al*. Genetic structure of *IDDM1*: two separate regions in the major histocompatibility complex contribute to susceptibility or protection. *Diabetes* 1998; **47**: 263–269.
29. Lie BA, Todd JA, Pociot F, *et al*. The predisposition to Type 1 diabetes linked to the human leukocyte antigen complex includes at least one non-class II gene. *Am J Hum Genet* 1999; **64**: 793–800.
30. Risch N. Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 1987; **40**: 1–14.
31. Noble JA, Valdes AM, Cook M, Klitz W, Thomson G, Erlich HA. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasians, multiplex families. *Am J Hum Genet* 1996; **59**: 1134–1148.
32. Monaco JJ, Cho S, Attaya M. Transport protein genes in the murine MHC: possible implications for antigen processing. *Science* 1990; **250**: 1723–1726.
33. Deng GY, Muir A, Maclaren NK, She JX. Association of LMP2 and LMP7 genes within the major histocompatibility complex with insulin-dependent diabetes mellitus: population and family studies. *Am J Hum Genet* 1995; **56**: 528–534.
34. Caplan NJ, Patel A, Millward A, *et al*. Complement C4 and heat shock protein 70 (HSP70) genotype and Type 1 diabetes mellitus. *Immunogenetics* 1990; **32**: 427–430.
35. Nakanishi K, Kobayashi T, Murase T, Kosaka K. Lack of association of the transporter associated with antigen processing with Japanese insulin-dependent diabetes mellitus. *Metabolism* 1994; **43**: 1013–1017.
36. Kawaguchi Y, Ikegami H, Fukuda Y, Fujioka Y, Shima K, Ogihara T. Polymorphism of HSP 70 gene is not associated with Type 1 (insulin-dependent) diabetes mellitus in Japanese. *Diabetes Res Clin Pract* 1993; **21**: 103–107.
37. Obayashi H, Hasegawa G, Fukui M, *et al*. Tumor necrosis factor microsatellite polymorphism influences the development of insulin dependency in adult-onset diabetes patients with the DRB1*1502-DQB1*0601 allele and anti-glutamic acid decarboxylase antibodies. *J Clin Endocrinol Metab* 2000; **85**: 3348–3351.
38. Fujisawa T, Ikegami H, Kawaguchi Y, *et al*. Class I HLA is associated with age-at-onset of IDDM, while class II HLA confers susceptibility to IDDM. *Diabetologia* 1995; **38**: 1493–1495.
39. Braud VM, Allan DJ, McMichael AJ. Functions of nonclassical MHC and non-MHC-encoded class I molecules. *Curr Opin Immunol* 1999; **11**: 100–108.
40. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. *Science* 1998; **279**: 1737–1740.
41. Kawabata Y, Ikegami H, Kawaguchi Y, *et al*. Age-related association of MHC class I chain-related gene A (MICA) with Type 1 (insulin-dependent) diabetes mellitus. *Hum Immunol* 2000; **61**: 624–629.
42. Park YS, Jospe E, Babu S, Eisenbarth GS. D6S2223 alleles associated with risk of IDDM in DR3-DQ2/DR4-DQ8 heterozygotes. *Diabetes* 1999; **48** (Suppl. 2): A183.
43. Park YS, Lee HK, Sanjeevi CR, Eisenbarth GS. MIC-A polymorphism is associated with type 1 diabetes in the Korean population. *Diabetes Care* 2001; **24**: 33–38.
44. Marron MP, Raffel LJ, Garchon H-J, *et al*. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum Mol Genet* 1997; **8**: 1275–1282.
45. Marron MP, Zeidler A, Raffel LJ, *et al*. Genetic and physical fine-mapping of a Type 1 diabetes susceptibility gene (IDDM12) to a 100-kb phagemid artificial chromosome clone containing D2S72-CTLA4-D2S105 on chromosome 2q33. *Diabetes* 2000; **49**: 492–499.
46. Matsushita M, Tsuchiya N, Shiota M, *et al*. Lack of a strong association of CTLA-4 exon 1 polymorphism with the susceptibility to rheumatoid arthritis and systemic lupus erythematosus in Japanese: an association study using a novel variation screening method. *Tissue Antigens* 1999; **54**: 578–584.
47. Lee YJ, Huang FY, Lo FS, *et al*. Association of CTLA4 gene A-G polymorphism with Type 1 diabetes in Chinese children. *Clin Endocrinol* 2000; **52**: 153–157.
48. Fu J, Ikegami H, Kawaguchi Y, *et al*. Association of distal chromosome 2q with IDDM in Japanese subjects. *Diabetologia* 1998; **41**: 228–232.
49. Park YS, Maes M, Fain P, Eisenbarth GS. IDDM13 polymorphisms in multiple Type 1A diabetic populations. *Diabetes* 1998; **47** (Suppl. 2): A217.
50. Tamimi R, Steingrimsson E, Copeland NG, *et al*. The NEURO D gene maps to human chromosome 2q32 and mouse chromosome 2. *Genomics* 1996; **34**: 418–421.
51. Iwata I, Nagafuchi S, Nakashima H, *et al*. Association of polymorphism in the NeuroD/BETA2 gene with Type 1 diabetes in the Japanese. *Diabetes* 1999; **48**: 416–419.
52. Marron MP, Hopkins DI, Park YS, She JX. NeuroD/Beta2 polymorphism is not associated with Type 1 diabetes in Chinese, Korean, or Caucasian populations. *Pediatric Research* (in press).
53. Tisch R, McDevitt H. Insulin-dependent diabetes mellitus. *Cell* 1996; **85**: 291–297.
54. Ikegami H, Makino S, Yamato E, *et al*. Identification of a new susceptibility locus for insulin-dependent diabetes mellitus by ancestral haplotype congenic mapping. *J Clin Invest* 1995; **96**: 1936–1942.
55. Nepom BS, Nepom GT, Coleman M, Kwok WK. Critical contribution of beta chain residue 57 in peptide binding ability of both HLA-DR and -DQ molecules. *Proc Natl Acad Sci U S A* 1996; **93**: 7202–7206.
56. Congia M, Patel S, Cope AP, De Virgiliis S, Sonderstrup G. T Cell epitopes of insulin defined in HLA-DR4 transgenic mice are derived from preproinsulin and proinsulin. *Proc Natl Acad Sci U S A* 1998; **95**: 3833–3838.
57. Wicker LS, Chen SL, Nepom GT, *et al*. Naturally processed T cell epitopes from human glutamic acid decarboxylase identified using mice transgenic for the Type 1 diabetes-associated human MHC class II allele, DRB1*0401. *J Clin Invest* 1996; **98**: 2597–2603.
58. Patel SD, Cope AP, Congia M, *et al*. Identification of immunodominant T cell epitopes of human glutamic acid decarboxylase65 by using HLA-DR (alpha1*0101, beta1*0401) transgenic mice. *Proc Natl Acad Sci U S A* 1997; **94**: 8082–8087.
59. Wen L, Wong FS, Tang J, *et al*. *In vivo* evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J Exp Med* 2000; **191**: 97–104.
60. Kristiansen OP, Pociot F, Johannesen J, *et al*. Linkage

 

disequilibrium testing of four interleukin-1 gene-cluster polymorphisms in Danish multiplex families with insulin-dependent diabetes mellitus. *Cytokine* 2000; **12**: 171–175.
61. Ito M, Tanimoto M, Kamura H, *et al*. Association of HLA-DR phenotypes and T-lymphocyte-receptor beta-chain-region RFLP with IDDM in Japanese. *Diabetes* 1988; **37**: 1633–1636.
62. Field LL, Dizier MH, Anderson CE, Spence MA, Rotter JI. HLA-dependent GM effects in insulin-dependent diabetes: evidence from pairs of affected siblings. *Am J Hum Genet* 1986; **39**: 640–647.
63. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**: 506–516.
64. Pugliese A, Zeller M, Fernandez Jr A, *et al*. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for Type 1 diabetes. *Nat Genet* 1997; **15**: 293–297.
65. Esposito L, Hill NJ, Pritchard LE, *et al*. Genetic analysis of chromosome 2 in Type 1 diabetes: analysis of putative loci *IDDM7*, *IDDM12*, and *IDDM13* and candidate genes NRAMP1 and IA-2 and the interleukin-1 gene cluster. IMDIAB Group. *Diabetes* 1998; **47**: 1797–1799.
66. Park YS, Kawasaki E, Keleman K, *et al*. Humoral autoreactivity to an alternatively spliced variant of ICA512/IA-2 in Type 1 diabetes. *Diabetologia* 2000; **43**: 1293–1301.
67. Diez J, Park YS, Zeller M, *et al*. Differential splicing of the IA-2 gene as a potential factor predisposing to autoimmune responses against the IA-2 molecule. *Autoimmunity* 1999; **27** (Suppl 1): A45.
68. Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated withGraves' disease in a Caucasian population. *J Clin Endocrinol Metab* 1995; **80**: 41–45.
69. Takahashi T, Tagami T, Yamazaki S, *et al*. Immunologic self-tolerance maintained by CD25(+) CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000; **192**: 303–310.
70. Park YJ, Chung HK, Park DJ, *et al*. Polymorphism in the promoter and exon 1 of the cytotoxic T lymphocyte antigen-4 gene associated with autoimmune thyroid disease in Koreans. *Thyroid* 2000; **10**: 453–459.
71. Takara M, Komiya I, Kinjo Y, *et al*. Association of CTLA-4 gene A/G polymorphism in Japanese Type 1 diabetic patients with younger age of onset and autoimmune thyroid disease. *Diabetes Care* 2000; **23**: 975–978.
72. Park YS, Yang SW, Bao F, *et al*. Differential expression of autoimmunity in IDDM patients of Korea compared to the US. *Diabetes* 1998; **47** (Suppl 2): A198.

 