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HLA-DQ Locus of the Human Leukocyte Antigen Complex and Type 1 Diabetes Mellitus: A HuGE Review*

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GENE

The human leukocyte antigen (HLA) complex is located on the short arm of chromosome 6 at p21.3 (1–4). It encompasses approximately 3,500 kilobases of DNA and contains at least 150 genes. It is the primary region of susceptibility for type 1 diabetes mellitus, as well as other autoimmune disorders. Recent genome screens have designated the class II subregion (i.e., the *HLA-DR*, *HLA-DQ*, and *HLA-DP* loci) *IDDM1*.

The *DQ* locus, which is the focus of this review, consists of two tightly linked genes (*DQA1* and *DQB1*) that encode α and β glycoproteins, respectively. These molecules combine noncovalently to form functional α-β heterodimers. The *DQA1* and *DQB1* genes are highly polymorphic. Allelic variation is observed primarily in the second exon, which corresponds to the peptide-binding cleft. *HLA-DQ* and other class II molecules present extracellular antigens to helper T cells and stimulate the body's immune response. They have a restricted tissue distribution and are located mainly on macrophages, B cells, and activated T cells.

Transcription of *DQA1* and *DQB1* is complex and involves *cis*- and *trans*-acting factors. Critical upstream regulatory sequences have been reported for *DQA1* and *DQB1*. Variation in promoter sequences affects gene expression and may also be involved in the pathogenesis of autoimmune disorders. In addition, posttranscription activities appear to influence disease risk. For example, functional *DQαβ* heterodimers can be formed from the noncovalent association of products of *DQA1* and *DQB1* genes in *cis* (5). Alternatively, the combined α and β glycoproteins can represent molecules encoded by genes in *trans*. Hybrid *DQ* molecules with *DR* or *DP* glycoproteins have also been observed.

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Abbreviations: HLA, human leukocyte antigen; TRIGR, Trial to Reduce Type 1 Diabetes in the Genetically At-Risk; WHO, World Health Organization.

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GENE VARIANTS

DQA1 alleles

There are eight major allelic variants of the *HLA-DQA1* gene (table 1). The data presented here are based on the World Health Organization (WHO) DiaMond Molecular Epidemiology Project (6). The populations included represent those with similar race-specific incidence rates. All recruited case patients were diagnosed between 1979 and 1990 and were identified from type 1 diabetes incidence registries established according to the WHO DiaMond Molecular Epidemiology Project protocol (7). Registration criteria included: 1) being diagnosed by a physician with type 1 diabetes, 2) being placed on daily insulin injection before one's 15th birthday, and 3) being a resident of the registration area at the time of the first insulin administration. The selected case patients were aged 18 years, on average, and approximately half were males.

Unrelated nondiabetic controls were identified for each participating center using a standardized computer program that selected a simple random sample stratified by age and sex. This method permitted the identification of representative controls who were at risk during the time the registry was established. They were recruited from consecutive hospital admissions for injuries/surgeries or from the local population. Because of ethical concerns, all controls were required to be aged 15 years or older at the time of evaluation. As a group, they were somewhat older than the cases (aged 22 years, on average). Approximately half were males. The target sample sizes for the case and control groups were 100.

Published frequencies from the 12th International Histocompatibility Workshop and Conference are also included in table 1 (8). These represent pooled estimates from approximately 20 Caucasian populations worldwide. Cases were defined according to National Diabetes Data Group criteria for type 1 diabetes and were not restricted by age at onset. Proportions of cases diagnosed before age 15 years, between the ages of 15 and 30 years, and after age 30 years were 66 percent, 23 percent, and 11 percent, respectively. Approximately half of the case patients were males. Controls were drawn from the same ethnic and geographic populations as the cases. However, eligibility and selection criteria were not defined, nor were they standardized across populations.

In addition to the information provided in table 1, there is a plethora of case-control studies regarding *HLA-DQ* associ-

TABLE 1. Frequencies of *HLA-DQA1*† alleles among case patients with type 1 diabetes and unrelated nondiabetic controls from the World Health Organization DiaMond Molecular Epidemiology Project (6) and the 12th International Histocompatibility Workshop and Conference (8)

DQA1 allele	DiaMond Molecular Epidemiology Project						12th workshop and conference	
	Caucasians‡		African Americans‡		Asians§			
	Cases (n = 163)	Controls (n = 192)	Cases (n = 99)	Controls (n = 152)	Cases (n = 207)	Controls (n = 168)	Cases (n = 1,820)	Controls (n = 1,936)
**0101*	0.10	0.15	0.09	0.16	0.08	0.11	0.07	0.14
**0102*	0.09*	0.22	0.12*	0.32	0.09*	0.19	0.06	0.19
**0103*	0.02	0.05	0.02	0.04	0.04*	0.14	0.02	0.08
**0201*	0.02*	0.12	0.03	0.07	0.02	0.02	0.04	0.10
**0301*¶	0.44*	0.16	0.42*	0.11	0.64*	0.37	0.40*	0.16
**0401*¶	0.04	0.05	0.03*	0.11	0.04	0.03	0.03	0.03
**0501*¶	0.28	0.25	0.29	0.19	0.08	0.12	0.37	0.28
**0601*¶						0.01	0.01	0.01

* $p < 0.05$ for cases versus controls (corrected for multiple comparisons).
† HLA, human leukocyte antigen.
‡ From Jefferson County, Alabama and Allegheny County, Pennsylvania.
§ From Hokkaido, Japan and Seoul, Korea.
¶ Allele with arginine in position 52.

ations and type 1 diabetes for Caucasian and other ethnic groups, many of which are not based on epidemiologic methods. Cases were generally selected from convenience samples (i.e., hospital clinics or medical practices). This can lead to an overrepresentation of multiplex families, as well as the inclusion of people with type 2 diabetes who use insulin in their treatment regimen. Control frequencies were often determined from blood donors or other groups that may not truly reflect the population at risk. These investigations also varied in terms of the approaches utilized for HLA molecular typing. Because of these inconsistencies, the results have not been included in table 1. The reader is referred elsewhere (9–18) for additional information regarding *HLA-DQ* and type 1 diabetes in non-Caucasian populations.

As table 1 illustrates, the frequencies of the *DQA1* alleles varied across ethnic groups. Significant differences were observed between type 1 diabetic and nondiabetic individuals from the same population. *DQA1* alleles coding for arginine at position 52 (Arg-52) have been associated with susceptibility to type 1 diabetes (19–21). Although there are four *DQA1*Arg-52* alleles, only *DQA1*0301* has been shown to have a significant independent effect on type 1 diabetes risk. In some populations, *DQA1*0501* is also associated with the disease. However, in other areas, this allele is neutral. Other than *DQA1*0102*, no *DQA1* variant is an independent protective marker for type 1 diabetes.

DQB1 alleles

Nineteen major *DQB1* alleles have been identified. Their frequency also varies by ethnicity, as is shown in table 2 for the WHO DiaMond Molecular Epidemiology Project (6) and the 12th International Histocompatibility Workshop and Conference (8). Significant differences between persons with type 1 diabetes and healthy unrelated controls have been observed. DNA sequences coding for an amino acid other than aspartic acid in position 57 (non-Asp-57) have been associated with type 1 diabetes in all ethnic groups (19–22) except the Japanese (22, 23). In particular, *DQB1*0302* and *DQB1*0201*, which are in linkage disequilibrium with *DR4* and *DR3*, respectively, have been consistent susceptibility markers for type 1 diabetes. Other non-Asp-57 alleles (i.e., *DQB1*0501*, **0502*, **0604*, and **0605*) were generally neutral. *DQB1*0602*, which codes for aspartic acid in position 57, was significantly less prevalent among cases than among controls.

DQA1-DQB1 haplotypes

Some researchers have suggested that *DQB1* polymorphisms may be more important than those in the *DQA1* gene for determining peptide binding specificity (24, 25). However, variation in both genes contributes to susceptibility to type 1 diabetes. In particular, the *DQA1*0501-DQB1*0201* and *DQA1*0301-DQB1*0302* haplotypes confer the highest type 1 diabetes risks. In combination, their effect is even stronger than that observed for individuals who are homozygous for *DQA1*0501-DQB1*0201* or *DQA1*0301-DQB1*0302*, which suggests that heterodimers formed from gene products in *trans* (i.e., *DQA1*0501* and *DQB1*0302*) may be particularly diabetogenic (5). Other high risk *DQ* haplotypes for type 1 diabetes include *DQA1*0301-DQB1*0201* in African Americans, *DQA1*0301-DQB1*0303* in the Japanese, and *DQA1*0301-DQB1*0401* in the Chinese (26). The *DQA1*0102-DQB1*0602* haplotype is protective and is associated with a reduced risk for type 1 diabetes in most populations.

DISEASE

Age, gender, and time

In the United States, the prevalence of type 1 diabetes is approximately 2 per 1,000 for children under age 20 years (27). This rate is higher than rates reported for other child-

TABLE 2. Frequencies of *HLA-DQB1*† alleles among case patients with type 1 diabetes and unrelated nondiabetic controls from the World Health Organization DiaMond Molecular Epidemiology Project (6) and the 12th International Histocompatibility Workshop and Conference (8)

DQB1 allele	DiaMond Molecular Epidemiology Project						12th workshop and conference	
	Caucasians‡		African Americans‡		Asians§			
	Cases (n = 163)	Controls (n = 192)	Cases (n = 99)	Controls (n = 152)	Cases (n = 207)	Controls (n = 168)	Cases (n = 1,820)	Controls (n = 1,936)
**0201*¶	0.30	0.22	0.46*	0.18	0.07	0.04	0.40*	0.22
**0301*	0.10*	0.17	0.09*	0.19	0.07*	0.18	0.06	0.20
**0302*¶	0.31*	0.09	0.18*	0.03	0.18	0.10	0.33*	0.08
**0303*	0.02	0.05	0.03	0.01	0.24*	0.10	0.02	0.04
**0401*					0.21*	0.10		
**0402*	0.04	0.04	0.01*	0.07	0.01	0.03	0.03	0.03
**0501*¶	0.10	0.13	0.11	0.16	0.08	0.08	0.07	0.12
**0502*¶	0.02	0.03	0.02	0.02	0.005	0.01	0.02	0.04
**0503*	0.003	0.03	0.01	0.01	0.01	0.03		0.04
**0601*		0.003			0.05*	0.16		0.01
**0602*	0.01*	0.13	0.05*	0.23	0.01*	0.07	0.01	0.10
**0603*	0.02	0.05	0.02	0.04	0.002	0.02	0.01	0.07
**0604*¶	0.06	0.03	0.02	0.03	0.05	0.06	0.03	0.04
**0605*¶	0.01	0.03	0.01	0.02	0.01	0.02		0.01

* $p < 0.05$ for cases versus controls (corrected for multiple comparisons).
† HLA, human leukocyte antigen.
‡ From Jefferson County, Alabama and Allegheny County, Pennsylvania.
§ From Hokkaido, Japan and Seoul, Korea.
¶ Allele with an amino acid other than aspartic acid in position 57 (non-Asp-57).

hood chronic disorders, such as cystic fibrosis, juvenile arthritis, etc. The onset of type 1 diabetes can occur at any age, but it is usually diagnosed during childhood and adolescence, with a peak incidence around the time of puberty. This pattern has been reported for most populations throughout the world.

Type 1 diabetes incidence rates are similar for males and females, although a female preponderance has been noted in low risk populations, such as the Japanese (28). An excess risk for males has been observed in some areas where the overall incidence is high, such as Finland. There is also a notable seasonal variation in the incidence of type 1 diabetes in most countries. Lower rates have been reported for late spring and summer, but rates are higher in the winter for populations in both the Northern and Southern hemispheres (29).

Temporal trends in incidence have recently been observed. A significant increase in type 1 diabetes incidence has been reported by many population-based registries in Northern and Central Europe (30, 31), as well as in Asian and Western Pacific populations (32, 33). In addition, epidemics of type 1 diabetes, such as the one that occurred in the Virgin Islands during the mid 1980s (34), have been observed.

Race and geography

Type 1 diabetes also exhibits dramatic geographic and ethnic variation. The highest incidence rates in the world (>35/100,000 population per year) have been reported for Finland and Sardinia, Italy (35). The lowest incidence rates are observed in Asian countries (<3/100,000 per year), including Japan, China, and Korea. Native American, Cuban, Chilean, and Mexican populations also have extremely low rates of type 1 diabetes. In most other Caucasian populations in Europe and the Americas, incidence rates are moderate (~10–20/100,000 per year).

Ethnic variations in risk for populations residing in the same geographic area have been observed for type 1 diabetes (35). African Americans and Hispanics generally have lower incidence rates than Caucasians living in the same community. Ethnic variations are also apparent in China, an extremely low risk country represented by more than 50 ethnic groups. In 1998, Yang et al. (36) reported annual incidence rates of 0.3/100,000 per year and 1.8/100,000 per year, respectively, for the Zhuang and Mongol ethnic groups. Similar findings were observed for Finland, an extremely high risk country, where the rates ranged from 4/100,000 per year to 245/100,000 per year (37). Unlike the population of China, the Finnish population is more genetically homogeneous. Reasons for these geographic and ethnic differences in type 1 diabetes incidence are currently being investigated; they appear to reflect differences in both genetic and environmental risk factors (6).

Risk factors

Viruses. Viruses have been implicated in the etiology of type 1 diabetes for the past several decades (38). They are thought to act as initiators, accelerators, or precipitators of the disease, and they may function through direct or indirect mechanisms. Viruses may attack and destroy the β cells of

the pancreas and directly cause diabetes, with or without autoimmunity (39). Alternatively, viruses may initiate or potentiate an autoimmune response against β cells through molecular mimicry or "bystander" autoimmune activation, the latter of which results from the induction of inflammatory cytokines after infection.

Many epidemiologic investigations have supported the involvement of Coxsackie virus B in the etiology of type 1 diabetes (40). The most recent studies were based on molecular analyses, and they revealed positive associations between the presence of enteroviral mRNA and the development of β cell autoimmunity (41) and type 1 diabetes (42, 43). Sequence homology between a highly conserved nonstructural Coxsackie virus B4 protein (P2C) and glutamic acid decarboxylase, a potential diabetes autoantigen, has been reported (44). Several investigations indicated that antibodies to P2C and glutamic acid decarboxylase cross-reacted (43, 44). However, P2C antibodies were also observed among healthy controls who were glutamic acid decarboxylase-negative (45), which suggests that the response was not specific for type 1 diabetes.

Other viruses have also been associated with type 1 diabetes. Finnish investigators observed an increase in the incidence of type 1 diabetes 2–4 years after a mumps epidemic (46). Although the type and variant of the virus is clearly important, age at exposure may also influence disease risk. Recent studies have shown that exposure to enteroviruses in utero increases the risk of developing the disease (47, 48). Moreover, 10–20 percent of children with congenital rubella, particularly those who carry high risk HLA alleles, develop autoimmune type 1 diabetes (49). Thus, early viral exposure appears to be particularly diabetogenic.

Infant nutrition. Ecologic analyses have shown positive correlations between type 1 diabetes incidence and average milk consumption/breastfeeding rates across populations (50). In addition, many case-control studies demonstrated weak positive associations between exposure to milk at an early age (<3 months) and type 1 diabetes (odds ratio from meta-analysis = 1.4; 95 percent confidence interval: 1.2, 1.6) (51). Although breastfeeding itself may be protective, it was hypothesized that the observed effect may indirectly reflect exposure to dietary proteins when the infant's gut is not completely developed and is still permeable by antigenic peptides (52).

In 1992, Karjalainen et al. (53) demonstrated that virtually all Finnish children with newly diagnosed diabetes had elevated levels of immunoglobulin G antibodies to the whey protein bovine serum albumin. Most of the antibodies were specific to the 17-amino-acid peptide ABBOS. The ABBOS peptide and p69, an islet cell surface protein, are similar in sequence. The authors therefore suggested that early exposure to cow's milk triggers an immune response that may lead to β cell autoimmunity because of molecular mimicry (53). However, the negative T cell proliferation studies in response to cow's milk antigen led Atkinson et al. (54) to question the molecular mimicry hypothesis. In addition, conflicting evidence came from recent reports of similar cellular responses to β casein among type 1 diabetic and nondiabetic subjects (55). Concern has also been raised by a short term natural history study that showed no association between infant feeding patterns and the development of β cell autoimmunity (56). Thus, the role of infant diet in type 1 diabetes is far from clear.

ASSOCIATIONS

One of the major issues in molecular epidemiology relates to the definition of genetic susceptibility. For type 1 diabetes, relevant questions include: What loci, alleles, and/or haplotypes should be considered high risk? How should they be determined? Are there protective and/or neutral alleles or haplotypes that should also be evaluated? These questions are not at all trivial; and for type 1 diabetes, they have been debated among epidemiologists, geneticists, immunologists, and clinicians for many years. Despite numerous discussions, there is no uniform agreement on the "best" definition of genetic susceptibility for type 1 diabetes. For the purposes of this review, we will outline the rationale for the strategy proposed for the WHO DiaMond Molecular Epidemiology Project (6, 57). This approach considered biologic significance, cost-effectiveness, and the statistical properties of the data.

Although the class II region has been designated *IDDM1*, the strong linkage disequilibrium between *DRB1* and *DQB1* has made it difficult to assess the contribution of *HLA-DQ* independent of that of *HLA-DR*. This issue has been addressed by examining case-control differences in linkage disequilibrium for haplotypes containing high risk *DQ* alleles (i.e., *DQA1*Arg-52*, *DQB1*non-Asp-57*) but low risk *DR* alleles (i.e., not *DR3* or *DR4*). Persons with type 1 diabetes had a significantly greater frequency of high risk *DQ* alleles in low risk *DR* haplotypes than controls, suggesting that *DQ* better defined susceptibility haplotypes than *DR* (58). Thus, the *HLA-DQ* locus has been considered to be the strongest single genetic marker for type 1 diabetes, particularly among Caucasians (59).

As indicated above, polymorphisms in the *DQA1* and *DQB1* genes appear to be of biologic importance and are probably involved in the etiology of the disease. However, analyses of allelic associations within ethnic groups revealed that not all *DQA1*Arg-52* and *DQB1*non-Asp-57* alleles increased type 1 diabetes risk. Moreover, in the Asian populations, *DQB1*Asp-57* alleles were significantly more common among cases with type 1 diabetes than among controls. Thus, defining type 1 diabetes susceptibility alleles by *DQA1*Arg-52* and *DQB1*non-Asp-57* was not accurate.

For the WHO DiaMond Molecular Epidemiology Project, therefore, genetic susceptibility was defined for each population and was based on the *DQA1* and *DQB1* alleles/haplotypes that were statistically significantly increased among cases compared with controls (tables 1 and 2). All other alleles were considered neutral/protective for that population. This method accounted, in part, for the contributions of specific *DRB1* alleles that were not directly evaluated but were known to be in linkage disequilibrium with high risk *DQA1-DQB1* haplotypes. It also permitted a more accurate classification of disease susceptibility genes than approaches previously employed. It was therefore established that *DQA1*

and *DQB1* alleles would be the minimum analysis required for the WHO DiaMond Molecular Epidemiology Project. However, *DRB1* typing was also recommended, particularly in non-Caucasian populations.

Associations of type 1 diabetes with *DQA1-DQB1* genotypes in the WHO DiaMond Molecular Epidemiology Project (6) are presented in table 3. Comparisons of odds ratios revealed statistically significant dose-response relationships (i.e., larger odds ratios for homozygotic susceptibles (S/S) than for heterozygotic susceptibles (S/P) relative to homozygotic nonsusceptibiles (P/P)). Although these data present useful information regarding the strength of disease associations, cumulative incidence rates (i.e., absolute risk estimates) for specific genotypes can be more meaningful from a clinical and public health perspective. They represent the actual risk of developing the disease during a specific time period and are determined directly from prospective studies. However, they also can be estimated from population-based case-control studies conducted in areas where the overall incidence of type 1 diabetes is known (60).

This approach was employed for the WHO DiaMond Molecular Epidemiology Project (6) (table 3). Caucasians and African Americans with the SS genotype had approximately a 3 percent chance of developing type 1 diabetes by age 30 years. Interestingly, this risk was similar to the rate observed for siblings of affected individuals, who are typically considered to be at high risk of developing the disease. Thus, using molecular *DQA1* and *DQB1* typing, one can identify a subgroup of individuals in the general Caucasian population who have a risk for type 1 diabetes similar to that of first degree relatives. These markers were much less predictive in the Asian groups, in which the disease is rare.

Population attributable fractions for type 1 diabetes also provide important information regarding potential public health implications for disease prevention strategies, since they reflect the proportion of the total population incidence that can be attributed to genetic susceptibility. Table 3 includes population attributable fractions for Caucasians, African Americans, and Asians from the WHO DiaMond Molecular Epidemiology Project. Population attributable fractions were lower for the Asian groups than for the Caucasian or African-American groups. This indicates that the *DQ* genotypes, as defined for the study, are better genetic markers in areas where the overall disease incidence is higher. Therefore, disease interventions in genetically susceptible individuals would probably have a greater impact among Caucasians or African Americans than among Asians.

INTERACTIONS

HLA-DQ and *HLA-DR*

Although recent studies have concluded that *DQA1-DQB1* haplotypes are the primary markers of susceptibility for type 1 diabetes, their effect can be modified by *DRB1*. Studies investigating this issue have examined differences in *DRB1*04* alleles among *DQA1*0301-DQB1*0302*-positive cases and controls. In Caucasians, the *DRB1*0401-DQA1*0301-DQB1*0302* haplotype has been shown to be increased in frequency among type 1 diabetes patients compared with controls (61). However, in combination with *DQA1*0301-DQB1*0301*, *DRB1*0401* was negatively associated with the disease. Thus, it is unlikely that *DRB1*0401* confers an independent risk for type 1 diabetes.

Studies of the *DRB1*0404-DQA1*0301-DQB1*0302* haplotype have yielded conflicting results. Positive (61, 62) and negative (63–65) associations have been observed. These discrepancies may be related to ethnic differences in linkage disequilibrium with B locus alleles, such as B39, which was significantly increased among *DRB1*0404-DQB1*0302* cases compared with controls in Estonia, Latvia, and Russia (64). *DRB1*0405-DQA1*0301-DQB1*0302* appears to be significantly more common among persons with type 1 diabetes than among controls in groups such as Mexican Americans (18). Variations in the peptide-binding motifs, which are reflected by the *DRB1*04* polymorphisms, may be the primary determinants of risk differences associated with the *DRB1*04-DQA1*0301-DQB1*0302* haplotype (66).

HLA-DQ and the insulin gene

In Caucasians, it has been demonstrated that the insulin gene region (*INS*), located on chromosome 11p15.5, contains the second major susceptibility locus for type 1 dia-

TABLE 3. Relative and absolute risks and population attributable fractions for homozygotic susceptibles (S/S), heterozygotic susceptibles (S/P), and homozygotic nonsusceptibles (P/P) from the World Health Organization DiaMond Molecular Epidemiology Project (6)

Population	Odds ratio			Absolute risk (%)†			Population attributable fraction (%)	
	S/S	S/P	P/P	S/S	S/P	P/P	S/S	S/S or S/P
Caucasians‡	15.9*	4.0	1.0	2.6	0.7	0.2	36.2	66.6
African Americans§	44.8*	7.3	1.0	3.1	0.5	0.1	43.5	74.9
Asians¶	10.7*	3.6	1.0	0.2	0.1	0.02	18.8	53.3

* $p < 0.0001$ (test for trend).
† Percentage through age 30 years.
‡ S = *DQA1*0301-DQB1*0302, DQA1*0501-DQB1*0201*, and *DQA1*0301-DQB1*0201*.
§ S = *DQA1*0301-DQB1*0302, DQA1*0501-DQB1*0201*, and *DQA1*0301-DQB1*0201*.
¶ S = *DQA1*0301-DQB1*0401* and *DQA1*0301-DQB1*0303*.

betes (i.e., *IDDM2*) (67). Positive associations have been observed with a nontranscribed minisatellite region (*VNTR*) in the 5′ flanking region. There are two common alleles; the shorter class I allele predisposes a person to type 1 diabetes, while the longer class III allele appears to be protective. The biologic plausibility of these associations may relate to the expression of insulin mRNA in the thymus (68). Class III alleles generate higher levels of insulin mRNA than class I alleles. These differences may contribute to a better immune tolerance for class III-positive individuals by increasing the likelihood of negative selection for autoreactive T cell clones.

The effect of *INS* appears to vary by ethnicity. Undlien et al. (69) found that class I *INS* alleles were significantly associated with type 1 diabetes in Caucasians, borderline significant in Tanzanian Blacks, and nonsignificant in Japanese. In contrast, Kawaguchi et al. (70) found a significant positive association between *INS* class I alleles and type 1 diabetes in the Japanese. Methodological differences, such as heterogenous case and control groups, and variations in allele frequencies in the general populations may be responsible for the inconsistencies in the literature.

Interaction between *INS* and *HLA-DR,DQ* has also been explored. Several groups observed an association between the *INS* class I alleles and type 1 diabetes, but only in the presence of low/moderate *DQ* genotypes (71, 72). However, other investigators observed no difference in risk for *INS* class I alleles in analysis stratified by *HLA-DQ* (73, 74). There is also a report of an association with the *INS* gene in the presence of *DR4* (75). These contradictory findings suggest that further investigation of the *INS* gene and type 1 diabetes is warranted.

HLA-DQ and infant nutrition

Most investigations of cow's milk exposure as a potential environmental trigger for type 1 diabetes were based on controls from the general population, many of whom were not genetically susceptible. Given the importance of allele-specific differences in immune response, failure to control for high risk HLA genotypes may obscure the effect of environmental risk factors. One of the first studies to address this issue was conducted in Denver, Colorado, where it was observed that exposure to cow's milk was associated with type 1 diabetes among individuals with high risk *DQB1* genotypes but not those with low risk genotypes (76). Individuals who were homozygous for *DQB1*non-Asp-57* alleles and were exposed to cow's milk at an early age had a markedly increased risk (odds ratio = 11.3, $p < 0.05$) compared with nonsusceptible, unexposed individuals. This was greater than the estimated relative risk reported for nonsusceptible but exposed persons (odds ratio = 3.7, $p > 0.05$). Subsequent reports revealed similar findings (77, 78). However, it remains to be determined whether the effect of breastfeeding is independent of or modified by high risk HLA genotypes.

LABORATORY TESTS

Serologic methods

Prior to the development of molecular techniques, serologic typing for *HLA-DR* and *HLA-DQ* was the standard for the field. This method required living B cells obtained from peripheral blood samples that were subsequently tested against known antisera. However, serologic evaluations were limited by unsuccessful or inaccurate results in approximately 11 percent of samples because of technical problems, lack of specific typing reagents, and cross-reactivity between alleles (79). These issues were resolved by the implementation of molecular typing methods. Compared with serologic approaches, molecular tests revealed discrepancies, which were, in part, allele-specific (80, 81). However, direct sequencing confirmed the results of the molecular studies. Reproducibility of molecular *DQB1* typing methods has also been demonstrated (82).

Molecular methods

Several techniques have been developed for *HLA-DQ* molecular typing based on genomic DNA, which can be easily isolated from a variety of sources (i.e., lymphocytes, dried blood spots, buccal brushes, etc.). These include restriction fragment length polymorphism analysis or methods based on polymerase chain reaction, the latter of which is most often utilized at the present time (81). Resolution with polymerase chain reaction methods depends on the amplification region (defined by the primers), as well as the number and specificity of the oligonucleotide probes used for hybridization. The WHO DiaMond Molecular Epidemiology Project employed sequence-specific oligonucleotide probes for *DQA1* and *DQB1* molecular typing (6), as utilized for the National Marrow Donor Program (81).

POPULATION TESTING

Screening in families

At present, there is no cure for type 1 diabetes, and lifelong insulin therapy is the only available treatment. However, several large clinical trials have begun to evaluate a variety of primary and secondary interventions in family members. Eligible persons are identified from families of affected probands using one of two general strategies. The first involves screening for high risk *HLA-DQ* alleles. The Trial to Reduce Type 1 Diabetes in the Genetically At-Risk (TRIGR) screens newborns for *DQB1*0302*, *DQB1*0201*, *DQB1*0602/*0603*, and *DQB1*0301* (83). Although this approach does not permit exact genotyping, it can be used to identify individuals at high risk (i.e., those who are positive for *DQB1*0302* and/or *DQB1*0201*) and low risk (i.e., those who are positive for *DQB1*0602/*0603* and/or *DQB1*0301*) for purposes of inclusion and exclusion, respectively. Persons at high risk are eligible for randomization. In TRIGR, the intervention group receives a casein hydrolysate formula and/or breast milk during the first 6 months of life. The control group receives a typical cow's milk formula.

The second screening approach in families is based on an initial evaluation of β cell autoantibodies (i.e., islet cell antibody, glutamic acid decarboxylase, IA2, insulin autoantibodies). Although these antibodies are rarely observed among first degree relatives (~2–5 percent) and not all antibody-positive individuals develop the disease, those who are positive for multiple autoantibodies appear to be at very high risk (84, 85). Some studies have estimated the positive predictive value associated with two and three autoantibodies as 65 percent and >90 percent, respectively. Thus, current investigations are adopting a two-stage screening strategy, with an initial test for one or two autoantibodies (86–88). Persons who test positive are subsequently evaluated for additional immunologic, metabolic, or genetic tests. Because the development of β cell autoantibodies is sequential, family members must be screened at regular follow-up intervals to detect the appearance of multiple autoantibodies.

Screening in the general population

Although most intervention trials are based on first degree relatives, approximately 90 percent of persons who develop type 1 diabetes have a negative family history. Thus, for interventions to have a public health impact, they must be based on the general population. Unfortunately, the genetic screening strategies described above are not as effective in the general population as they are in families of affected probands (89). For example, in Finland, sensitivity estimates for high risk alleles for siblings and the general population were 38 percent and 24 percent, respectively. Corresponding figures for specificity were 86 percent and 39 percent, respectively. However, one clinical trial based on screening in the general population was recently initiated.

The Diabetes Prediction and Prevention Project is designed to determine whether it is possible to delay the clinical manifestations of type 1 diabetes by at least 3 years in high risk Finnish newborns through the administration of nasal insulin (90). This is the only intervention currently being targeted toward the general population, and the screening strategy employed by the Diabetes Prediction and Prevention Project is based on that developed for TRIGR. Persons at high genetic risk are randomized. The treatment group receives daily intranasal insulin at a dose of 1 IU/kg. The control group is observed and receives no intervention. Recruitment is expected to be complete by 2002, with final results available in 2005.

The Diabetes Autoimmunity Study in the Young is a natural history study which is also based on newborn screening for high risk HLA class II alleles (91). This investigation involves families of varied ethnic backgrounds (Hispanic, non-Hispanic White, Black, and Asian). The susceptibility alleles required for inclusion were defined as *DRB1*03*, *DRB1*04*, and *DQB1*0302*; *DRB1*15/*16* (*DR2*) was considered an exclusion criterion. Since the study is in its early phases, the sensitivity and specificity of the screening have yet to be determined.

In summary, *HLA-DQ* screening for type 1 diabetes is now being conducted in high risk families and the general population for intervention trials and natural history studies. Thus, there is a critical need to reconsider the risks, benefits, ethics, and legal and social issues regarding genetic and/or autoantibody testing for type 1 diabetes. In addition, population-based risk factor-specific incidence rates are urgently needed for all ethnic groups. Translating research findings for prediction and prevention outside a research environment also requires genetic counseling and genetic education programs for type 1 diabetes family members, as well as health care professionals. During the next millennium, these issues should be among the top priorities in type 1 diabetes research.

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APPENDIX TABLE. Internet sites pertaining to the *HLA-DQ* locus of the human leukocyte antigen (HLA) complex and type 1 diabetes mellitus

World Wide Web URL	Description of site
HLA-DQ locus	
http://depts.washington.edu/rhwlab/dq/intro.html	Contains a description of the *HLA-DQ* molecule and its function, and excellent graphics of its structure.
http://www.whfreeman.com/immunology/CH09/kuby09.htm	Contains information on the major histocompatibility complex.
http://www-ermm.cbcu.cam.ac.uk/smc/fig004smc.htm	Contains a description and figure outlining degradation and transport of the antigens that bind MHC class II molecules.
http://www.ultranet.com/~jkimball/BiologyPages/H/HLA.html	Contains a description of HLA class I and class II molecules, with links to a glossary of useful immunogenetic terms and models.
http://www.anthonynolan.com/HIG	Home page of the HLA Informatics Group. Contains information regarding HLA sequences and nomenclature, and full lists of assigned alleles. This web site is updated monthly.
http://www.umds.ac.uk/tissue/bshi1.html	Home page of the British Society for Histocompatibility and Immunogenetics. Includes useful links to standards, sequence data, laboratories, etc.
http://www.cbi.pku.edu.cn/GenomeWeb/prot-antibodies.html	Contains a collection of links related to HLAs, histocompatibility, immunogenetics, etc.
http://www.swmed.edu/home_pages/ASHI/ashi.htm	Home page of the American Society for Histocompatibility and Immunogenetics.
http://www.bmdw.org	Home page of Bone Marrow Donors Worldwide.
http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?146880	Contains information on *HLA-DQ* from the Online Mendelian Inheritance in Man (OMIM) database.
Type 1 diabetes mellitus	
http://www.diabetes.org	Home page of the American Diabetes Association.
http://www.diabetes.about.com/health/diabetes	Contains information on type 1 and type 2 diabetes.
http://www.jdf.org/index.html	Home page of the Juvenile Diabetes Foundation International.
http://methodisthealth.com/diabetes/type1.htm	Contains statistics on type 1 diabetes.
http://www.medscape.com/Home/Topics/endocrinology/endocrinology.html	Contains current risk information and articles concerning the etiology and prevention of type 1 diabetes.
http://diabetes-in-america.s-3.com/	Contains the online version of *Diabetes in America* (27), published by the National Institute of Diabetes and Digestive and Kidney Diseases.
http://www.niddk.nih.gov/	Home page of the National Institute of Diabetes and Digestive and Kidney Diseases.
http://www.pitt.edu/~iml1/diabetes/general.html	Contains the diabetes home page of the Global Health Network.
http://www.cdc.gov/nccdphp/diabetes.htm	Contains the diabetes home page of the Centers for Disease Control and Prevention.
http://diabetes.org/ada/dpt-1.asp	Home page for the Diabetes Prevention Trial.