

Genetics of Type 1 Diabetes

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BACKGROUND: Type 1 diabetes, a multifactorial disease with a strong genetic component, is caused by the autoimmune destruction of pancreatic β cells. The major susceptibility locus maps to the HLA class II genes at 6p21, although more than 40 non-HLA susceptibility gene markers have been confirmed.

CONTENT: Although HLA class II alleles account for up to 30%–50% of genetic type 1 diabetes risk, multiple non-MHC loci contribute to disease risk with smaller effects. These include the insulin, *PTPN22*, *CTLA4*, *IL2RA*, *IFIH1*, and other recently discovered loci. Genomewide association studies performed with high-density single-nucleotide-polymorphism genotyping platforms have provided evidence for a number of novel loci, although fine mapping and characterization of these new regions remain to be performed.

Children born with the high-risk genotype HLADR3/4-DQ8 comprise almost 50% of children who develop antiislet autoimmunity by the age of 5 years. Genetic risk for type 1 diabetes can be further stratified by selection of children with susceptible genotypes at other diabetes genes, by selection of children with a multiple family history of diabetes, and/or by selection of relatives that are HLA identical to the proband.

SUMMARY: Children with the HLA-risk genotypes DR3/4-DQ8 or DR4/DR4 who have a family history of type 1 diabetes have more than a 1 in 5 risk for developing islet autoantibodies during childhood, and children with the same HLA-risk genotype but no family history have approximately a 1 in 20 risk. Determining extreme genetic risk is a prerequisite for the implementation of primary prevention trials, which are now underway for relatives of individuals with type 1 diabetes.

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Type 1 diabetes (T1D),² a multifactorial disease with a strong genetic component, is caused by the autoimmune destruction of pancreatic β cells. The major T1D susceptibility locus maps to the HLA class II genes at 6p21 and accounts for up to 30%–50% of genetic T1D risk (1). Other non-HLA T1D loci in combination have smaller effects on disease risk compared to HLA. These include the insulin gene (*INS*)³ on chromosome 11p15 (2), the polymorphic, cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*) gene on chromosome 2q33 (3), the protein tyrosine phosphatase, non-receptor type 22 (lymphoid) (*PTPN22*) gene on chromosome 1p13 (4), the interleukin 2 receptor, alpha (*IL2RA*) and interferon induced with helicase C domain 1 (*IFIH1*) genes, and other recently discovered loci from genomewide association (GWA) studies (5) (Fig. 1).

Technological progress has facilitated the study of the genome to map disease-susceptibility genes for multifactorial diseases, including the increasing availability of microsatellite markers, single-nucleotide polymorphisms (SNPs), automated typing technology (6), and recently, whole-genome SNP analysis (7). In the case of T1D, genome scans for T1D susceptibility loci have been facilitated by the availability of large collections of families with affected sib pairs, including those in the Human Biological Data Interchange, the Warren repositories of the British Diabetic Association (8), and recently, the Type 1 Diabetes Genetics Consortium.

Family History of Type 1 Diabetes

Although more than 85% of patients with T1D lack a positive family history for the disease, there is high familial clustering with a mean prevalence of 6% in siblings compared to 0.4% in the US white population. The familial clustering (λ s) can be calculated as the ratio of the risk to siblings over the disease prevalence

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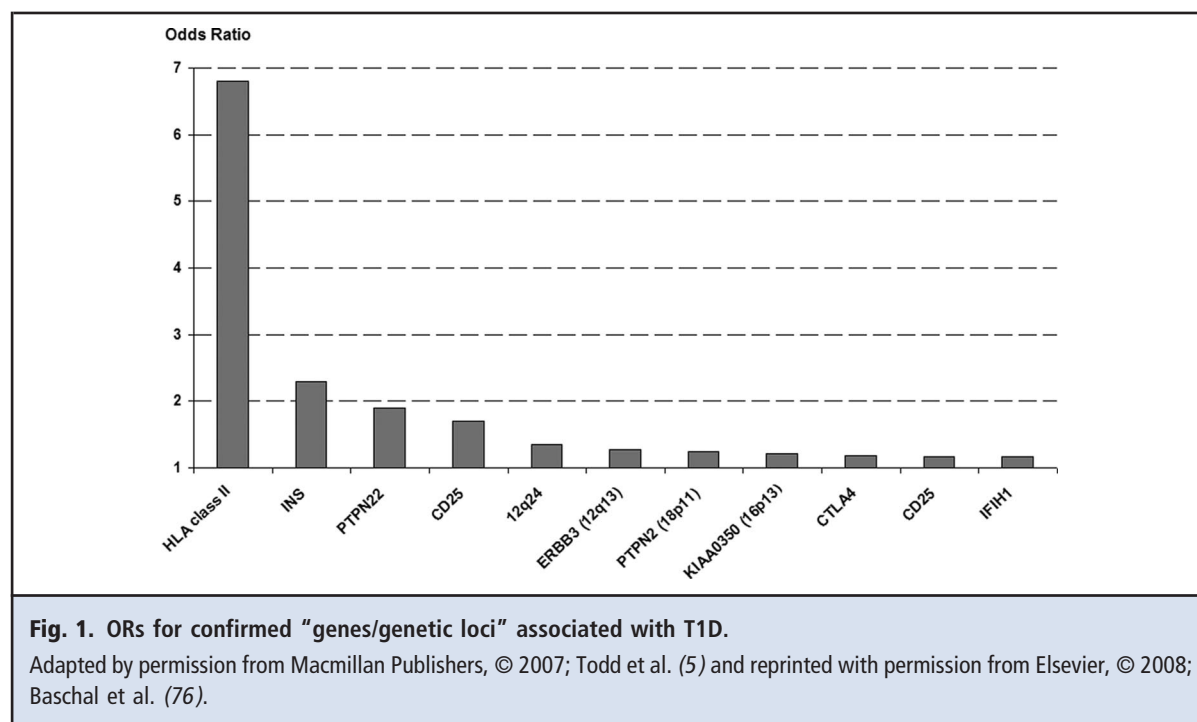
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² Nonstandard abbreviations: T1D, type 1 diabetes; GWA, genomewide association; SNP, single-nucleotide polymorphism; DR3, *DR3-DQA1*0501-DQB1*0201*; DR4, *DR4-DQA1*0301-DQB1*0302*; OR, odds ratio; DAISY, Diabetes Autoimmunity Study in the Young.

³ Human genes: *INS*, insulin; *CTLA4*, cytotoxic T-lymphocyte-associated protein 4; *PTPN22*, protein tyrosine phosphatase, non-receptor type 22 (lymphoid); *IL2RA*, interleukin 2 receptor, alpha; *IFIH1*, interferon induced with helicase C domain 1.



in the general population, and thus $\lambda_s = 6/0.4 = 15$ (9, 10).

The risk for T1D in siblings of patients is 15-fold higher than the risk for T1D in the general population, which suggests that genetic factors play an important role in disease susceptibility. The pattern of inheritance is complex, and the development of disease is thought to be determined by an interaction between genetic predisposition and environmental triggers. Concordance rates for T1D in monozygous twins with long-term follow-up is >50%, compared to 6%–10% in dizygous twins, which is similar to what is found in nontwin siblings. With long-term (>30 years) follow-up, at least two-thirds of initially discordant monozygous twins show evidence of persistent β -cell autoantibodies and/or diabetes (Fig. 2) (11).

Among first-degree relatives, siblings are at a higher risk (5%–10% risk by age 20) than offspring; offspring of diabetic fathers are at a higher risk (approximately 12%) than offspring of diabetic mothers (approximately 6%) (12). In addition, DR3/4 siblings who share both HLA haplotypes identical by descent with their diabetic DR3/4 sibling are at a higher risk than those sharing 1 or no haplotypes (13).

When the first twin of a twin pair develops T1D after age 25 years, the risk of the second monozygotic twin developing T1D is <5% with long-term follow-up (14), whereas when the first twin develops diabetes before the age of 6 years the risk of the second twin developing diabetes is at least 60%. For monozy-

gotic twins of patients with T1D, expression of antiislet autoantibodies directly correlates with progression to overt diabetes. Essentially all such twins who express "biochemical" antiislet autoantibodies progress to diabetes, even though this may not occur until after decades of follow-up (14, 15). Similar results were found in a population-based twin cohort of 22 650 twin pairs from Finland (9). With longer follow-up, the cumulative incidence of diabetes among monozygotic twins who were initially discordant for diabetes was 65%, and persistent autoantibody positivity or T1D developed in 78% by age 60 years (Fig. 2) (11).

Linkage and Association Studies

The 2 primary approaches used to identify risk loci for T1D have been linkage studies and association studies. Linkage studies, typically using affected sibling pairs, can identify regions of the genome that are shared more frequently among affected relatives. Linkage studies use markers spanning the genome at a modest density and are the method of choice when the risk factors have large effect sizes but are relatively rare. They provide broad information on chromosomal regions that may contribute to T1D risk. The most important and consistent evidence of linkage for T1D has been in the HLA region on chromosome 6p21 (10, 16). A metaanalysis done by the Type 1 Diabetes Genetics Consortium provided a total sample of 1435 families with 1636 affected sibling pairs (17). In addition to the HLA re-

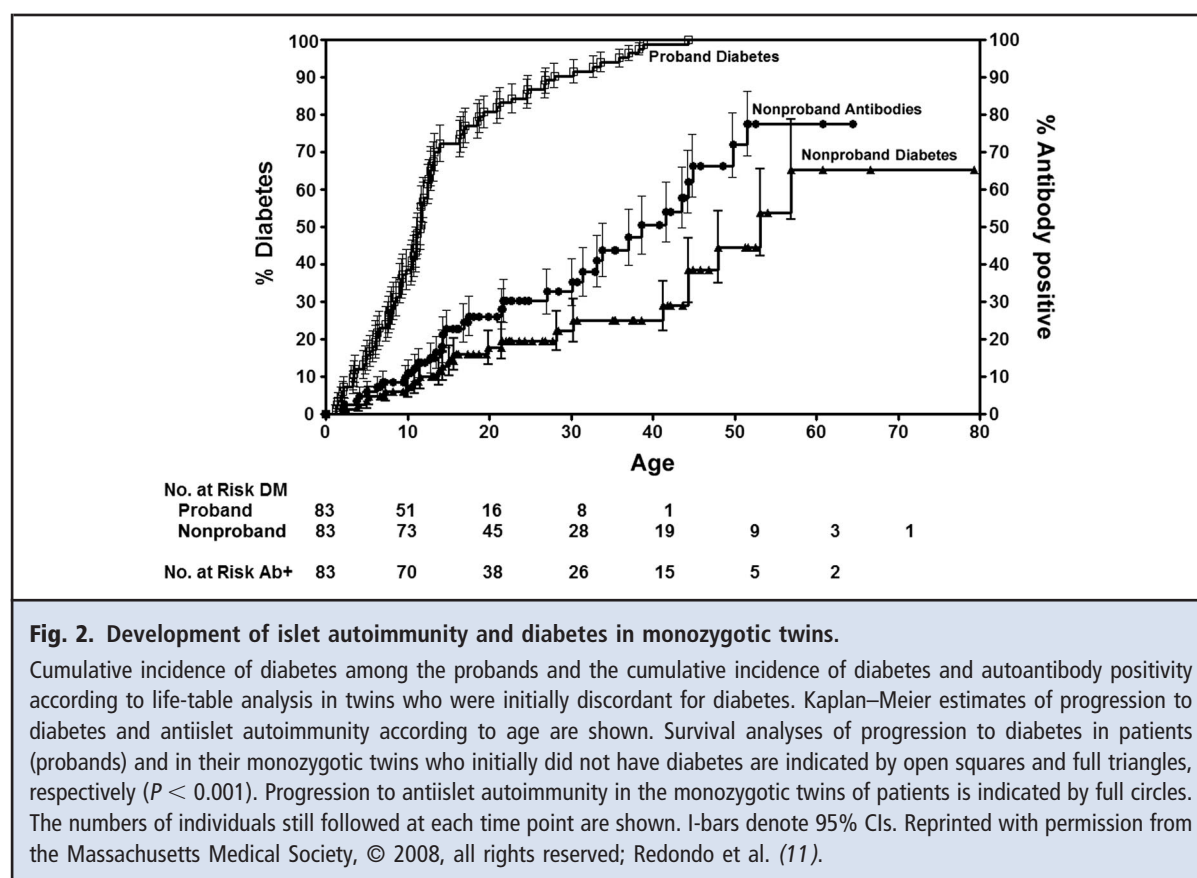


Fig. 2. Development of islet autoimmunity and diabetes in monozygotic twins.

Cumulative incidence of diabetes among the probands and the cumulative incidence of diabetes and autoantibody positivity according to life-table analysis in twins who were initially discordant for diabetes. Kaplan–Meier estimates of progression to diabetes and antiislet autoimmunity according to age are shown. Survival analyses of progression to diabetes in patients (probands) and in their monozygotic twins who initially did not have diabetes are indicated by open squares and full triangles, respectively ($P < 0.001$). Progression to antiislet autoimmunity in the monozygotic twins of patients is indicated by full circles. The numbers of individuals still followed at each time point are shown. I-bars denote 95% CIs. Reprinted with permission from the Massachusetts Medical Society, © 2008, all rights reserved; Redondo et al. (11).

gion (nominal $P = 2.0 \times 10^{-52}$), 9 non-HLA-linked regions showed some evidence of linkage to T1D (nominal $P < 0.01$), including 3 at (or near) genomewide significance ($P < 0.05$): 2q31-q33, 10p14-q11, and 16q22-q24.

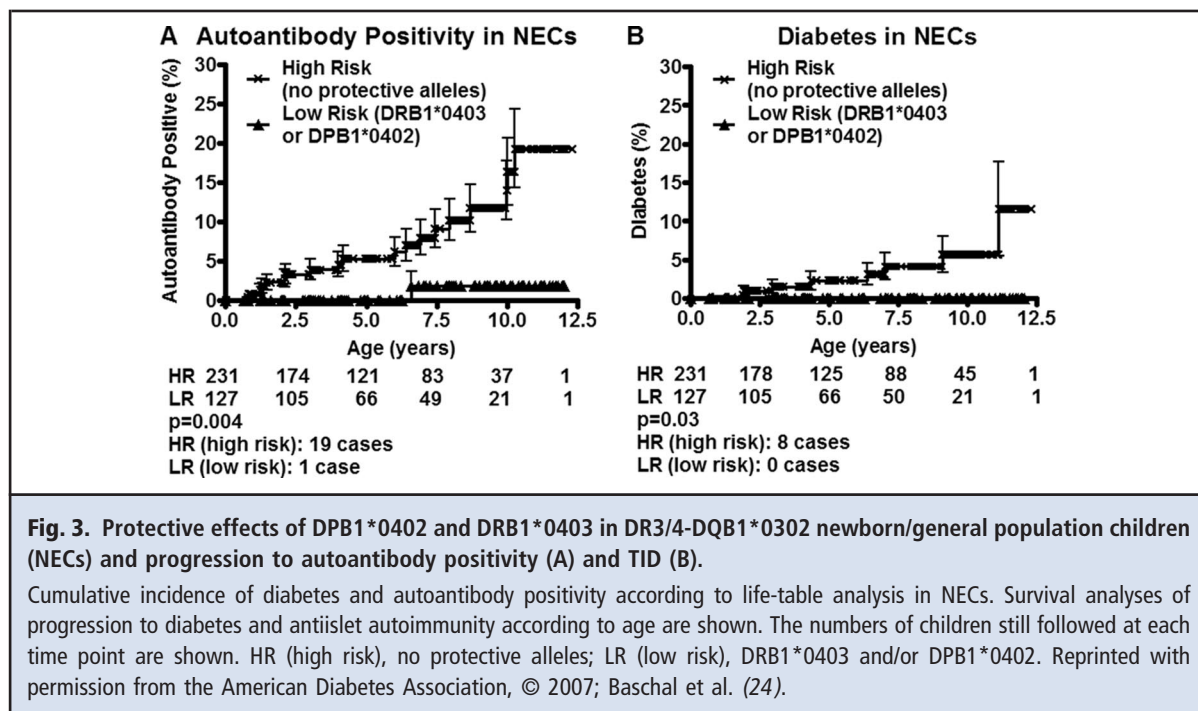
In contrast to linkage studies, association studies can detect alleles with much more modest effects on risk as long as those alleles are relatively common. Association studies use specifically selected markers in genes of interest that are genotyped in case patients and unaffected control individuals or, in some studies, in case patients and their parents. Association studies in T1D have initially focused on candidate genes. All of the 4 well-established risk loci, including HLA, *INS*, *CTLA4*, and *PTPN22*, were identified in candidate gene association studies. Recently, GWA studies have revolutionized genetic studies by the development of new, high-throughput SNP genotyping platforms and dense maps of SNPs.

HLA Class II Alleles

The major T1D susceptibility locus maps to the class II loci *HLA-DRB1* and *HLA-DQB1* on chromosome 6p21. The highest risk *DR/DQ* haplotypes for T1D

are *DR3-DQA1*0501-DQB1*0201* (DR3) and *DR4-DQA1*0301-DQB1*0302* (DR4), and these alleles account for 30%–50% of genetic T1D risk (1). Although 40% of whites in the US have an HLA-DR3 or -DR4 allele, at least 1 of these alleles is present in 95% of patients with T1D. The estimated risk of developing T1D for the general population in children who have the HLA-DR3/4 genotype is approximately 1 in 15 to 1 in 25 vs a risk of 1 in 300 in the general population (18). Only 2.4% of the general population carries this genotype compared to 30%–40% of T1D patients. In siblings, HLA-DR3/4 heterozygosity identifies higher risk of T1D than HLA identity (19). Deschamps and co-workers examined the predictive value of HLA typing in a study of 536 siblings of diabetic probands in France (20). The risk of T1D after 8 years, estimated by life-table analysis, was 10% for siblings who were HLA identical with the probands, 3%–4% for siblings with either DR3 or DR4, and 16% for those with the HLA-DR3/DR4 genotype.

Although this genotype confers extremely high risk, there is a spectrum of risk associated with HLA-DR/DQ genotypes—from increased, to neutral, to protective. For instance, the *HLA-DQA1*0102*, *DQB1*0602* haplotype confers dominant protection



from T1D, even in the presence of islet autoantibodies (21). Too few HLA-DQB1*0602 individuals expressing multiple islet autoantibodies have been studied to know the exact magnitude of protection, but 1% of new-onset patients expressing islet autoantibodies have DQB1*0602 vs 20% of the general US population.

Risk of diabetes is also influenced by *DRB1*04* variants and *DQ* alleles on *DR4* haplotypes, with higher risk from *DRB1*0405* [odds ratio (OR) = 11.4], *DRB1*0401* (OR = 8.4), *DRB1*0402* (OR = 3.6), and *DRB1*0404* (OR = 1.6), whereas *DRB1*0403* is protective (OR = 0.27) (6). Similarly, for *DRB1*0401*, variation of *DQB1* influences risk, because haplotypes with *DQB1*0302* (OR = 8.4) are highly susceptible, whereas those with *DQB1*0301* (OR = 0.35) are modestly protective.

The incidence of T1D has been increasing worldwide by approximately 3% per year (7), with the highest increase occurring in young children (8). Interestingly, however, as T1D incidence increases, the percentage of those cases with the high-risk HLA-DR3/4 genotype seems to be decreasing (22, 23). These temporal changes in HLA genotypes suggest increased environmental pressure with higher disease progression rate in individuals with lower-risk HLA genotypes and/or contribution of other non-HLA class II alleles or non-MHC-related alleles to T1D risk.

Several investigators have reported some association of *HLA-DPB1* alleles with T1D (24, 25). Results of these studies have shown that the frequency of the

*DPB1*0402* allele is lower in patients than in control groups and that transmission of *DPB1*0402* to T1D patients is decreased. On average, the relative risk for *DPB1*0402* overall from these studies is 0.56. *DPB1*0301* and *DPB1*0202* have been reported to be associated with a predisposition to T1D. Among HLA DR3/DR4-DQB1*0302 children in the general population, we can now identify a risk of 20% of activating islet autoimmunity by excluding both the protective alleles *DP*0402* and *DRB1*0403* (Fig. 3) (24).

HLA Class I and Class III Alleles

Recently, evidence has been found for susceptibility loci within or linked to the MHC independent of HLA-DR/DQ, such as HLA class I alleles (26–28). In the Diabetes Autoimmunity Study in the Young (DAISY) cohort, siblings of children with T1D who have HLA DR3/DR4-DQB1*0302 and are identical by descent for both HLA haplotypes with their diabetic proband sibling were observed to have a 65% risk for developing islet autoantibodies by age 7 years and a 50% risk of developing diabetes by age 10 years (13). These findings suggest that additional MHC-linked genes determine T1D risk.

HLA-A is associated with T1D independent of *DR/DQ* (29). Allele A24 has been associated with younger age of onset; allele A30 is higher risk whereas allele A1 is lower risk than other DR3 haplotypes. *HLA-B* and *HLA-C* alleles have also been associated

with T1D independent of *DR/DQ* (26). After adjustment for linkage disequilibrium with *DR/DQ*, the alleles *HLA-B18*, *B39*, *B44*, *C3*, *C8*, and *C16* remained associated with T1D (26). In a study by Nejentsev et al., both *HLA-A* and *HLA-B* survived correction for *HLA-DR* and *DQ*, and in particular, *HLA-B39* was observed to confer high risk for T1D and was associated with lower age of onset (27).

MIC-A is an MHC class I-like molecule that activates certain T cells, natural killer cells. *MIC-A* allele 5.1 introduces a stop codon into the molecule, interrupting production of the membrane-spanning portion of the molecule, and thus a functional change. *MIC-A* has been reported to be associated with T1D when the *DR/DQ* genotype is fixed (within DR3/4 first-degree relatives) (30).

Non-MHC Genetic Factors

More than 40 non-HLA-susceptibility gene markers have been confirmed (5, 31). At present, polymorphisms of the *INS* gene and *PTPN22* genes contribute most to diabetes risk after HLA alleles. Adding high-risk alleles of these genetic markers to HLA class II genotyping can improve risk prediction, but the effect is small even for the strongest loci, with ORs between 1.7 and 2.0.

INS GENE

The *INS* gene located on chromosome 11p15.5 confers about 10% of the genetic susceptibility to T1D. Both a variable number of tandem repeats located approximately 0.5 kb upstream of *INS* (2) and other polymorphisms in tight linkage disequilibrium such as -23HphI and +1140A/C (32) have been implicated as etiological factors. All of the polymorphisms lie outside coding sequences, suggesting that diabetes susceptibility derives from modulation of *INS* transcription (33). The short class I variable number of tandem repeats alleles (26–63 repeats) were associated with predisposition to T1D, whereas class III alleles (140–210 repeats) were dominantly protective. The allelic variant associated with protection from T1D has been associated with greater expression of insulin messenger RNA within the thymus (33, 34). Results of studies in the NOD (nonobese diabetic) mouse model of T1D have implicated insulin as the primary autoantigen, where decreased insulin expression in the thymus also correlates with risk (35).

PTPN22 Gene

The lymphoid-specific phosphatase LYP, encoded by the *PTPN22* gene on chromosome 1p13, is involved in preventing spontaneous T cell activation. An association of a nonsynonymous SNP in *PTPN22* at position

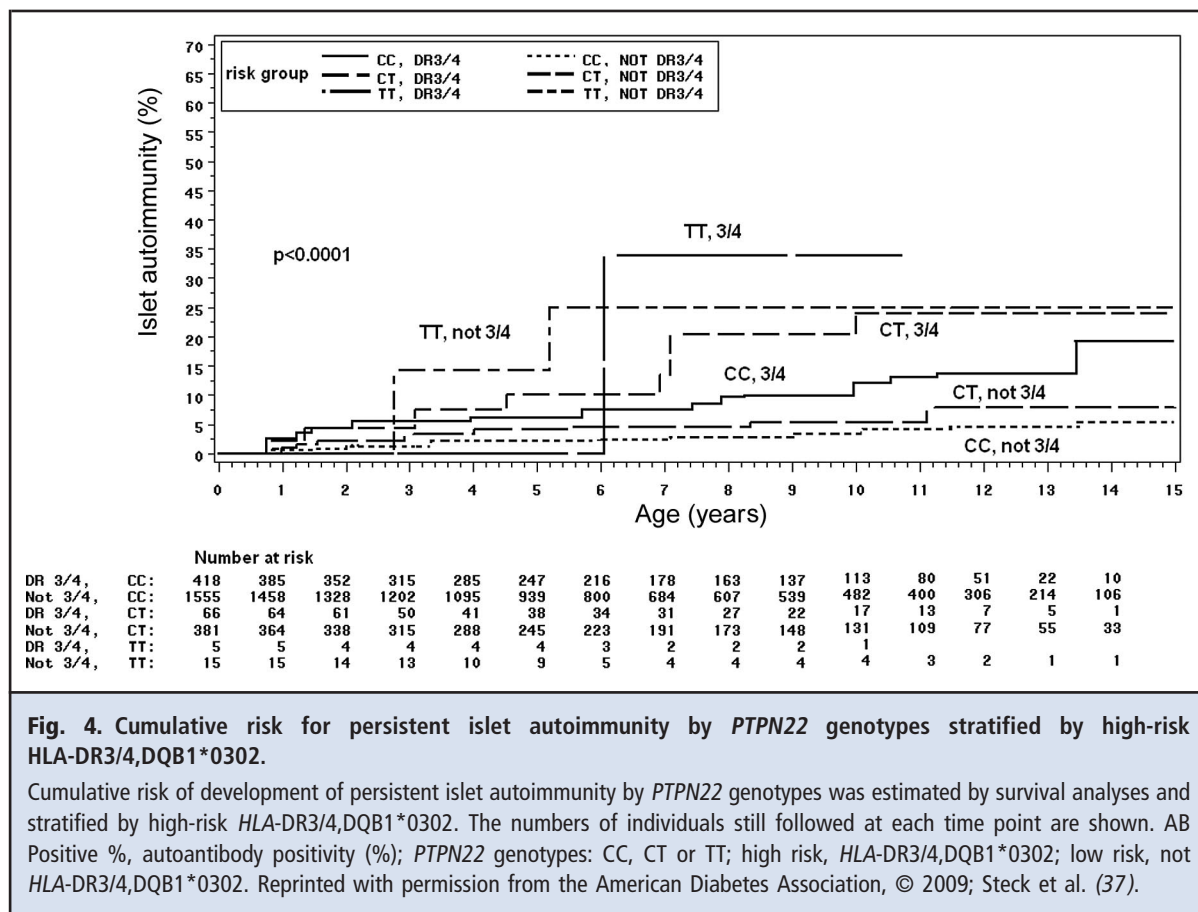
1858 with T1D has been reported from many populations, with an OR of 2–3 for the homozygous TT genotype (4, 36–38) (Fig. 4). The C1858T SNP results in a missense mutation (R620W) that changes an arginine at position 620 to a tryptophan and thereby abrogates the ability of the molecule to bind to the signaling molecule Csk (4,39–41). The polymorphism has been associated with a gain-of-function mutation, apparently decreasing T-cell-receptor signaling (40). The Trp620 variant is associated with other autoimmune disorders including Graves disease (42), rheumatoid arthritis (39), and systemic lupus erythematosus (43).

CTLA4 Gene

The *CTLA4* gene encodes a molecule that functions as a negative regulator of T-cell activation. The G allele of the first exon (Ala17Thr) has been most consistently associated with T1D (44) and reduced control of T-cell proliferation (45). The G/G variant in the first exon has been associated with decreased expression of a soluble variant of *CTLA4* that may have an influence on immune function, especially in light of *CTLA4* polymorphism associated with diabetes of the NOD mouse (46), Graves disease (47), and Addison disease (48). The (AT)_n microsatellite marker in the 3' untranslated region, in strong linkage disequilibrium with Ala17Thr, is also associated with T1D (49) and Graves disease (50). *CTLA4*, located at 2q33, has an OR of 1.1–1.2, but the association has been replicated in multiple studies (50, 51). The locus is associated primarily with patients with both thyroid autoimmunity and T1D, concordant with its stronger association with thyroid autoimmunity.

Other Defined Loci, GWA, and Microarrays

Besides these well-established non-HLA loci, a number of other associations with T1D have been reported for *IL2RA/CD25* (52, 53), *SUMO4* (small ubiquitinlike modifier 4) (54) and *IFIH1* (55, 56). Of these 3 genes, the clearest confirmation is for *IL2RA* (10p15), with the finding of more than 1 SNP associated with T1D. The *IL2RA/CD25* locus is implicated in a number of autoimmune disorders including multiple sclerosis, but in that case a different SNP is associated with increased risk. The *IL2RA/CD25* SNPs associated with risk for T1D have been shown to have different circulating concentrations of CD25; despite extensive overlap between concentrations, this variation in concentration is highly significant when a large number of individuals are studied (53). The mechanism by which the SNPs contribute to diabetes risk is currently unknown, and may be complex given 2 associated SNPs in the same locus, neither of which alters the coding sequence.

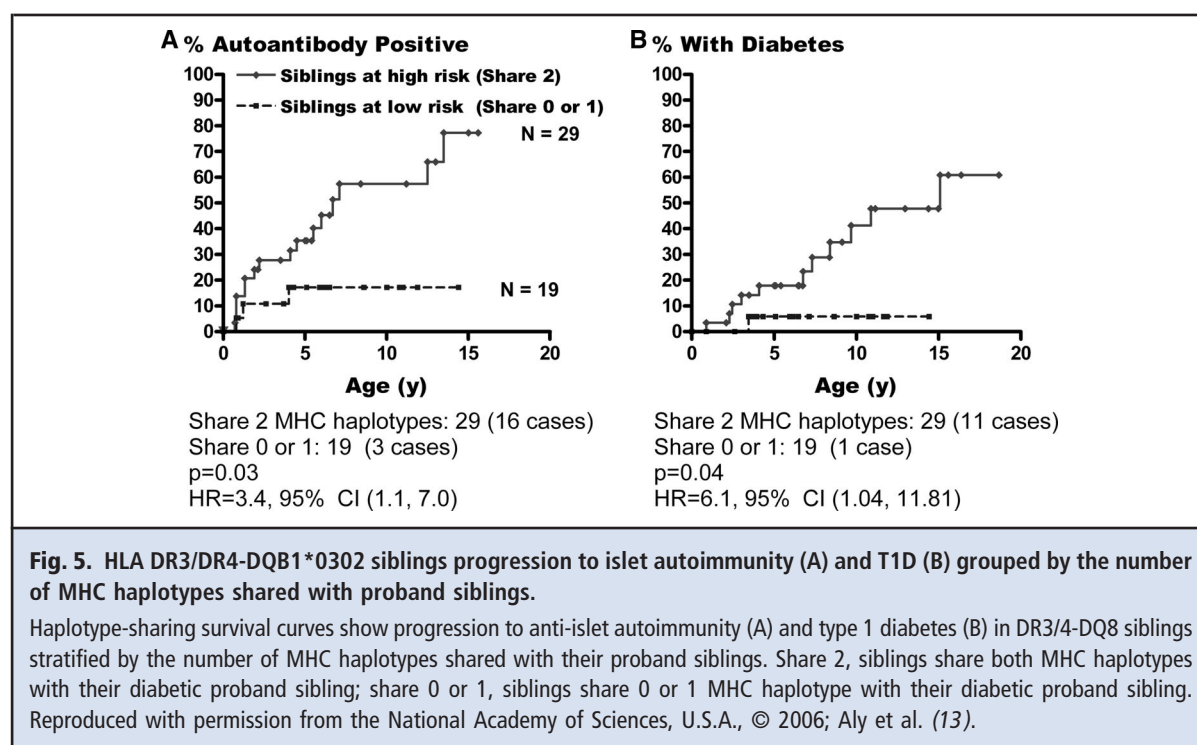


The minor allele of rs1990760 in the *IFIH1* gene was reported to be associated with T1D, with a risk ratio of 0.86 in a large study of 4253 cases and 5842 controls, and with an additional analysis of 2134 parent-child trios (55). The Wellcome Trust analysis used a different SNP (rs3788964) and revealed a very modest association with T1D (31). The gene (on chromosome 2q24) is of particular interest in that it may relate the innate immune system to the development of disease, presumably mediated by the adaptive immune system, and animal models are available in which activation of innate immunity, and interferon α , are associated with induction of autoimmune diabetes (57).

Several GWA studies of T1D have now been performed and reported (58–60). A GWA study for T1D was completed in 2007 by the Wellcome Trust Case Control Consortium, which reported signals at known loci [6p21 (MHC region), 11q15 (*INS*), 2q33 (*CTLA4*), 1p13 (*PTPN22*), 10p15 (*IL2RA*) and 2q24 (*IFIH1*)] and also described signals at several novel loci (12q13, 12q24, 16p13, 4q27, 12p13, and 18p11) (31). The associations at 12q24, 12q13, 16p13, and 18p11 were confirmed in other recent independent studies

(5, 59, 61–64). Additional follow-up studies (65) and metaanalyses of GWA (60, 66) have increased the list of new T1D loci to more than 40.

Microarray technology and bioinformatics allow the comparison of gene expression profiles (or transcriptomes). This new technology seems promising because it enables investigators to study the behavior of many genes simultaneously. Studies in humans with T1D have been rather small so far and limited to peripheral blood mononuclear cells (67). Some authors (68) have reported an overexpression of IL-1-regulated genes in both type 1 and type 2 diabetic patients, whereas others (69) have reported on an expression signature that includes IL-1 cytokine family members and chemokines in both new-onset T1D patients and at-risk siblings who later developed the disease. Gene expression profiles in pancreatic lymph nodes have been described in the NOD mouse. Future research in humans might be possible with new collaborative efforts such as the international Network for Pancreatic Organ Donors with Diabetes (www.jdrfnpod.org).



Genetic Markers in Prediction of T1D

A number of large population-based studies have been carried out stratifying individuals at birth by HLA genotype and insulin gene polymorphisms. Children born in Denver with the highest risk genotype DR3/4-DQ8 comprise 2.4% of newborns and almost 50% of children developing antiislet autoimmunity by age 5 (DAISY study) (18, 70). The BabyDiab study of offspring of patients with T1D in Germany and the Diabetes Prediction and Prevention study from Finland provide similar information concerning the risk associated with specific HLA genotypes and insulin gene polymorphisms (71, 72). Risk can be further stratified by selection of children with susceptible genotypes at other diabetes genes (71), by selection of children with a multiple family history of diabetes (73), and by selection of relatives that are HLA identical to the proband (13). In the DAISY cohort, siblings of children with T1D who have the highest risk HLA DR3/DR4-DQ8 and are identical by descent for both HLA haplotypes with their diabetic proband sibling were found to have a 65% risk for developing islet autoantibodies by age 7 years and a 50% risk of developing diabetes by age 10 years (13) (Fig. 5). These findings suggest that additional MHC-linked genes determine T1D risk.

More than 90% of T1D patients have no affected relatives, so to have a major impact preventive efforts will require inclusion of the general population. Among HLA

DR3/DR4-DQB1*0302 general-population children, we can now identify a risk of 20% of activating islet autoantibodies by excluding both *DP*0402* and *DRB1*0403* alleles (Fig. 4) (24). This risk of 20% is, however, much lower than the risk of 80% conferred for siblings who have DR3/DR4-DQB1*0302 identical by descent with their sibling proband and is similar to a 20% risk for those DR3/DR4-DQB1*0302 siblings sharing only 1 (or no) haplotype identical by descent (13).

Conclusions

The major T1D susceptibility locus maps to the class II loci *HLA-DRB1* and *HLA-DQB1* on chromosome 6p21. With the advent of whole-genome SNP genotyping studies in the past several years, many additional non-MHC loci have been identified that contribute to T1D risk. A majority of these loci appear to have effects in the immune system, particularly on T cells. In addition, non-DR/DQ loci within the MHC have been identified that contribute to prediction of T1D in the general population (i.e., *HLA-DP*).

Preventive therapies can be applied before diabetes onset or even before the appearance of islet autoimmunity in individuals who are genetically at risk for T1D. Children who have a family history of T1D and the HLA risk genotypes DR3/4-DQ8 or DR4/DR4 have a more than 1 in 5 risk for developing islet autoanti-

bodies during childhood (71, 74) and approximately a 1 in 20 risk without a relative with T1D (74). Primary prevention trials such as Pre-POINT (Primary Oral INsulin Trial) are currently underway in siblings with high genetic risk for T1D. The Pre-POINT trial will determine the feasibility of performing a primary autoantigen vaccination trial in children with high genetic risk for T1D in a dose-escalation primary-intervention pilot study (75). In this study oral insulin will be administered to high-risk siblings who have not yet developed islet autoantibodies to provide protection before islet autoimmunity starts. Eligible children are those who have multiple first-degree relatives with T1D or those who have the HLA-DR3/DR4-DQ8 genotype inherited identical by descent with a sibling proband; such siblings have T1D risk as high as 80% (13). This multicenter, placebo-controlled, double-blinded trial will enroll up to 25 children randomized to receive increasing doses of oral insulin (2.5, 7.5, 22.5, or 67.5 mg per day) to determine a dose that is safe and bioavailable to the immune system. Children will be monitored for the development of islet autoantibodies, diabetes, and protective immune responses to insulin. Pre-POINT will proceed to the POINT study if and when the dose-finding committee identifies a dose that can be tested for efficacy.

The oral insulin study conducted by the Type 1 Diabetes TrialNet consortium is currently enrolling first-degree relatives ages 3–45 years and second-degree relatives ages 3–20 years. The primary objective is to determine whether administration of oral insulin will prevent or delay the development of T1D in individuals at risk. Eligible individuals have to be insulin-

autoantibody positive on 2 samples, with at least 1 other islet autoantibody present, have oral glucose tolerance test results within reference intervals, and meet certain criteria on first-phase insulin response by intravenous glucose tolerance test.

However, only 10% of new-onset T1D patients have a relative with T1D. About 90% of new cases of T1D occur in the general population. Determining extreme genetic risk in the general population is a prerequisite for the implementation of primary prevention trials in the general population, where most new cases of T1D arise.

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