

# Type 1 diabetes genetic susceptibility markers and their functional implications

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by selective destruction of pancreatic  $\beta$ -cells resulting in insulin deficiency. The genetic determinants of T1D susceptibility have been linked to several loci, in particular to the human leukocyte antigen (HLA) region, which accounts for 50% of the genetic risk of developing T1D. Multiple genes in the HLA region, which are in strong linkage disequilibrium, are thought to be involved. Another important locus, with a smaller effect on genetic predisposition to T1D, is the insulin gene. The advent of numerous single nucleotide polymorphism markers and genome screening has enabled the identification of dozens of new T1D susceptibility loci. Some of them appear to predispose to T1D independently of the HLA and may be important in families with T1D who lack strong HLA susceptibility. Other loci may interact with each other to cause susceptibility. The autoimmune response against  $\beta$ -cells can also be triggered by environmental factors in the presence of a predisposing genetic background. Deciphering the environmental and genetic factors involved should help to understand the origin of T1D and aid in the design of individualized prevention programs.

**Key words:** Type 1 diabetes mellitus, Autoimmune diseases, Genetics, HLA antigens.

## Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by destruction of the insulin-producing  $\beta$ -cells of the pancreas. Although the American Diabetes Association recommends an etiological classification, diabetes resulting from deficient insulin secretion, rather than deficient insulin action, is usually classified as T1D. The prevalence of T1D ranges from <5 in every 100,000 individuals in Asian countries to as many as 39.9 per 100,000 in European and other Western countries [1]. A distinguishing feature of T1D is the presence of anti-islet autoantibodies, including antibodies against glutamic acid decarboxylase (GAD) 65, against insulinoma-associated antigen (IA-2), against insulin, against zinc transporter 8 (ZNT8), and

against islet cell autoantigen (ICA). More than 90% of individuals with T1D test positive for at least one such autoantibody, and the presence of autoantibodies is used to identify the relatives of patients who are at increased risk of the disease [2-4].

Although genetic factors are strong determinants of the disease, more than 90% of individuals who present with new-onset diabetes do not have a relative with T1D [3]. The overall risk of T1D for the siblings of T1D patients is approximately 6%, which is 15 fold higher than in the general population [5]. The most important susceptibility alleles are found within the human leukocyte antigen (HLA) region, which has an odds ratio for disease of approximately 6.8 [6].

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## Inheritance of Immunologic Markers and Development of Diabetes

About half of monozygotic twins of T1D patients go on to develop diabetes [5]. There is a large difference between the concordance rates for T1D in monozygotic (50%) and dizygotic (~5%) twins, which demonstrates the importance of genetic factors in the development of T1D. However, since the rate is not 100%, other environmental factors clearly play an important role.

The non-obese diabetic (NOD) mouse is an inbred strain that develops insulinitis and diabetes that closely resembles human T1D. Autoimmune diabetes in NOD mice and in humans shares many genetic and pathophysiological characteristics [7,8]. In both species, genes within and outside the major histocompatibility complex (MHC, whose homolog in humans

is HLA) contribute to disease susceptibility. The odds associated with the non-MHC genes are considerably smaller than with the MHC genes. A number of non-MHC T1D-associated genetic variants, out of which the insulin gene is most important, are thought to increase disease susceptibility in humans [9–11]. A comprehensive list of the susceptibility loci, derived both from the syntenic candidate genes of mouse homologs and from linkage analysis in humans can be found in Table 1, together with LOD scores from the 2005 T1D Genetics Consortium (T1DGC) scan [12,13]. Although as many as 18 genetic loci have been implicated in T1D susceptibility, more than 50% of the genetic risk of developing diabetes is thought to be conferred by the HLA region alone [6,10,12,14].

**Table 1.** Genetic contributions of the known susceptibility loci derived from candidate gene and linkage analyses to the risk of type 1 diabetes

Locus	Chromosome	Candidate genes	Markers	LOD <sup>a</sup>
<i>IDDM1</i>	6p21.3	<i>HLA-DR/DQ</i>	TNFA	116.38
<i>IDDM2</i>	11p15.5	<i>Insulin VNTR</i>	D11S922	1.87
<i>PTPN22</i>	1p13	<i>PTPN22 (Lyp)</i>	SNP=R620W	NR
<i>IDDM3</i>	15q26		D15S107	NR
<i>IDDM4</i>	11q13.3	<i>MDU1, ZFM1, RT6, ICE, LRP5, FADD, CD3</i>	FGF3, D11S1917	NR
<i>IDDM5</i>	6q25	<i>SUMO4, MnSOD</i>	ESR, a046Xa9, SNP=M55VA allele 163 (G)	NR
<i>IDDM6</i>	18q12-q21	<i>JK (Kidd), ZNF236</i>	D18S487, D18S64	NR
<i>IDDM7</i>	2q31-33	<i>NEUROD</i>	D2S152, D251391	3.34
<i>IDDM8</i>	6q25-27		D6S281, D6S264, D6S446	NR
<i>IDDM9</i>	3q21-25		D3S1303, D3S1589, D3S3606	NR
<i>IDDM10</i>	10p11-q11	<i>SDF-1</i>	D10S565, D10S193	3.21
<i>IDDM11</i>	14q24.3-q31	<i>ENSA, SEL-1L</i>	D14S67	NR
<i>IDDM12</i>	2q33	<i>CTLA-4</i>	(AT)n 3' UTR, A/G Exon 1	3.34
<i>IDDM13</i>	2q34	<i>IGFBP2, IGFBP5, NEUROD, HOXD8</i>	D2S137, D2S164, D2S1471	NR
<i>IDDM15</i>	6q21		D6S283, D6S434, D6S1580	22.39
<i>IDDM16</i>	14q32	<i>IGH</i>		NR
<i>IDDM17</i>	10q25		D10S1750, D10S1773	NR
<i>IDDM18</i>	5Q31.1-33.1	<i>IL12B</i>	IL12B	NR
	1q42		D1S1617	NR
	16p12-q11.1		D16S3131	1.88
	16q22-q24		D16S504	2.64
	17q25			NR
	19q11			NR
	3p13-p14		D3S1261	1.52
	9q33-q34		D9S260	2.20
	12q14-q12		D12S375	1.66
	19p13.3-p.13.2		INSR	1.92

NR, not reported in the 2005 T1DGC scan [12], which applies to loci with no significant evidence for linkage in that study.

<sup>a</sup>According to the criteria of Lander and Kruglyak (1995) [13], an LOD  $\geq 3.6$  is considered significant.

## Major Histocompatibility Complex

Several studies have now shown that both the HLA-DQ and the HLA-DR genes influence T1D susceptibility. It has also become evident that susceptibility as well as protective alleles can be found at the DRB1, DQA1, and DQB1 loci. Studies have shown that HLA-DR3 or -DR4, or both alleles, strongly increase susceptibility to T1D, whereas HLA-DR15 has a protective effect across several populations [6,7,9]. In Asian populations, studies identified a positive association of T1D with HLA-DRB1\*09, and a negative association with HLA-DRB1\*11(12) [10,11,14,15]. DQ2 (DQB1\*0201-DQA1\*0501) and DQ8 (DQB1\*0302-DQA1\*0301), which occur in strong linkage disequilibrium (LD) with DR3 and DR4, respectively, are strongly associated with the disease not only in Caucasian but also in Asian populations, especially when present in DR3/DR4 heterozygotes [10-12,14-16]. In T1D, the HLA-DR3-DQ2 and -DR4 -DQ8 haplotypes are referred to as 'high-risk haplotypes'. HLA-DR15 is in LD with DQ6.2 (DQA1\*0102-DQB1\*0602) and is known as 'a protective haplotype' [11,17]. The protection conferred by the DQ6.2 haplotype is assumed to be dominant, as even among autoantibody(+) relatives, this haplotype protects from the development of diabetes.

In many countries, however, the incidence of T1D has been increasing in young children at a faster rate than can be explained by genetic change alone, highlighting the role of environmental factors [9,18,19]. There is evidence of an increasing trend over the past 20 years in the incidence of T1D, and especially in individuals with the protective HLA haplotypes, such as HLA-DQB1\*0602. These findings are alarming, and they are driving the search for environmental factors that may be responsible for triggering T1D, such as changes in exposure to infectious, environmental, or nutritional agents.

## HLA Molecules in a Trimolecular Complex Determine T1D Susceptibility

In addition to the high-risk and protective variants, there are other, less common, HLA haplotypes that influence T1D susceptibility [17]. The peptide-binding selectivity of HLA class II molecules was found to be influenced by their genetic polymorphisms, and the overall risk of T1D associated with the HLA-DR and -DQ molecules is determined by a combination of alleles (haplotypes and genotypes) in any individual. Studies of peptide binding to the HLA antigen have indicated that different HLA alleles may bind different peptide epitopes from a single

candidate diabetes antigen, such as GAD65 or insulin [20]. Consistent with this observation is the finding that the disease process is affected by multiple peptides. This, in turn, may mean that multiple amino-acid residues in class II molecules (i.e., alleles) are also involved. The antigen-specific pathogenic T cell response is triggered either by several  $\beta$  cell-derived peptides at the same time, or by a single peptide, with subsequent inter- and intramolecular spreading of the autoimmune response and expansion of the T cell repertoire [21].

Some studies have reported a modulatory effect of HLA-DPB1 alleles, as well as class I molecules, on the DR/DQ-associated risk of T1D. These studies suggest that susceptibility of the DP locus is conferred primarily by the DPB1\*0301 and DPB1\*0202 alleles, while protection is conferred by DPB1\*0402 [22,23].

## MHC Class I Chain-related Gene A

MHC class I chain-related gene A (MICA) also resides in the human MHC locus. The trinucleotide repeat (GCT)<sub>n</sub> microsatellite polymorphisms within exon 5 code for polyalanine in the transmembrane (TM) region of the MICA molecule. In most populations, 5 MICA microsatellite alleles have been identified, consisting of 4, 5, 6, and 9 GCT repetitions, and 5 repetitions with an additional nucleotide insertion (GCT to GGCT). Accordingly, these alleles have been named A4, A5, A6, A9 and A5.1. There are few studies describing the functional consequences of these polymorphisms. It has been suggested that the dihydrophobic Leu-Val tandem sequence at position 344-345 in the cytoplasmic tail of MICA is responsible for targeting the protein to the basolateral plasma membrane of gut epithelial cells, which is the prime site of contact with effector NK and intraepithelial T cells. In MICA-A5.1, insertion of an additional G creates a frame-shift mutation resulting in a premature termination codon within the TM region, as a result of which the MICA-A5.1 molecule is not translated all the way to the Leu-Val sequence at position 344-345. The Leu-Val sorting signal is thus removed, which results in MICA-A5.1 molecules being localized to the apical plasma membrane instead of the basolateral membrane [24].

A number of studies have reported an association between the MICA alleles and several autoimmune disorders, such as juvenile rheumatoid arthritis, Addison's disease, psoriasis and ankylosing spondylitis. Likewise, there are reports describing an association between different MICA alleles and T1D in several populations [25,26]. MICA-A9 was found to be positively associated with T1D in a Chinese population, whereas in Koreans, MICA-A4

was found to be positively associated, and MICA-A6 negatively associated with T1D.

## The Variable Number of Tandem Repeats (VNTR) Locus of the Insulin Gene

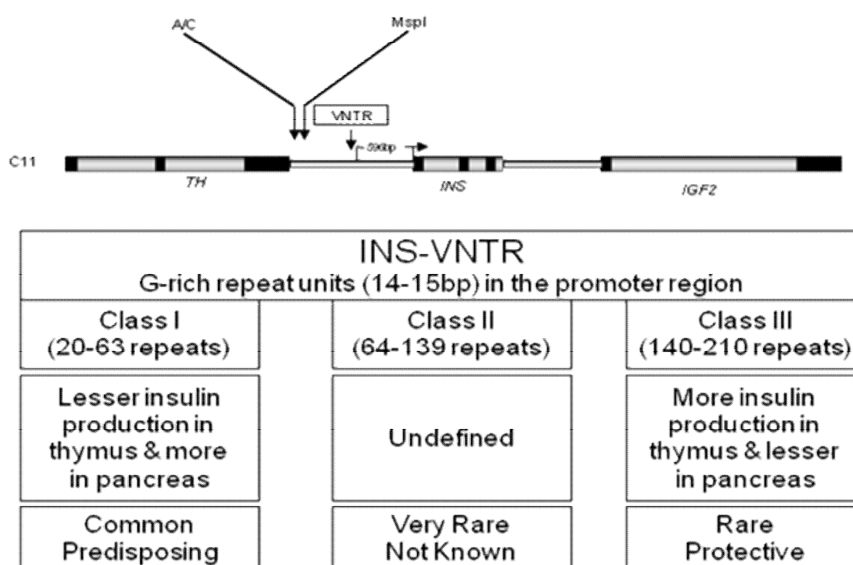
Several studies have shown a strong association between T1D and the variable number of tandem repeats (VNTR) locus, found approximately 596 bases upstream of the transcription start site of the insulin gene on chromosome 11p15.5 (Fig. 1). This locus is thought to contribute about 10% of the variation in T1D susceptibility. Based on the number of tandem repeats of 14–15 bp G-rich sequences, the VNTR alleles have been grouped into 3 classes: class I alleles (20–63 repeats), class II alleles (64–139 repeats) and class III alleles (140–210 repeats). The short, class I alleles are generally predisposing, especially in the homozygous state, with a relative risk >2. Conversely, class III alleles are associated with dominant protection. Among Caucasians, class I alleles are the most common, with a frequency of nearly 80%; class III alleles are less frequent (nearly 20%), and class II are the least common variants [9,27]. By contrast, class I alleles occur in more than 90% of the Asian population [10,11,14].

The exact mechanism by which these alleles influence the risk of T1D is not well understood. As the VNTR locus is in a non-

coding region, its allelic variants may exert their influence by modulating the expression levels of insulin in the thymus and pancreas. On the one hand, increased transcription of the insulin gene in the thymus is associated with the protective, class III alleles, while, on the other, low levels of transcription are found in the presence of the predisposing, class I alleles. However, the situation is reversed in the pancreas, where higher insulin mRNA expression is associated with class I alleles [28]. These findings suggest that the negative selection of autoreactive thymocytes is dose-dependent, and that elevated levels of insulin in the thymus may promote efficient deletion of autoreactive T cells for insulin and its precursors. This, in turn, would induce central immune tolerance to this important autoantigen involved in the pathogenesis of T1D.

## The Cytotoxic T Lymphocyte Associated Antigen 4 Gene

The cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) gene on chromosome 2 (2q33) has been associated with many autoimmune diseases (Fig. 2) [29]. Susceptibility to T1D was found to be linked to a single nucleotide polymorphism (SNP) at the 5' end of CTLA-4 rather than a polymorphism in the 3'UTR [30]. A combined analysis of families from several European popula-



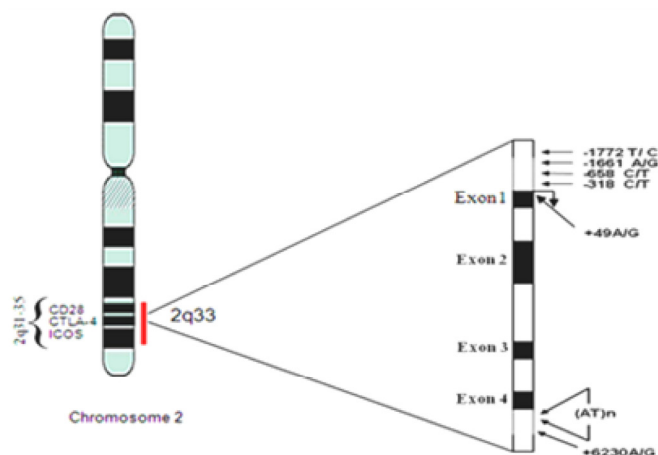
**Fig. 1.** Schematic presentation of the insulin gene (INS) and variable numbers of tandem repeats (VNTR) locus, with special emphasis on the association of differential expression pattern of VNTR in thymus and pancreas with type 1 diabetes (T1D) susceptibility. VNTRs associated with T1D are located 596 bp upstream of the transcription site in the promoter region of the gene. The lower half of the figure explains the correlation between INS-VNTR alleles and insulin production that leads to T1D susceptibility or resistance. Dark-shaded boxes are exons; light-shaded boxes are introns.

tions revealed linkage with T1D and autoimmune thyroid disease in the 6.1 kb region of CTLA-4. The +49G-CT60G haplotype was identified as predisposing and the +49A-CT60A as protective.

CTLA-4, expressed on the surface of activated T cells, binds to ligand B7, expressed on the surface of antigen presenting cells (APCs), and down-regulates the expression of the interleukin (IL)-2 receptor, which is followed by decreased synthesis of IL-2. CTLA-4 is also a molecule through which CD4+CD25+ regulatory T cells exert their suppressive activity. There are 2 known CTLA-4 isoforms: the main isoform is a full-length TM glycoprotein expressed on the surface of activated T cells, and the other is a soluble form (sCTLA-4). The latter is generated by alternative splicing of the TM domain and is mainly expressed by inactivated T cells. Allelic variation at the CTLA-4 locus determines the expression level of the soluble isoform [30]. The latter may increase the inhibitory effects of CTLA-4 by competing with CD28 for binding to costimulatory B7 molecules on the APCs. The ratio of sCTLA-4 to full-length CTLA-4 transcripts in unstimulated CD4 T cells was found to be 50% lower in individuals with a predisposing haplotype. Therefore, the level of CTLA-4, especially of the soluble form, may affect the risk of developing autoimmune diseases, including T1D.

## The Protein Tyrosine Phosphatase N22 Gene

The protein tyrosine phosphatase N22 (PTPN22) gene on chromosome 1p13.2 encodes a lymphoid-specific phosphatase known as lymphoid tyrosine phosphatase (Lyp), which plays an



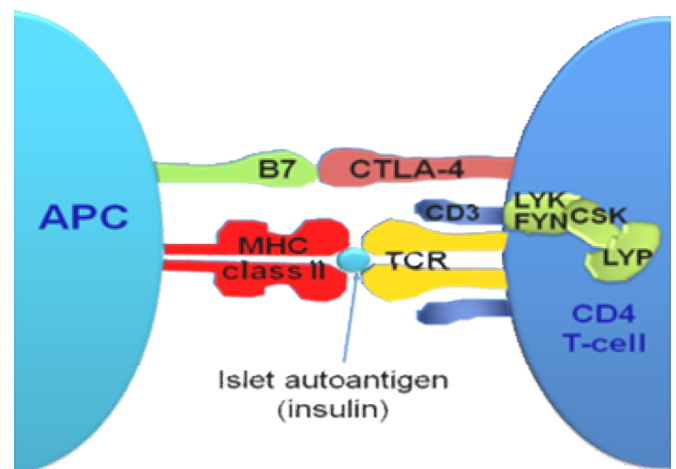
**Fig. 2.** Schematic presentation of the genetic structure of the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) on chromosome 2q33 is shown. Various polymorphisms in the CTLA-4 gene in the 5'UTR- and 3'UTR-regulatory regions and exon 1 have been studied extensively in patients with type 1 diabetes. Dark-shaded boxes are exons; light-shaded boxes are introns.

important role in the autoimmune response (Fig. 3). It dephosphorylates the Lck, Fyn, and ZAP-10 proteins, which are known to be important in T cell signaling. Lyp binds to C-terminal Src kinase (Csk) and to the adaptor molecule Grb2 (growth factor receptor-bound protein 2), resulting in down-regulation of activated T cells [31].

Recently, a positive association was found in several populations between a nonsynonymous SNP C1858T in codon 620 of the PTPN22 gene and T1D [32,33]. 1858T is a rare allele that changes the amino acid from arginine (R) to tryptophan (W) at position 620, resulting in disruption of the proline-rich binding motif 'PxxPxxR', which is important for Lyp binding to both Csk and Grb2. As a result, the reduced binding capacity of W620 Lyp to Csk is believed to disrupt the function of Csk as a down-regulator of T cell activation. The 1858T allele has been positively associated with other autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus. However, several studies found that in Asian populations it was not polymorphic, and was not associated with T1D [14].

## Other Candidate Markers and Lessons from the Recent Genome-wide Association Studies

During the past 3 decades many genetic studies have been devoted to the search for other T1D susceptibility loci, either by



**Fig. 3.** Schematic presentation of the lymphoid tyrosine phosphatase (Lyp) and its interaction with other signaling molecules are shown. An islet  $\beta$ -cell autoantigen (insulin peptide) is presented to CD4 T cells by the human leukocyte antigen (HLA) class II molecules on an antigen presenting cell (APC), leading to T cell activation. Like the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), LYP is an inhibitor of T cell activation and is encoded by the protein tyrosine phosphatase N22 (PTPN22) gene. The LYP-CSK (C-terminal Src kinase) complex inhibits Lck signaling after HLA-peptide-TCR engagement. MHC, major histocompatibility complex; TCR, T cell receptor.

linkage or by association with candidate genes. Although fine-mapping of the intervals has not yet determined the identity of the putative susceptibility genes, important candidate genes have been identified [10-12,34,35]. A susceptibility locus close to the IL12B gene was identified and mapped to chromosome 5q31.1- q33.1. IL12B encodes the IL-12 p40 subunit, and IL-12 affects the T cell response, which may be important in T1D pathogenesis. LD is confined to a 30 kb region carrying IL12B, the only known gene in the region. Several polymorphisms have been identified in IL12B, with the A allele of C1159A showing strong LD with T1D susceptibility [35]. Fine-mapping in T1D patients have also narrowed the relevant region on chromosome 10q15, applying a multilocus test using tag SNPs [36]. The biological candidate gene IL2RA (CD25) in that region, which encodes a subunit (IL-2R $\alpha$ ) of the high-affinity IL-2 receptor complex was found to be associated with T1D. This study indicated the utility of tag SNPs in a chromosome-regional test of disease association and justify future fine mapping of the causal variant in the region. Moreover, the T1DGC has also assembled and genotyped a large collection of multiplex families. By utilizing genome-wide linkage scan data and family-based association methods, they confirmed previously reported T1D associations at the insulin gene [27], a region on 2q24 near interferon induced with helicase C domain 1 (IFIH1) [37], and a region on 16p13 near C-type lectin domain family 16, member A (CLEC16A/KIAA0350) [38] and identified an additional disease association on chromosome 21q22.3 in the ubiquitin associated and SH3 domain-containing protein A (UBASH3A) locus [39].

Although there is a large genetic component to T1D susceptibility, uncovering the non-HLA genes contributing to T1D has proven difficult. It was not the advent of genome-wide association studies (GWAS), until only 5 loci (the insulin, the PTPN22, the CTLA-4, the IL2RA, and the KIAA0350) had been fully established to be associated with T1D [39]. The majority of the other reported associations in the pre-GWAS era [40,41] were found to be doubtful, where an initial report of association could not be replicated in subsequent studies by other investigators. Inconsistent findings might be attributed to small sample sizes. The candidate gene approach (where genomic regions were studied based on biological reasoning) and family-based linkage methodologies, which are limited in terms of hypothesis and power are no longer popular and can be replaced by the recent unbiased GWAS. They allow investigators to scan the genome for variants conferring risk for disease without prior hypotheses. They have also leveraged large, well-powered sample sizes combined with sound statistical analyses.

Such GWAS have revealed multiple regions of the genome to be consistently associated with T1D [42,43]. More recent reports have been a consequence of combining of multiple datasets from independent investigators in meta-analyses, which have more power to pick up additional variants contributing to T1D susceptibility. In addition to the repertoire of over 40 loci already demonstrated to be associated, in a meta-analysis of T1D GWAS, which combines 6 large studies, 3 new signals residing at the chromosomal locations 13q22, 2p23, and 6q27 were found to be replicated in independent samples [44]. Further work will be required to fully validate the role of these loci in the pathogenesis of T1D. These latest associated regions may add to the growing repertoire of gene networks predisposing to T1D.

## Genetic susceptibility to T1D in Koreans

Although all T1D cases have common traits and selective destruction of pancreatic  $\beta$ -cells is always observed, the clinical and immunologic characteristics of T1D differ considerably between Asian and Caucasian populations [1]. While reporting a low incidence of T1D in Asia, researchers were unclear about the prevalence of islet-specific autoantibodies in Asian T1D patients [1,4,14]. A subsequent study, using a radioligand binding assay for GAD and IA-2 in young (<15 years at diagnosis) Korean T1D patients, found that the prevalences of IA-2, GAD65, and ICA autoantibodies in patients with recent-onset disease (diagnosed <1 year before) were comparable to those reported for Caucasians [4,14].

T1D is much less frequent in Asian countries than in countries with predominantly Caucasian populations, which might suggest that Asians are genetically protected against T1D. However, the  $\lambda$ s value, indicating familial clustering, was found to be high despite the low prevalence [45]. It could, therefore, be argued that if a genetic factor is responsible for the high  $\lambda$ s when general prevalence is low, then susceptibility genes (whose frequencies are very low in the general population) may be segregating in T1D families.

T1D in Koreans is thought to be strongly associated with specific HLA alleles, namely DR and DQ [15]. A case-control study evaluating the DRB1-DQB1 haplotypes found that there was a difference of over 200-fold in the risk of T1D between the least susceptible and the most susceptible haplotypes. Although the risk associated with individual alleles seems to vary between populations, when comparing identical DRB1-DQB1 haplotypes the association and transmission to diabetic offspring was

similar for Koreans and Caucasians, suggesting that these haplotypes transcend ethnic boundaries [17].

Importantly, having autoantibodies does not necessarily mean being diabetic. As expected, concordant sibs or T1D patients and multiple autoantibody (+) sibs appeared to share the same 2 HLA DR-DQ haplotypes. However, a typical 1:2:1 haplotype sharing was observed between patients with T1D and single antibody (+) sibs [14].

The contribution of the DQ molecule to overall disease susceptibility is thought to be genotype dependent [16]. Although the prevalence of the HLA-DR4-DQB1\*0302 haplotype did not differ between T1D patients and controls, the HLA-DR3/4-DQB1\*0302 genotype was linked to an increased risk, indicating a synergistic effect. This synergism may be explained by the finding that the transmission of DQB1\*0302 varied depending on whether the homologous haplotype was DR3 or not; DQB1\*0302 was more frequently transmitted to the affected sibling when there was DR3 on the other chromosome. Thus, a significant association of DQB1\*0302 with the DR3/4 genotype must exist. The situation was the reverse for the DQB1\*0401 haplotype, which is also positively associated with T1D; DQB1\*0401 was not frequently transmitted to diabetic offspring in the presence of DR3, suggesting a significant association of DQB1\*0401 with DR4/X.

The frequencies of T1D-associated HLA antigens correlate well with T1D incidence rates worldwide [14,46]. Generally, the DR3 and DR4 haplotypes are common in Caucasians, which means that the prevalence of T1D is likely to be higher due to easy formation of the DQ0201/0302 dimer. Conversely, the frequency of the DRB1 genotypes among Korean T1D patients points to a much lower contribution of DR3 and DR4, especially the DR3/4 genotype. In Koreans, it appears, moderate-risk haplotypes are more frequent whereas susceptible haplotypes are rare. When determining population-specific susceptibility, rare genotypes are important: among the DR genotypes, that includes DR3/9, DR9/9, and DR3/X (X: other than 3, 4), none of which are susceptible in Caucasians. As the contribution of HLA-DR3/4 is relatively minor in Korea, it may be just the tip of an iceberg, with many more minor genes (including non-HLA) and less frequent genotypes at play. Moreover, when we assessed the association of the different DR4 subtypes with susceptible DQ8 and neutral DQ4 (DQA1\*0301-DQB1\*0401) haplotypes in Korean general population, DQ8 is usually combined with protective DR4 subtypes (DRB1\*0403, \*0406), while DQ4 is in LD with susceptible DRB1\*0405. High-risk DR4 subtypes (DRB1\*0401, \*0402, \*0405) were predominant in DR4/X, whereas protective DR4 subtypes (DRB1\*0403, \*0406) were observed mainly in the

DR3/4 genotype [46]. In this sense, the low incidence rate in the Korean population may be explained by the counterbalancing effect of the DRB1 and DQB1 alleles in the control population. A different genetic susceptibility including a low frequency of high-risk HLA alleles could explain the lower prevalence of islet-specific autoantibodies and the low incidence of T1D, or different genetic and environmental interactions might be involved in the etiology of T1D.

## Clinical Heterogeneity of Diabetes in Young Asian Patients

In Asia, diabetes among young patients has complex causation. The relative proportion of type 2 diabetes (T2D) among all diabetic cases is quite high. There is also a variable incidence of non-autoimmune types of T1D associated with insulin deficiency (T1b), such as fulminant T1D (FT1D) and virus-induced T1D. Imagawa et al. [47] reported a high prevalence (up to 20%) of FT1D among cases of acute onset T1D in Japan, but little is known about the situation in other countries. Our own data suggest that, although T1a is the major type of childhood diabetes, FT1D exists as a hyperacute subtype of T1D that affects older children, without there being any evidence of autoimmunity. Our patients showed a complete loss of  $\beta$ -cell secretory capacity without evidence of recovery, necessitating long-term treatment with insulin [48]. In this context, early diagnosis and classification of patients with acute-onset diabetes may be crucial in order to minimize the formidable complications.

Latent autoimmune diabetes in adults (LADA) is a form of autoimmune-mediated diabetes, usually diagnosed based on GAD autoantibody positivity. Although several epidemiological surveys of LADA have been conducted in Caucasian and Asian populations, their reported incidence and prevalence rates vary due to the use of different diagnostic criteria. Applying comparable criteria and a similar level of ascertainment, a cross-sectional analysis was performed on 193 LADA patients diagnosed in Italy and 39 LADA patients in Korea. Using GAD autoantibody positivity as the diagnostic criterion, the prevalence of LADA was identical in Italy and in Korea (4.4%) [49]. For Korea, this was considerably higher than the previously reported prevalence of 1.7% in a population-based T2D survey [1]. However, as the GAD antibodies were mostly low-affinity and low-titer, this higher prevalence may not necessarily lead to a significant increase in subsequent insulin use. Furthermore, the prevalence of many other autoantibodies was also low in this population. After

36 months of follow-up, only 3 of the 39 patients initially diagnosed with LADA had become insulin-dependent, and they were all positive for multiple autoantibodies (GAD, IA-2 and ZnT8 antibody) [49]. Importantly, this demonstrates that true insulin dependency, in the beginning indicated by multiple antibody positivity, has not increased in the Korean population.

## Conclusion

T1D is a complex, polygenic, organ-specific autoimmune disease, with both genetic and environmental factors contributing to its development. At present, HLA genes, including the HLA-DQ and -DR haplotypes, are considered to be its strongest risk factors. The non-HLA genetic susceptibility is influenced by multiple genes and, under certain conditions, may modify the risk contributed by the HLA genes. Fine mapping and functional studies are revealing the complex mechanisms whereby immune tolerance is lost, involving multiple aspects of adaptive immunity. The triggering of these events by dysregulation of the innate immunity has also been suggested by genetic evidences. At this stage, pathway-directed or system biology-aided functional studies are necessary to understand their exact influence on overall disease susceptibility and/or protection. Research also needs to focus on the autoantigenic peptides that are of critical importance for diabetes, their binding affinity with particular MHCs, and the type and specificity of the T cells involved in the immunological cascade that leads to damage to the pancreatic  $\beta$  cells (especially via the interaction with candidate antigens). Other contributors to disease susceptibility that may warrant further study include differential expression of candidate antigens and their variants in the thymus, as well as non-germline genes and environmental factors [50].

In addition to the HLA locus, at least 40 other loci are now known to influence susceptibility to T1D. Some of these appear to predispose individuals to T1D independently of HLA, and they may play an important role in families who lack strong HLA susceptibility. The effect of non-HLA genes on T1D susceptibility appears to be modest. However, studying their involvement in various population groups may provide a vital clue to the pathways involved in the development of T1D, and lead to effective methods of intervention. GWAS revealed genomic risk loci that potentially have an impact on disease and phenotypic traits. This extensive resource will guide us to novel directions for personalized medicine, including disease risk prediction, prevention and intervention. To make the initial identification of an association contribute to precision

treatment of patients, the integrative analysis of system-wide data on genome variation, gene expression, transcription factor binding, epigenetic profiles and chromatin conformation is crucial in understanding the biological mechanism that underlie the association. The generation of these data mainly relies on next-generation sequencing. Mass spectrometry-based proteomics now offers additional possibilities for the identification of functional genome variants and, in particular, for the identification and characterization of (differentially) bound protein complexes as well as physiological target genes. Such data will also be crucial in developing personalized molecular medicine solutions based on individuals' genetic make-up.

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