

Genetics and pathogenesis of type 1 diabetes: prospects for prevention and intervention

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ABSTRACT

Type 1 diabetes is etiologically a multifactorial disease caused by a complex interaction of genetic and environmental factors, with the former consisting of multiple susceptibility genes. Identification of genes conferring susceptibility to type 1 diabetes would clarify etiological pathways in the development and progression of type 1 diabetes, leading to the establishment of effective methods for prevention and intervention of the disease. Among multiple susceptibility genes, *HLA* and *INS* are particularly important because of their contribution to tissue specificity in the autoimmune process. *DRB1*04:05-DQB1*04:01* is associated with autoimmune type 1 diabetes, idiopathic fulminant type 1 diabetes and anti-islet autoimmunity in autoimmune thyroid diseases, suggesting that this haplotype is associated with beta-cell specificity in autoimmune diseases. Genes involved in the expression of insulin in the thymus contribute to beta-cell-specific autoimmune mechanisms in type 1 diabetes. These genes and pathways are important targets for tissue-specific prevention and intervention of type 1 diabetes. (*J Diabetes Invest*, doi: 10.1111/j.2040-1124.2011.00176.x, 2011)

KEY WORDS: Autoimmune disease, Genetics, Type 1 diabetes

INTRODUCTION

Type 1 diabetes is caused by destruction of insulin-producing beta-cells of the pancreas in genetically susceptible individuals. Etiologically, type 1 diabetes is classified into two major subtypes, autoimmune (type 1A) and idiopathic (type 1B). The etiologic factors and pathogenesis of idiopathic type 1 diabetes are still unknown, but recent studies suggested that fulminant type 1 diabetes may belong to this subtype^{1,2}. Type 1A diabetes is an organ-specific autoimmune disease in which beta-cells of the pancreas are the target organ of the autoimmune attack.

Type 1 diabetes is a multifactorial disease caused by a complex interaction of genetic and environmental factors, with the former consisting of multiple susceptibility genes. Identification of genes conferring susceptibility to type 1 diabetes would clarify the etiological pathways in the development and progression of type 1 diabetes, leading to the establishment of effective methods for prevention and intervention of the disease. In this review, clinical problems in the treatment of type 1 diabetes are summarized in order to help understand the reason why identification of genes conferring susceptibility to type 1 diabetes is necessary, and then the current status of the molecular genetics of type 1 diabetes is reviewed with special emphasis on genes that contribute to tissue specificity of autoimmune mechanisms.

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WHY GENES?

Among patients with type 1 diabetes, heterogeneity of residual beta-cell function is observed. Some patients completely lack endogenous insulin secretion, while others have preservation of minimal insulin secretory capacity. Complete lack of endogenous insulin secretion in type 1 diabetes is associated with unstable glycemic control, so-called brittle diabetes, as evidenced by our previous studies showing an inverse correlation between unstable glycemic control and minimal residual beta-cell function in type 1 diabetes³. These data have recently been confirmed in fulminant diabetes⁴. This can be explained by the buffering action of endogenous insulin, whose secretion, even in a small amount, is automatically adjusted to the body's need on a minute-to-minute basis. Excess exogenous insulin can be adjusted by a decrease in endogenous insulin, whereas deficiency of insulin can be adjusted by a small increase in endogenous insulin. Type 1 diabetic patients with no residual beta-cell function lack this buffering action of endogenous insulin, and therefore have difficulty maintaining stable glycemic control, even with continuous subcutaneous insulin infusion (CSII). At the moment, pre-programmable CSII may be the only way to achieve glycemic control in such patients. Figure 1 shows the basal insulin infusion rate of pre-programmable CSII to achieve stable glycemic control in five patients with type 1 diabetes with complete lack of endogenous insulin. To achieve stable glycemic control, very dynamic adjustment of basal insulin infusion was required, with a decrease in infusion rate to avoid nocturnal hypoglycemia and an increase in infusion rate to overcome the dawn phenomenon. This in turn suggests that type 1 diabetic patients, particularly those with complete lack of endogenous

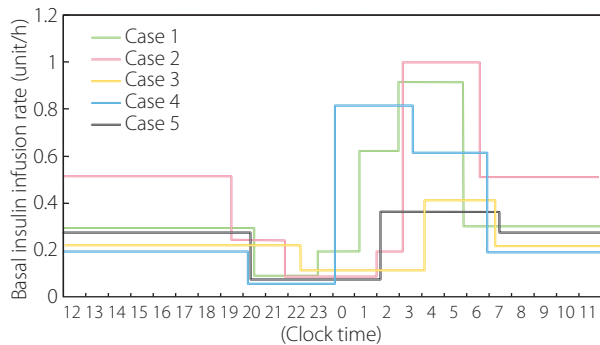


Figure 1 | Basal insulin infusion rate in type 1 diabetics with complete lack of endogenous insulin. C-peptide-negative patients with type 1 diabetes ($n = 5$) were treated with continuous subcutaneous insulin infusion (CSII) with a pre-programmable insulin pump. The infusion rate of basal insulin at night was adjusted to achieve target glycemic control with near-normal glycemia at bedtime and before breakfast, and no hypoglycemia (<70 mg/dL) at 3:00 AM. Very dynamic changes in basal insulin infusion rate were required. To avoid nocturnal hypoglycemia, a decrease in infusion rate to a level as low as 1/5 the daytime infusion rate was required, whereas an increase to as great as four times the daytime infusion rate was required to overcome the 'dawn phenomenon'.

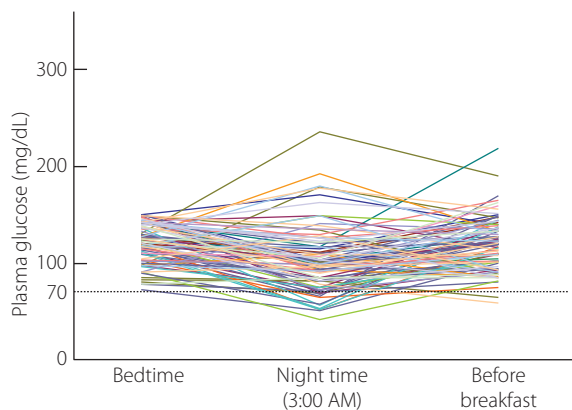


Figure 2 | Plasma glucose level at 3:00 AM compared with bedtime and before breakfast. Plasma glucose levels were monitored at bedtime (9:00 PM), during the night (3:00 AM) and before breakfast (7:00 AM) in diabetic inpatients ($n = 87$) treated with bedtime NPH insulin or a long-acting insulin analogue, with relatively stable glycemic control (bedtime glucose level 70–150 mg/dL). It is evident that the night time glucose level cannot be estimated from glucose levels at bedtime and/or before breakfast in some patients. Nocturnal hypoglycemia is seen in some patients, while in others an increase in glucose level with a nocturnal peak is observed.

insulin, are at high risk of nocturnal hypoglycemia when treated with bedtime NPH insulin or a long-acting insulin analogue. In fact, when plasma glucose level was measured at 3:00 AM in diabetic inpatients ($n = 87$) who had relatively stable glycemic control with multiple insulin injections of bedtime NPH insulin

or a long-acting insulin analogue, marked variation in the glucose level was noticed (Figure 2), with nocturnal hypoglycemia occurring in 18% of patients. Nocturnal hypoglycemia was significantly more frequent in type 1 diabetic patients than in type 2 diabetic patients (31 vs 12%, $P = 0.03$). These data indicate the importance of preservation, or possibly regeneration, of beta-cells in type 1 diabetes. At the clinical onset of type 1 diabetes, beta-cells are not completely destroyed, and low, but significant, secretory capacity of insulin still remains in most patients. To preserve residual beta cells at an early stage of type 1 diabetes and protect regenerating beta cells from recurrent autoimmune attack, the molecular mechanisms of autoimmune beta-cell destruction must be clarified in order to establish effective methods for prevention and intervention. Identification of genes conferring susceptibility to type 1 diabetes is thus important because molecular pathways can be clarified by studying the function of genes identified.

WHICH GENES?

Initially, susceptibility genes for type 1 diabetes were studied by the candidate gene approach, and several important genes, such as *HLA* and insulin gene (*INS*), have been identified^{5–9}. The random marker approach was then adopted initially in multiplex families, and more recently in a large number of cases and controls with hundreds of thousands of single nucleotide polymorphisms (SNPs), termed genome-wide association studies (GWAS) (10–12). By using these approaches, more than 40 susceptibility loci have been mapped in Caucasian populations (Figure 3). Most of them, however, were loci, and responsible genes are yet to be identified. To clarify the etiological pathway in order to develop effective methods for prevention and intervention, responsible genes must be identified.

Among multiple susceptibility genes, at least five genes, *HLA*, *INS*, *CTLA4*, *PTPN22* and *IL2RA* (*CD25*), have been shown to be responsible for type 1 diabetes susceptibility in Caucasian populations^{5–12}. Although the incidence of type 1 diabetes is markedly different between Japanese and Caucasian populations, the association of candidate genes with type 1 diabetes is generally similar in both populations, and there are good reasons for the apparent differences in the genes associated with type 1 diabetes between Japanese and Caucasians^{13–19}.

Among these genes, *HLA* shows particularly strong susceptibility in both Japanese and Caucasian populations^{20–22}. The contribution of insulin gene (*INS*) to susceptibility to type 1 diabetes is well established in Caucasian populations, but its contribution in Japanese is not as clear as that in Caucasians due to the very high frequency of risk haplotype in the Japanese general population²³. Recent studies demonstrated that *INS* is associated with type 1 diabetes in Japanese¹⁸. These two genes appear to contribute to type 1 diabetes at different steps in the etiological pathway, but as discussed below, several lines of evidence suggest that not only *INS*, but also class II *HLA* may contribute to tissue specificity of autoimmune destruction.

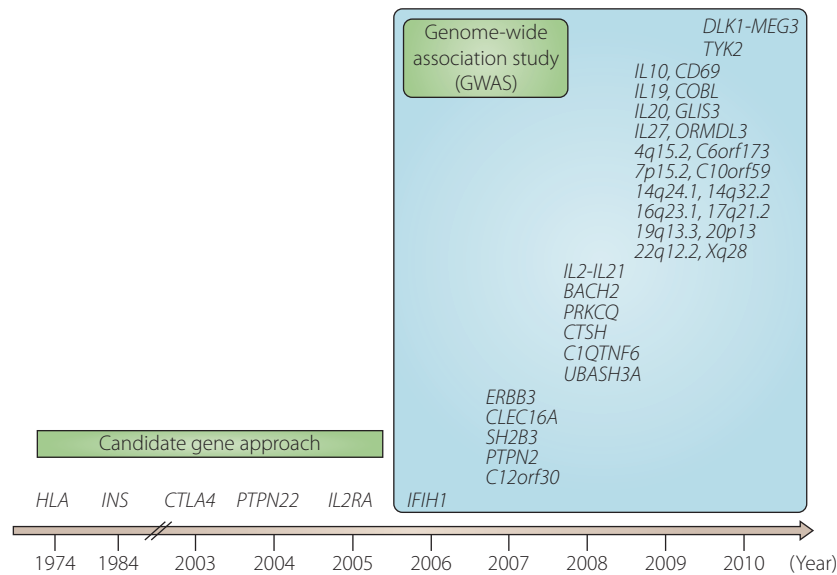


Figure 3 | Susceptibility genes or loci for type 1 diabetes identified by candidate gene approach and/or genome-wide association study (GWAS). Genes or loci for type 1 diabetes are shown relative to the year when convincing evidence was reported. *HLA*, *INS*, *CTLA4*, *PTPN22* and *IL2RA* (*CD25*) were identified by candidate gene approach. *IFIH1* was identified by genome-wide association study (GWAS) with non-synonymous SNPs, and the rest were identified by GWAS. Note that most gene symbols reported by GWAS are markers associated with the disease, but not necessarily actual genes or causal variants responsible for the disease.

MAJOR SUSCEPTIBILITY GENE: *HLA*

Class II *HLA*, *DRB1* and *DQB1*, have been consistently reported to be associated with type 1 diabetes in almost all ethnic groups. Differences, however, in alleles and haplotypes associated with type 1 diabetes have been reported among different ethnic groups. The DR3 (*DRB1*03:01-DQB1*02:01*) and DR4 (*DRB1*04:01-DQB1*03:02*) haplotypes are positively associated with type 1 diabetes in Caucasian populations, whereas the DR4 (*DRB1*04:05-DQB1*04:01*) and DR9 (*DRB1*09:01-DQB1*03:03*) haplotypes are associated with the disease in Japanese and most east-Asian populations^{20–22}. The difference in *HLA* haplotypes associated with type 1 diabetes between Japanese and Caucasian populations can be explained by the presence or absence of haplotypes in each population^{13,14}.

HLA in Rare Multiplex Family

Type 1 diabetes clusters in families, not only in Western countries²⁴ but also in Japan, as evidenced by the much higher frequency in siblings of type 1 diabetic probands than in the general population^{13,14,25}. Although the incidence of type 1 diabetes is much lower in Japan²⁶, the frequency of type 1 diabetes in siblings of type 1 diabetic probands is similar to that in white populations of European descent^{13,14,25}. As a consequence, the ratio of frequencies in siblings and the general population, termed λ , which is often used to express the degree of familial clustering of a disease, is much higher in Japanese than in Caucasian populations^{13,14,24,25}. A high λ value with low incidence in the general population suggests

two possibilities: rare variants, that is susceptibility variants with low frequencies, but with high penetrance, cluster in families, and the shared environment within families contributes to familial clustering.

To study the former possibility, we studied a rare multiplex family in which three out of four sisters developed type 1 diabetes and the fourth sister was found to be positive for anti-GAD and anti-IA-2 antibodies²⁷, suggesting that the type 1 disease process existed in all four sisters, with three of them having developed the disease clinically. In this family, all four sisters shared the same *HLA* genotypes, *DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02*²⁷. This genotype has a very low frequency in the Japanese general population, but was previously reported to show a very high odds ratio for type 1 diabetes^{21,28}, suggesting that this *HLA* genotype is a rare variant and is one of the reasons for clustering of type 1 diabetes in this family. Both the *DRB1*04:05-DQB1*04:01* and *DRB1*08:02-DQB1*03:02* haplotypes are very rare in Caucasian populations, and as a consequence, the *DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02* genotype is almost absent in Caucasian populations. However, a rare *DRB1*04:05-DQB1*03:02* haplotype does exist in Caucasian populations and was reported to be the highest risk haplotype for type 1 diabetes in Caucasians²⁹, although its frequency is low in the general population. These data suggest that the combination of *DRB1*04:05* and *DQB1*03:02* in either trans (Japanese) or cis (Caucasians) acts as a kind of rare variant and confers very high susceptibility to type 1 diabetes (Table 1).

Table 1 | Combination of *DRB1*04:05* and *DQB1*03:02* as rare variant with low frequency, but high penetrance for type 1 diabetes

| Population | HLA haplotype or genotype | Frequency in controls (%) | Odds ratio for type 1 diabetes | Reference |
|------------|--|---------------------------|--------------------------------|-----------|
| Caucasian | <i>DRB1*04:05-DQB1*03:02</i> | 0.2 | 11.4 | 29 |
| Japanese | <i>DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02</i> | 0.3 | 42.7 | 28 |

HLA in Fulminant Type 1 Diabetes

In contrast to autoimmune type 1A diabetes, idiopathic type 1B diabetes is not well characterized, but recent studies suggest that fulminant type 1 diabetes belongs to this subtype^{1,2}. Fulminant type 1 diabetes is characterized by a markedly acute onset of diabetes and an absence of islet-related autoantibodies¹, accounting for up to 20% of type 1 diabetes in Japan² and 7% in Korea³⁰. In contrast to the relatively high frequencies in Asian populations, fulminant type 1 diabetes appears to be very rare in Caucasian and other non-Asian populations. The reason for the difference is still unknown, but one possibility may be the difference in the frequencies of risk HLAs in general populations. *DRB1*04:05-DQB1*04:01*, in particular in the homozygous form, is strongly associated with fulminant type 1 diabetes²⁸. The frequency of fulminant type 1 diabetes appears to correlate with the frequency of the *DRB1*04:05-DQB1*04:01* haplotype, in that fulminant type 1 diabetes is common in Japanese and most East Asian populations, where the *DRB1*04:05-DQB1*04:01* haplotype is common in the general population, but is absent or extremely rare in Caucasian populations, where the *DRB1*04:05-DQB1*04:01* haplotype is also absent or very rare.

HLA in Autoimmune Thyroid Diseases Complicated with Islet Autoimmunity

Patients with type 1 diabetes frequently develop other organ-specific autoimmune diseases, of which autoimmune thyroid diseases (AITD) are the most frequent disorder^{31,32}. In contrast to the large number of studies on autoimmunity against the thyroid gland in patients with type 1 diabetes, little is known about the anti-islet autoimmune status in patients with AITD. We recently studied the anti-islet autoimmune status in patients with AITD, and the clinical and genetic characteristics of AITD patients with anti-islet autoimmunity³³. The prevalence of anti-islet autoimmunity as assessed by GAD Ab was significantly higher in patients with AITD than in normal control subjects. AITD patients with GAD Ab showed a significantly higher frequency of diabetes than did those without GAD Ab, and this was more pronounced in patients with a high titer of GAD Ab. Diabetes in AITD patients with GAD Ab was characterized by younger age-at-onset, lower BMI, higher HbA1c and higher frequency of insulin treatment than that in patients without GAD Ab, suggesting that diabetes in AITD patients positive for GAD Ab shows the clinical features of type 1 diabetes.

The *DRB1*04:05-DQB1*04:01* haplotype, which confers susceptibility to type 1 diabetes, was associated with AITD positive for GAD Ab, but not with AITD negative for GAD Ab³³, suggesting that the *DRB1*04:05-DQB1*04:01* haplotype is associated with anti-islet autoimmunity in subjects with as well as without AITD. In contrast, the *DRB1*08:03-DQB1*06:01* haplotype was associated with AITD without GAD Ab, but not with AITD with GAD Ab³³, suggesting that the *DRB1*08:03-DQB1*06:01* haplotype confers susceptibility to autoimmunity against the thyroid gland, but not anti-islet autoimmunity. These data suggest the contribution of HLA haplotypes not only to immune regulation, but also to organ specificity in autoimmune diseases, with *DRB1*04:05-DQB1*04:01* contributing to beta-cell specificity of the destructive process by an autoimmune mechanism in type 1A diabetes and in AITD with anti-islet autoimmunity, as well as an idiopathic mechanism in type 1B (fulminant) diabetes.

INSULIN GENE-RELATED PATHWAY

Cis Regulatory Region: *INS*-VNTR

Accumulating lines of evidence suggest that insulin is a primary autoantigen in type 1 diabetes³⁴⁻³⁷. Association of the insulin gene region with type 1 diabetes has been repeatedly reported in Caucasian populations^{6,38-41}. Allelic variation in the variable number of tandem repeats (VNTR) located in the 5' upstream region of *INS* has been suggested to be responsible for disease susceptibility^{39,40}. In the Japanese population, the markedly high frequency (>90%) of disease-susceptible haplotype in the general population made it difficult to demonstrate the contribution of *INS* to disease susceptibility²³. Recent large scale studies, however, demonstrated that *INS*-VNTR is associated with type 1 diabetes in Japanese as well as in Caucasian populations¹⁸. *INS*-VNTR is thought to contribute to type 1 diabetes susceptibility through reduced expression of insulin in the thymus, leading to impaired negative selection of insulin-specific autoreactive T-cells^{41,42}.

Trans-acting factor

In contrast to the association of the cis-regulatory region of *INS* with type 1 diabetes in humans, such variants have not been identified in the NOD mouse, an animal model of type 1 diabetes. Since expression of insulin is regulated not only by cis-regulatory elements, but also trans-acting factors, we studied the expression of beta-cell specific transcription factors in the thymus⁴³. Among beta-cell-specific transcription factors, such as Pdx-1, Neurod 1, and MafA, only MafA was expressed in the thymus⁴³. Functional polymorphisms of MafA were newly identified in the NOD mouse, which were associated with reduced expression of insulin in the thymus and susceptibility to type 1 diabetes in the NOD mouse⁴³. Functional polymorphisms of human MAFA were also identified and shown to be associated with type 1 diabetes⁴³, suggesting that antigen-specific transcriptional factors play a critical role in induction of central tolerance to self antigens, and abnormality in such regulation may lead to organ-specific autoimmune diseases (Figure 4).

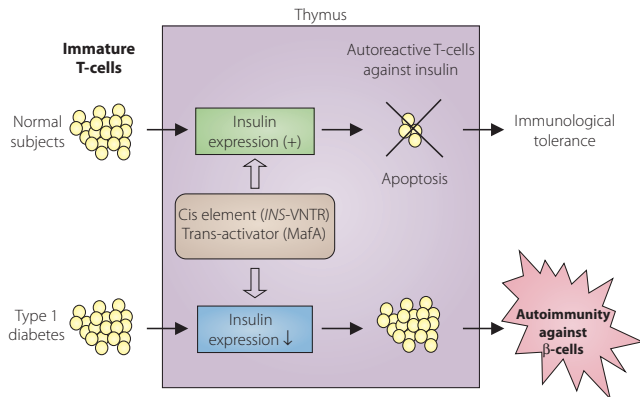


Figure 4 | Expression of insulin in thymus and negative selection of autoreactive T-cells against insulin. In subjects whose expression level of insulin is normal in the thymus, apoptosis is induced in insulin-specific T-cells upon recognition of insulin in the thymus, leading to negative selection of autoreactive T-cells and induction of central tolerance to insulin. In subjects with reduced expression of insulin in the thymus, negative selection of insulin-specific T cells is impaired, resulting in autoimmune attack against insulin-producing beta-cells of the pancreas and development of type 1 diabetes. Intra-thymic expression of insulin is regulated by cis-regulatory elements, such as *INS-VNTR*, and trans-acting factors, such as MafA. Functional variants in these elements or factors thus cause autoimmunity against pancreatic beta-cells through impaired negative selection of insulin-specific T-cells.

CONCLUSIONS

Identification of genes conferring susceptibility to type 1 diabetes is important, even if the effect of each gene is small, because each gene contributes to a step or steps in the etiological pathway, and modification of the function of the gene or gene product could contribute to prevention and intervention of the disease. In particular, genes that contribute to tissue specificity of the autoimmune process, as in the case of genes involved in the regulatory pathway of intra-thymic expression of insulin, are important because they are targets for tissue-specific prevention and intervention of autoimmunity in type 1 diabetes. Although a large number of loci have been mapped in Caucasian populations by GWAS, most of them are still loci, but not responsible genes. It will be a formidable challenge to identify genes responsible for susceptibility loci mapped by GWAS because of the relatively small effect of each locus and the multifactorial nature of the disease. To overcome this, GWAS must be performed in a population possessing haplotypes different from those in Caucasians, and genes should be identified by trans-racial studies. Such studies are now underway as a nationwide effort by the Committee on Type 1 Diabetes of the Japan Diabetes Society.

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