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Genetic Markers, Serological Auto Antibodies and Prediction of Type 1 Diabetes

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1. Introduction

The type 1 diabetes mellitus (formerly known as insulin-dependent diabetes mellitus) is a chronic autoimmune disorder that precipitates in genetically susceptible individuals by environmental factors (Atkinson and Eisenbarth 2001). The body's own immune system attacks the beta-cells in the islets of Langerhans of the pancreas, destroying or damaging them sufficiently to reduce and eventually eliminate insulin production.

The increase in understanding of the pathogenesis of type 1 diabetes has made it possible to consider intervention to slow the autoimmune disease process in an attempt to delay or even prevent the onset of hyperglycemia but varying in terms of their genetic, environmental, and amphopometric measures (2003).

Can we really predict type 1 diabetes? This is question has been a major target of diabetes research over the last decade. The aims have been to find a way of identifying individuals at risk and to accurately define their degrees of risk. Subjects who are at high risk for type 1 diabetes (T1D) can be identified using a combination of immune, genetic, and metabolic markers, For example, prediction of T1D among relatives can be quite accurate, by combining screening of relatives by measurement of islet cell autoantibodies with subsequent assessment of insulin autoantibodies (IAAs), first-phase insulin response to intravenous glucose, and oral glucose tolerance, while excluding those relatives with the known protective genetic allele HLA-DQB1-0602 (Pugliese, Gianani et al. 1995).

Using this combination approach and screening approximately 100,000 relatives, it was possible to identify accurately two cohorts of relatives, one (339 individuals) with a projected 5-year risk of greater than 50% and actual 5-year risk of 60%, (2002) and another (372 individuals) with a projected 5-year risk of 25–50% and actual 5-year risk of 35% (Skyler, Krischer et al. 2005).

The seminal research discovery of islet cell cytoplasmic autoantibodies (ICA) in 1974 not only offered clues to the autoimmune basis for type 1 diabetes but in addition, aided in providing some degree of clarity to the aforementioned difficulties associated with disease classification and diagnosis (Bottazzo, Florin-Christensen et al. 1974). The discovery of autoantibodies in T1D also supported the formation of a series of studies defining the natural history of metabolic and immunologic events underling the formation of this disease, the biochemical nature of islet cell autoantigens in the disorder, and trials attempting to predict as well as prevent the formation of T1D.

2. Animal models of type 1 diabetes

The availability of two animal models of type 1 diabetes has made it possible to evaluate plausible therapeutic strategies before starting human trials. Non-obese diabetic (NOD) mice and BioBreeding (BB) rats are in-bred strains that spontaneously develop autoimmune insulitis and diabetes with striking similarities to type 1 diabetes in humans (Mordes, Desemone et al. 1987; Elias and Cohen 1994).

The present landscape of basic and translational research in animal models of T1D is characterized by overuse of the NOD mouse. This scenario has some historic reasons that are understandable, foremost among those being the fact that NOD mice and humans share several susceptibility-related genes, including genes encoding the MHC class II homologs. But it is now also known that there is considerable complexity and heterogeneity in both the disease and in the genetics of the disease, and a singular focus on the NOD model generates too narrow a perspective.

The cumulative incidence of type 1 diabetes in these animals is high, and the onset of insulitis as well as hyperglycemia can be readily detected. Several interventions have been tested in these animals, often at a very early stage in the autoimmune disease process before the onset of insulitis. Examples include subcutaneous and oral insulin, nicotinamide, and the ß-cell antigen glutamic acid decarboxylase. Of note, many interventions have been effective in the murine models when applied before the development of hyperglycemia; however, very few interventions have reversed established diabetes.

3. Natural history of preclinical type 1 diabetes in humans

Type 1 diabetes is usually caused by autoimmune destruction of the insulin-producing ß-cells in the islets of Langerhans (Atkinson and Maclaren 1994). In the new classification of diabetes, immune mediated type 1 diabetes is called type 1A to distinguish it from some rarer cases in which an autoimmune etiology cannot be determined (type 1B); the latter are said to be idiopathic (1997).

Type 1 diabetes (T1D) occurs in genetically susceptible subjects. However many agree that an individual's genetic predisposition to this disease modified by environmental factors likely form a key facet for development (Knip 2003) (figure 1).

Indeed, the genetic predisposition for T1D, like most autoimmune disorders, in large part resides within genes controlling the immune response, principal amongst these being the major histocompatiblity complex (MHC). However, susceptibility and resistance for T1D does not reside in the MHC alone as more than two dozen additional loci outside of the MHC complex have been identified to influence risk for this disease (Melanitou, Fain et al. 2003; Atkinson 2005). Among the many potential candidate genes residing in such loci are BCL2, CD28, CTLA-4, CXCL12, interleukin-2 receptor and INS genes (Atkinson 2005).

The potential influence of environmental factors in T1D development has been suggested through multiple observations, the primary ones being the 500-fold variance in disease incidence based on geographic locale, seasonal variance in disease onset, and somewhat dramatic increases in the frequency of this disease, particularly over the last half-century. While environmental factors influencing T1D development have remained elusive (i.e., none have specifically been isolated), epidemiological studies have associated infant diet, viruses and perhaps increased hygiene as contributing events to this disease (Knip 2003). In terms of how they might contribute to disease, without specific identification, such models remain

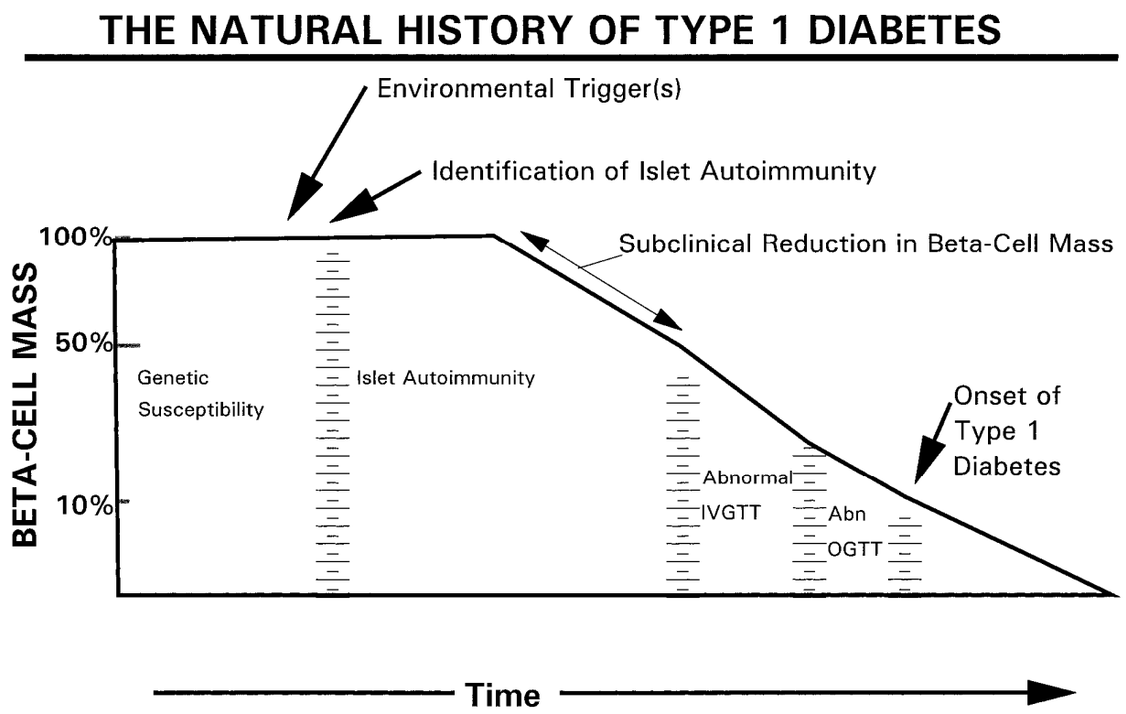

Fig. 1. The natural history of type 1 diabetes is depicted. In individuals with genetic predisposition to beta-cell autoimmunity, exposure to an environmental trigger or triggers is believed to initiate beta-cell autoimmunity, first evidenced in the appearance of islet autoantibodies. If beta-cell necrosis ensues as a consequence of destructive betacell cellular autoimmunity, a subclinical reduction in beta-cell mass ensues. The first clinical evidence of beta-cell dysfunction is an abnormal IVGTT result. With continued damage to the beta-cells, the OGTT will become abnormal. Within 1–2 years, frank symptoms of type 1 diabetes usually evolve (Winter, Harris et al. 2002).

hypothetical, but it is currently speculated that once the pathway to T1D has been initiated, either through as yet unknown triggers or natural physiological processes, various aberrant immune responses begin to play a role. It is important to note that many theories for environmental contributions to T1D development (e.g., viral molecular mimicry, β-cell trophic viruses, cow milk consumption, childhood vaccines, etc.), while popular in terms of their notion, have not proven themselves reproducible across a variety of study populations over time (Litherland, Xie et al. 1999; Atkinson 2005).

Despite genetics and environment forming knowledge voids for T1D pathogenesis, models for T1D development have been proposed that include these factors in combination with other findings (e.g., immunoregulatory defects, rate of metabolic loss, formation of anti-islet antibodies, etc.) which have been subject to better and more detailed description (Litherland, Xie et al. 1999). Under one such natural history model, defects in antigen presentation as well as antigen presenting cell maturation imparts an unnatural arrest in clearance of immune cells from the islets. The additional inability to regulate this response to self-antigens, a facet largely controlled by genetic susceptibility, would ultimately lead to a destructive islet cell inflammation (i.e., insulitis), with death of the insulin secreting β-cells (Brusko, Wasserfall et al. 2005). As the total β-cell mass declines, a critical point is reached

where glucose homeostasis is lost and eventually exogenous insulin is an absolute requirement for survival.

Aside from model systems, thoughts continue that T-lymphocytes play the predominant role either directly (cytotoxicity) or indirectly (cytokine mediated β-cell apoptosis) in the destruction of islets. Adoptive transfer experiments in animal models have shown that T-lymphocytes transfer disease and serum does not. At the same time, recent studies have suggested key roles for B-lymphocytes in this process as one mouse model for T1D, the NOD mouse, when rendered deficient in these cells (either through genetic manipulation or through antibody treatment) fail to develop overt disease.

Hence, at one level, it is clear that B-lymphocytes are also involved inasmuch as they do produce autoantibodies (Brusko, Wasserfall et al. 2005) and in reality, it has been hypothesized that antigens presented by B lymphocytes may represent a critical feature to the development of T1D.

However, several aspects of the natural history of preclinical type 1 diabetes remain unclear, including the rate of progression and the changes in and predictive value of genetic and metabolic markers.

4. Biomarkers of susceptibility - tools for disease prediction

4.1 Genetic markers

Genetic markers may be helpful in assessing the risk of type 1 diabetes in close relatives of a patient with type 1 diabetes. The risk is markedly increased in these relatives, averaging about 6 percent in offspring and 5 percent in siblings (versus 0.4 percent in subjects with no family history) (Atkinson and Maclaren 1994).

The major genetic determinants of type 1 diabetes reside in the HLA region within the major histocompatibility complex (MHC) on the short arm of chromosome 6. An association between HLA class I alleles and type 1 diabetes was first described in the early 1970s (Nerup, Platz et al. 1974). More recent observations have indicated that the genes in the HLA-DQ region are even more closely associated with type 1 diabetes than the DR genes (Morel, Dorman et al. 1988). Over 90 percent of patients with type 1 diabetes carry DR4, DQB*0302 and/or DR3, DQB*0201. Thus, if the proband is heterozygous for DR3 and DR4 (the highest risk combination), the incidence of type 1 diabetes in a sibling who shares these two haplotypes rises to 19 percent. On the other hand, the absence of the above alleles makes type 1 diabetes very unlikely, especially if the subject carries a protective allele such as DQB*0301, *0602 (Pugliese, Gianani et al. 1995), DRB*0403, or *0406.

Use of genetic markers plus the family history make it possible to estimate the risk of type 1 diabetes as being as low as one in 5000 (no susceptibility alleles or family history) to as high as one in four (two susceptibility alleles and a positive family history). However, the prevalence of the HLA susceptibility genes is relatively high in whites. As a result, the predictive value of HLA typing is much lower in population screening than it is among families in which one or more members have type 1 diabetes (Bingley, Bonifacio et al. 1993) (Table 1).

In one study, the risk for islet autoimmunity drastically increased in DR3/4-DQ2/DQ8 siblings who shared both HLA hapl9otypes identical by descent with their diabetic proband sibling (63 and 85 percent by ages 7 and 15, respectively) as compared to siblings who did not share both HLA haplotypes with their diabetic proband sibling (Aly, Ide et al. 2006). These data suggest that HLA genotyping at birth may identify individuals at very high risk of developing type 1 diabetes before the occurrence of clear signs of islet autoimmunity and

type 1 diabetes onset. Rapid automated assays make it possible to do large-scale population screening for HLA easily, even in newborns (Ilonen, Reijonen et al. 1996; Rewers, Bugawan et al. 1996)

Population	Type 1 Diabetes Risk (%)
Low Risk	
No affected FDR plus HLA protective genes	0.01
No affected FDR	0.4
Affected FDR plus HLA protective genes	0.3
Intermediate Risk	
No affected FDR plus HLA risk genes	4
One affected FDR	5
Mother with T1D	3
Father with T1D	5
Sibling with T1D	8
High Risk	
One affected FDR plus HLA high risk genes	10–20
Multiple affected FDRs	20–25
Very High Risk	
Identical twin affected	30–70
Multiple affected FDRs plus HLA risk genes	50
Sibling affected plus HLA risk genes, identical by descent	30–70

FDR, first-degree relative; HLA risk genes, *HLA DRB1*03,*04;DQB1*0302*; HLA protective genes, *HLA DQB1*0602*.

Table 1. Type 1 Diabetes Risk Stratification by T1D Family History and HLA Genotyping.

In general, the additional measurement of 2 HLA-DQ high-risk haplotypes does not increase the predictive value of combined autoantibody assays. However, in relatives who are seronegative for conventional islet autoantibodies, the presence of two HLA-DQ high-risk haplotypes is associated with an increased risk of progression to type 1 diabetes (Pietropaolo, Becker et al. 2002). This observation suggests that unidentified autoimmune phenomena may be present in seronegative relatives who carry the 2 HLA-DQ high-risk haplotypes.

Furthermore, specific allelic combinations of variants in the insulin gene (INS), the cytotoxic T lymphocyte antigen-4 gene (CTLA4) and the protein tyrosine phosphatase, non-receptor type 22 gene (PTPN22) have been repeatedly associated with type 1 diabetes susceptibility (Undlien, Lie et al. 2001; Pugliese and Miceli 2002; Ueda, Howson et al. 2003) using different approaches and increasing the number of susceptibility loci considered simultaneously generally increases the predictive value for T1D disease. The receiver operating characteristic (ROC) curve analysis confirms that, despite the higher absolute risk for those few with combinations of several risk markers, adding non-HLA genetic markers only marginally increases the utility of the prediction over that of HLA alone.

Despite near-multiplicative effects for most loci, and the fact that groups with very high relative risk of type 1 diabetes can be identified by testing for multiple susceptibility genes, only a small proportion of the population (and cases with type 1 diabetes) simultaneously carry HLA and multiple non-HLA susceptibility genotypes.

4.2 Immunologic markers

4.2.1 Islet autoantibodies:

The most important change in the T1D risk status of a child occurs when islet autoantibodies develop. Several clinically useful serum autoantibodies can be detected during the preclinical period of type 1 diabetes, including islet-cell antibodies (ICA), insulin

autoantibodies (IAA), antibodies to glutamic acid decarboxylase (GAD), and antibodies to tyrosine phosphatase-like proteins such as insulinoma associated protein (IA-2, ICA512).
Furthermore, only 8 to 10% of all cases of type 1 diabetes have a family history, while 90% of cases occur sporadically (Dahlquist, Blom et al. 1985). It was therefore important to examine the prevalence and the prognostic value of autoantibodies in the general population.
ICA is polyclonal autoantibodies that react with all cells of the islet (i.e., α, β, δ, and pancreatic polypeptide cells). The next major discovery in terms of humoral autoimmunity in T1D was that of autoantibodies to insulin or IAA (Williams, Bingley et al. 1999).
The presence of ICA was associated with an increased risk of diabetes, particularly if the ICA titer was high, ICA were persistently detected, or ICA were present in combination with IAA or GAD antibodies (Verge, Stenger et al. 1998).
Similar findings have been reported with IA-2. In one study of first-degree relatives of type 1 diabetic probands, those with IA-2 autoantibodies in the upper three quartiles were at higher risk than relatives with an IA-2 autoantibody titer in the lowest quartile (Achenbach, Bonifacio et al. 2008).
In another study, an autoantibody response directed to the extracellular domain of IA-2 was associated with very high risk of type 1 diabetes progression, suggesting the presence of new antigenic determinants within the extracellular domain of IA-2 (Morran, Casu et al.). This has considerable implications not only for stratifying high type 1 diabetes risk, but also to facilitate the search for pathogenic epitopes to enable the design of peptide-based immunotherapies, which may prevent the progression to overt type 1 diabetes at its preclinical stages.
Unlike NOD mice, an animal model for type 1 diabetes, humans exhibit any combination of ICA, IAA, GAD, and IA-2 antibodies (Kaufman, Clare-Salzler et al. 1993; Tisch, Yang et al. 1993; Greenbaum, Sears et al. 1999). The risk of type 1 diabetes is relatively low with IAA alone, but is higher with the presence of multiple autoantibodies against islet antigens (insulin, GAD, IA-2 and ICA) (Bingley 1996; Pietropaolo and Eisenbarth 2001). Antibodies to GAD are predictive of progression to hyperglycemia even in the absence of ICA or IAA (Verge, Stenger et al. 1998). As with IAA, however, the risk is higher in subjects who are ICA-positive.
Parallel studies have shown the presence of these autoantibodies in the sera of individuals prior to the onset of T1D (Bingley 1996; Bingley, Bonifacio et al. 2001; Achenbach, Warncke et al. 2004). At the onset of disease using GADA, IA–2A and IAA in combination offers N85% sensitivity and 98% specificity (Bingley, Bonifacio et al. 2001). The sensitivity at onset of T1D for ICA is 70–90%, GADA 70–80%, IA–2A 50–70% and IAA 30–50% respectively, with the variances in the ranges reflecting the population differences between studies. In terms of prediction, multiple large intervention trials, while failing to prevent T1D, have validated the predictive value of these autoantibodies for T1D (Gale, Bingley et al. 2004) (Figure 2).
The titter of IAA has been used to predict the time to onset of type 1 diabetes, particularly in children younger than five years of age (Ziegler, Ziegler et al. 1989). In a prospective, cohort study of 1353 offspring of parents with type 1 diabetes, antibodies detected in the first six months were derived by placental transfer from the mother. Autoantibodies began to appear by nine months and frequently persisted. IAA were almost always the first to appear, with other antibodies (ICA, GAD, or IA-2) appearing later. By age five years, nine (1.8 percent) children had developed type 1 diabetes, and all had one or more autoantibodies beforehand. Fifty percent of children who had two or more antibodies

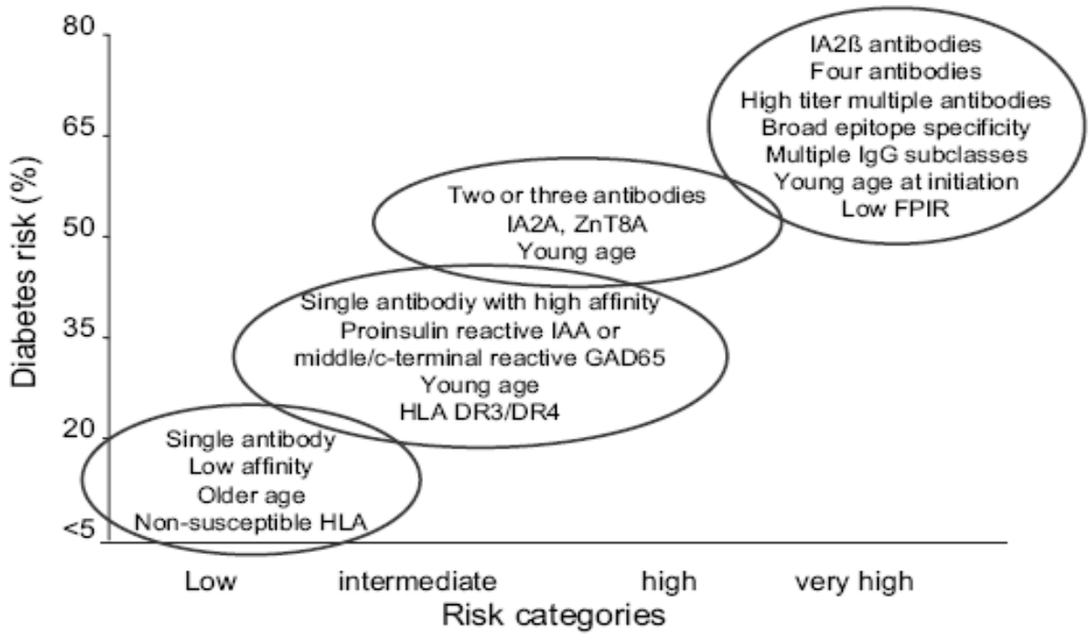

Fig. 2. Type 1 diabetes (T1D) risk stratification by islet autoantibody characteristics. Characteristics associated with low, intermediate, high, and very high risk are grouped from left to right on the abscissa with corresponding T1D risks on the ordinate. Increase in T1D risk is associated with progression of islet autoantibodies from single to multiple autoantibodies. HLA genotype discriminates risk in single antibody–positive children, but multiple antibody–positive children have high risk regardless of HLA genotype.

present by two years had diabetes by age five years (Ziegler, Hummel et al. 1999). In a follow-up report of a slightly larger cohort (1610 offspring), the following results were seen (Hummel, Bonifacio et al. 2004):

- By age five years, the frequencies of islet autoantibodies, multiple autoantibodies, and type 1 diabetes were 5.9, 3.5, and 1.5 percent, respectively.
- The risk of diabetes was highest in those with multiple autoantibodies (40 percent within five years versus 3 percent in those with single autoantibodies).
- Progression to multiple islet autoantibodies was fastest in children who developed their first autoantibody by age two years.
- The risk of progression to diabetes was inversely related to the age of positivity for multiple islet autoantibodies (50 percent of children who had multiple positivity before age 9 months developed diabetes within two years, compared to 7 percent in those who had multiple autoantibodies at age five years).

Thus, children with the earliest evidence of autoimmunity are at greatest risk for and progress more quickly to the development of type 1 diabetes. Periodic testing for islet autoantibodies appears to help assess the risk of diabetes in children of parents with type 1 diabetes.

In another study of 81 Swedish children who later developed type 1 diabetes, 14 (17 percent) had at least one autoantibody present at birth (either GAD, IAA, or ICA512), as compared with 12 of 320 (4 percent) control children (Lindberg, Ivarsson et al. 1999). Four percent had

more than one autoantibody present, compared with none of the control children. This study suggests that the autoimmune process may start in utero, but that this is rare.
In addition to identifying subjects at risk for type 1 diabetes, the presence of ICA and GAD antibodies can also identify late-onset type 1 diabetes in adults thought to have type 2 diabetes. In a study of 97 Swedish diabetic patients who were initially thought to have type 2 or unclassifiable diabetes, 70 became insulin-dependent after six years of follow-up. Among these 70 patients, 60 percent were positive for either ICA or GAD at diagnosis, compared with only 2 percent of the 27 patients who remained responsive to oral therapy.
The presence of these serological markers was closely correlated with histological evidence of insulitis in a study of 29 patients with recently diagnosed type 1 diabetes (Imagawa, Hanafusa et al. 2001). Pancreatic biopsies obtained within these months of diagnosis revealed a T-cell infiltration of pancreatic islets and hyperexpression of HLA class I antigens on islet cells. These features were much more evident among patients with high serum ICA concentration, or the presence of GAD, IAA, or multiple antibodies.
The absence of islet autoantibodies, however, does not exclude type 1 diabetes (House and Winter 1997). The appearance of islet autoantibodies in pancreas transplant recipients predicts recurrence of type 1 diabetes (Bosi, Braghi et al. 2001). Type 1 diabetes can occur after organ donation, and thus living kidney donors from families with histories of type 1 diabetes should be screened for islet autoantibodies (Riley, Maclaren et al. 1990).

4.2.2 Zinc transporter antibodies

In 2007, zinc transporter-8 (ZnT8) was identified as a novel diabetes autoantigen (Wenzlau, Juhl et al. 2007). Autoantibodies against the cation efflux zinc transporter (ZnT8A) have also been identified as a candidate type 1 diabetes autoantigen and proposed as additional markers of rapid disease progression (Wenzlau, Juhl et al. 2007). This study demonstrated that ZnT8 antibodies (ZnTA) were found in 26% of T1D subjects classified as autoantibody-negative on the basis of existing markers (GADA, IA2A, IAA, and ICA). In addition, sixty to 80 percent of patients with newly diagnosed type 1 diabetes have ZnT8 autoantibodies.
The function of this transporter is unknown. But, the combined measurement of ZnT8A, GADA, IA2A, and IAA raised autoimmunity detection rates to 98% at disease onset, a level that approaches that needed to detect prediabetes in a general pediatric population.
A recent study was examined the added value of measuring both IA-2βA and ZnT8A for prediction of impending diabetes in siblings or offspring of type 1 diabetic patients. It confirms the association of IA-2A, IA-2βA and ZnT8A with rapid disease progression and demonstrates that IA-2A and ZnT8A represent the most sensitive combination of two markers to identify relatives with a high progression rate.

4.3 Metabolic markers

Efficacious prevention of T1D will require detection of the earliest events in the process. Autoimmunity is likely the predominant effector mechanism in T1D, but it is possibly not its primary cause. A recent interesting report by Oresic et al. (Oresic, Simell et al. 2008) (see sect. **V***A*) showed that elevated serum concentrations of lysophosphatidyl choline precede the appearance of each islet autoantibody, and thus overt autoimmunity, in T1D. If these results are validated in other well-characterized cohorts, like the German BABYDIAB (Achenbach, Koczwara et al. 2004; Baschal, Aly et al. 2007), the United States-based DAISY (Baschal, Aly et al. 2007) and PANDA (Carmichael, Johnson et al. 2003) studies, and the

multinational TEDDY study (Hagopian, Lernmark et al. 2006), metabolome screening could be added to the screening panel to effectively identify pre-diabetic individuals for preventive treatments.

Although glucose tolerance remains normal until close to the onset of hyperglycemia (Atkinson, Maclaren et al. 1990), the acute insulin response to several secretagogues (glucose, arginine, glucagon and isoproterenol) decreases progressively during the preclinical period (Aanstoot, Sigurdsson et al. 1994). The most useful and widely performed test is the acute (or "first phase") insulin response to glucose (FPIR) during an intravenous glucose tolerance test (IVGTT); in this test the rise in serum insulin above baseline is measured during the first 10 minutes after an intravenous glucose challenge; the response correlates with the functioning ß-cell mass (figure 3). The IVGTT has been standardized to allow easier comparison between centers (McCulloch, Bingley et al. 1993). In first-degree relatives of patients with type 1 diabetes, for example, an FPIR below the first percentile of normal is a strong predictor of type 1 diabetes.

In the Diabetes Prevention Trial-Type 1 Diabetes (DPT-1), subjects at high risk for developing diabetes were followed with serial IVGTTs and oral glucose tolerance tests (OGTTs), and in a subsequent study, the metabolic factors associated with progression to diabetes were evaluated (Barker, McFann et al. 2007).

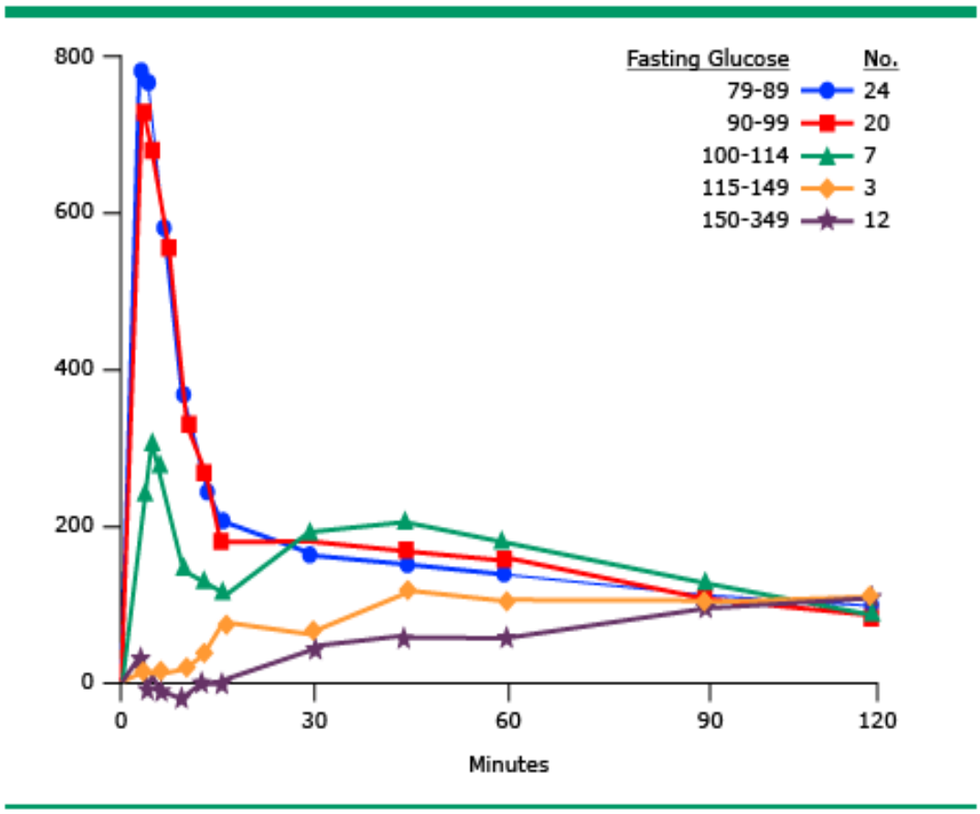

Fig. 3. Relative acute insulin response to IV glucose. Reproduced with permission from Brunzell et al. (Brunzell, Robertson et al. 1976) http://jcem.endojournals.org. Copyright © 1976 The Endocrine Society.

Abnormalities of FPIR and two-hour glucose during OGTT had similar sensitivities for diabetes prediction within six months of diagnosis (76 percent for OGTT [95% CI 60-83%] and 73 percent for FPIR [95% CI 60-83%]). Sensitivity was better when both tests were performed, and the vast majority of these individuals (97 percent) had abnormal IVGTTs and/or OGTTs before the development of the overt diabetes. In contrast, fasting blood glucose levels were a poor predictor of diabetes.

The more important as pathogenetical significance was the finding of increased proinsulin in first degree relatives or descendants from diabetic parents, both with type 1 (Roder, Knip et al. 1994; Truyen, De Pauw et al. 2005). Truyen *et al.* (Truyen, De Pauw et al. 2005) considered that the increased plasma proinsulin levels can be an additional marker for the prediction of type 1 diabetes. A simpler test that may prove useful is to measure the fasting serum concentration of proinsulin, the precursor of insulin. In normal subjects, proinsulin accounts for approximately 15 percent of serum immunoreactive insulin. This proportion rises as ß-cell function declines. One report, as an example, found that serum proinsulin concentrations were three to four times higher among ICA-positive relatives of type 1 diabetes patients as compared with ICA-negative relatives. However, prospective studies are needed to determine whether elevated serum proinsulin values will help in predicting the development of type 1 diabetes.

4.4 Combining risk biomarkers

Since genetic susceptibility is only part of the risk factors for common diseases, genetic markers alone usually do not have adequate specificity. There is growing evidence that combining multiple genetic and clinical markers is the best way to develop a molecular test with clinically useful predictive power.

Various combinations can be used to obtain similar overall risk, and for most combinations the risk can be calculated empirically. Risk can be stratified from less than 0.1% to greater than 70%. Current approaches use a stepwise decision tree (Krischer, Cuthbertson et al. 2003) in which genetic risk is usually the first applied in the form of family history or HLA DR-DQ genotype. Autoantibodies are measured in those individuals who are considered to have sufficient genetic risk to warrant autoantibody testing. Because the risk of developing multiple islet autoantibodies is strongly linked to major histocompatibility complex (MHC) class II genotypes,(Walter, Albert et al. 2003) further typing is unlikely to be helpful in a child who has an armada of islet autoantibodies. Finally, beta cell function is measured in islet autoantibody–positive individuals using either the ability of the beta cell to secrete insulin in response to an intravenous glucose challenge or the ability of the individual to clear glucose after a meal challenge where low insulin secretion (eg, less than the first percentile) or impaired glucose tolerance are indicators of late-stage preclinical T1D.

Although the decision tree approach is logical, it may be expected that once effective preventative therapies become available, the decision tree approach could be replaced by the population-wide application of all the previously mentioned markers in a public health prevention manner. Clinicians may eventually move toward introducing a risk score based on the combination of all markers. This would represent a paradigm shift after years of increasingly complex layers of decisions in screening.

Finally, the T1D risk of an individual is not static throughout life. This is true even for genetically defined risk. Risk in a child who has no family history of T1D at birth increases more than 10-fold if his or her sibling develops T1D, and if the child has an identical twin

who develops T1D, risk immediately increases 100-fold to around 50% (Redondo, Jeffrey et al. 2008). Risk calculated from the autoantibody status usually increases over time as autoantibodies appear and their number rises. Younger age is associated with increased risk than older age. Beta cell function measures are expected to show decrease the closer someone is to disease onset. Over the lifespan of an individual, the calculated T1D risk on the basis of genes, autoantibody, age, and beta cell function change.

5. Conclusions

Type 1 diabetes is an immunemediated disease leading to chronic insulin deficiency due to extensive and selective -cell destruction in subjects with increased genetic disease susceptibility (Atkinson and Eisenbarth 2001). As far as diagnosis of T1D versus other forms of diabetes the autoantibodies and genetic markers are of great value. There remains a subset of patients that are autoantibody negative at onset. This subset may at times present a diagnostic challenge and it is of importance for treatment to know if T1D (absolute requirement for insulin) or type 2 diabetes with a relative insulin resistance is present.

The occurrence of multiple antibodies against islet autoantigens serves as a surrogate marker of disease in primary or secondary intervention strategies aimed at halting the disease process (Pietropaolo and Eisenbarth 2001). Genetic typing for susceptibility or protective HLA alleles can also be performed.

In a research setting, the following approach may be used (McCulloch and Palmer 1991):

- Test individuals at risk for type 1 diabetes progression for GAD65 and IA-2 autoantibodies.
- If they are present and confirmed in a subsequent sample, tests for insulin and islet cell antibodies can be done and the FPIR determined.

Nonetheless, the studies performed to date have given us tremendous insight into the natural history of T1Ds (Eisenbarth 2004; Achenbach, Bonifacio et al. 2005; Sherry, Tsai et al. 2005). As a consequence, at present we can predict the development of T1D. Ideally, we would like to couple such prediction with prevention, but unfortunately we do not yet have a safe and effective preventive therapy.

Successful prevention depends on *1*) a good prediction/ identification of at-risk individuals and *2*) a very safe intervention that causes no harm in those individuals who would have never developed T1D. Knowledge of the primary cause of T1D might not be crucial, even at the preventive stage. This statement is based on the fact that immune modulation appears to work in a variety of T1D models and at different stages of the disease. However, many preventive trials are based on data from the NOD mouse model which has improved our understanding of disease pathophysiology. A comprehensive analysis by Shoda et al. (Shoda, Young et al. 2005) concluded that "some popular tenets regarding NOD interventions were not confirmed: all treatments do not prevent disease, treatment dose and timing strongly influence efficacy, and several therapies have successfully treated overtly diabetic mice." So, the good news is that some preventive strategies appear to have a good chance to cure the disease, even during an advanced status of beta-cell destruction. Examples of succesful treatments in NOD mice are ATG, anti-CD3, hsp, and proinsulin DNA vaccine.

Ideally, the balance between therapeutic efficacy and disease stage should be known prior to human trials.

A major problem with preventive trials is that it takes many years before conclusions can be drawn. As can be seen in Table 2, preventive trials divide in two main classes.

Agent	Target/Mechanism	Development Phase, Clinical ID, Organizer
Vitamin D_3	Treg induction	Pilot, NCT00141986*, CDA
Omega-3 fatty acids	Anti-inflammatory	Phase II, NCT00333554*, NIDDK
Hydrolyzed cow's milk	Abnormal handling of intact foreign protein	TRIGR, Phase II, NCT00179777*, CHEO
Oral insulin	Ag-specific tolerance (oral)	Phase II, NCT00419562*, NIDDK
Nasal insulin	Ag-specific tolerance (mucosal)	Phase III, NCT00223613*, University of Turku
		Phase II, NCT00850161*, CPEX Pharma
		Phase II, NCT00336674*, Melbourne Health

- * More information is available at http://www.clinicaltrials.gov/. CDA, Canadian Diabetes Association; CHEO, Children's Hospital of Eastern Ontario; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.

Table 2. Prevention trials in T1D.

6. Reference

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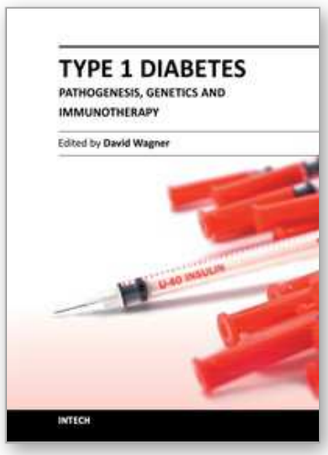

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This book is a compilation of reviews about the pathogenesis of Type 1 Diabetes. T1D is a classic autoimmune disease. Genetic factors are clearly determinant but cannot explain the rapid, even overwhelming expanse of this disease. Understanding etiology and pathogenesis of this disease is essential. A number of experts in the field have covered a range of topics for consideration that are applicable to researcher and clinician alike. This book provides apt descriptions of cutting edge technologies and applications in the ever going search for treatments and cure for diabetes. Areas including T cell development, innate immune responses, imaging of pancreata, potential viral initiators, etc. are considered.

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