Preclinical Type 1 Diabetes: Natural Course and Predictive Characteristics in Siblings of Affected Children

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Helsinki 2005

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SAMY MRENA

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in the Niilo Hallman Auditorium of the Hospital for Children and Adolescents, on November, 11th, 2005, at 12 noon

HELSINKI 2005

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To my family

1 ABSTRACT

Type 1 diabetes (T1D) is one of the most common chronic disorders among children and adolescents. It is perceived as an immune-mediated disease with a subclinical prodromal period characterized by the selective loss of insulin-producing beta cells in the pancreatic islets in genetically susceptible subjects. The most important genes contributing to disease susceptibility are located in the HLA class II locus on the short arm of chromosome 6. This work aimed at (i) assessing whether it is clinically relevant to classify individuals with signs of beta-cell autoimmunity into various stages of preclinical T1D, (ii) evaluating the natural history of preclinical diabetes in subjects at risk, (iii) assessing whether HLA-conferred disease susceptibility modifies the risk associated with different stages of preclinical T1D, and (iv) establishing predictive models for T1D that integrate sociodemographic, genetic, immunological and metabolic markers and testing their utility for the prediction of T1D in siblings of children affected by T1D.

The population, derived from the "Childhood Diabetes in Finland" Study, comprised more than 700 initially unaffected siblings of the index cases with newly diagnosed T1D. The mean age of these siblings at the initial sampling was 9.9 years (range 0.8-19.7 years). The first three objectives were addressed based on observation of the siblings for progression to overt T1D for an average of 9 years, during which time 35 of them (4.6%) presented with clinical disease. The last objective was approached through observation up to the end of 2002, i.e. for an average of 15 years. Twelve additional siblings developed clinical T1D during that time, resulting in a total number of 47 progressors (6.7%). All four diabetes-associated autoantibodies [islet cell antibodies (ICA), insulin autoantibodies (IAA) and antibodies to the 65 kD isoform of glutamic acid decarboxylasze (GADA) and to islet antigen 2 (IA-2A)] were analyzed in the initial sample taken from each sibling close to the time of diagnosis in the index case. Eighty-three autoantibody-positive siblings underwent an intravenous glucose tolerance test (IVGTT) to assess the first-phase insulin response (FPIR). In addition, a homeostasis model assessment of insulin resistance (HOMA-IR) was made based on fasting insulin and blood glucose concentrations. HLA DR and DQ typing was performed in the majority of the subjects. The siblings were divided into four categories of preclinical T1D as follows: no prediabetes (no autoantibodies), early prediabetes (one autoantibody reactivity), advanced prediabetes (two autoantibodies) and late prediabetes (at least three autoantibodies) in **classification 1**. **Classification 2** included information on FPIR and employed the categories no prediabetes (no autoantibodies), early prediabetes (one autoantibody reactivity, normal FPIR), advanced prediabetes (two or more autoantibodies, normal FPIR) and late prediabetes (at least one autoantibody, reduced FPIR). The data for the analysis of the predictive models included age at first sampling, sex, HLA-conferred disease susceptibility, autoantibody positivity and titers, age at diagnosis and sex of the index case, the number of children in the family and the number of first-degree relatives affected by T1D. In the smaller series comprising the autoantibody-positive siblings who had undergone an IVGTT, data on the FPIR, glucose elimination rate (K_g), HOMA-IR and HOMA-IR/FPIR ratio were also included in the analyses.

The first paper showed that the risk of progression to clinical T1D was clearly associated with the stage of prediabetes. The odds ratio (OR) for progression to T1D was 7.1 in early

prediabetes, 32.8 in advanced prediabetes and 209 in late prediabetes according to **classification 1** and 7.8 in early prediabetes, 38.5 in advanced prediabetes and 1310 in late prediabetes based on **classification 2**. The time to diagnosis was significantly shorter in those with late prediabetes initially than in those with no signs of prediabetes. According to **classification 1**, 36% of the siblings with signs of prediabetes progressed, 27% remained stable and 37% regressed in relation to their initial prediabetic stage during prospective observation for an average of 3.6 years, as reported in the second paper. The siblings who progressed were younger and had a higher initial number of detectable autoantibodies and higher initial autoantibody levels except for IAA, but lower FPIR and K_g , than those who regressed. More than half of the siblings with initial preclinical T1D (56%) progressed, 34% remained stable and only 10% regressed during the observation period according to **classification 2**. The third paper showed that there was a higher proportion of siblings with late prediabetes (17%) among those with strong HLA-conferred disease susceptibility than among those with a weaker genetic predisposition (0.5%), while there was a higher proportion of siblings with no signs of prediabetes among the genotypes conferring a decreased risk (91% *vs* 70%). Autoantibodies alone were more sensitive for the prediction of future diabetes in siblings than autoantibodies combined with HLA-defined susceptibility. Genetic susceptibility played a role in determining whether progression took place from the initial prediabetic stage and whether T1D became manifest or not. The fourth paper revealed that young age, an increasing number of detectable diabetes-associated autoantibodies at initial sampling, an increased number of affected first-degree relatives and HLA DR-conferred disease susceptibility predicted progression to T1D. There was a subgroup of 77 autoantibody-positive siblings in whom young age, HLA DR-conferred susceptibility, an increasing number of autoantibodies, reduced FPIR and decreased insulin sensitivity in relation to FPIR were associated with an increased risk of progression to T1D. Age at diagnosis was predicted by age, IA-2 antibody levels and number of autoantibodies at initial sampling (R^2=0.76; P<0.001). First-phase insulin response and HLA DR-conferred susceptibility were additional predictors of age at diagnosis in a smaller cohort of autoantibody-positive subjects.

The above observations imply that it is feasible to grade siblings of children with newly diagnosed T1D into categories with significant differences in the subsequent risk of overt T1D and in time to diagnosis. Almost half of the siblings with signs of prediabetes at the time of diagnosis of the index case progressed further in their preclinical disease process during the period of prospective observation. Advanced and late prediabetes seem to represent a point of no return, as regression from such stages is extremely rare. HLA-conferred diabetes susceptibility has an impact on both the initiation and progression of the autoimmune process leading to clinical diabetes in siblings of affected children. Information on autoantibody status and levels, HLA-conferred disease susceptibility and insulin secretion and sensitivity seems to be useful in addition to age and the family history of T1D when assessing the risk of progression to T1D and time to diagnosis in siblings of children with newly diagnosed T1D.

ACKNOWLEDGEMENTS

This work was carried out at the Hospital for Children and Adolescents, University of Helsinki, Helsinki, and the Department of Pediatrics and Research Unit, Tampere University Hospital, Tampere, during the years 1996-2005.

I would like to thank my supervisor, Professor Mikael Knip, MD, PhD, for his friendship, never-ending enthusiasm, personal advice and probably the best tutoring in the world –and for believing in me for all these years. I was already in 1996 convinced, and I still am, that there is nothing Mikael doesn't know about type 1 diabetes.

I would like to thank Docent Tiinamaija Tuomi, MD, PhD, and Docent Kirsti Näntö-Salonen, MD, PhD, for their abundant supportive criticism and remarks regarding my work.

I would like to thank my co-authors Professor Hans Åkerblom, MD, PhD, Professor Jorma Ilonen, MD, PhD, Petri Kulmala, MD, PhD, Kaisa Savola, MD, PhD, and Helena Reijonen, PhD for their numerous and stimulating expert ideas and comments, although they were not always in line with my views.

I would like to thank Professor Suvi Virtanen, MD, PhD, late Professor Pekka Laippala, PhD, and Marja-Leena Hannila, MSc, for their statistical expertise, without which I would have been lost. We will never forget your brilliant ideas and endless fascinating stories, Pekka.

I am also grateful to Malcolm Hicks, MSc, for his excellent revision of the English language of this thesis and for his comments.

Thank you, Matti Kontkanen, MD, PhD, for your friendship and for making it possible to continue this work in Helsinki even from as far away as Joensuu. I will always remember you all in the Department of Ophthalmolgy at the North Carelia Central Hospital and the pleasant times we had at work and after work (except the emergency duties "Kolmospäivystykset" which I will never forget…).

Many thanks also go to all the friendly staff of the Department of Ophthalmology at Helsinki University Central Hospital for making it possible to take time off to finish my thesis, immediately after beginning my work there.

I would like to thank the members of the Society of Card-playing Academic Gentlemen for their excellent skill of taking my mind off work and always inventing new social events (which was also the reason why it took so many years to complete this thesis). I hope I will have more time to play now.

I would also like to thank my brother Erik for his excellent computer skills, without which I would still be writing this thesis, and also my brother Roderik and his wife Johanna for their witty medical comments, which have been irreplaceable. Thank you, Igor and Olga, for always making me feel welcome at home, and for your patience and advice when I was (and

still am) growing up. You are truly the best parents in the world. Thank you all for being there for me in any and every situation.

Thank you, my faithful dog Jeeda, for many relaxing moments to take my mind off work, and for not eating the manuscript.

And finally I wish to thank Johanna, my Love, for her patience, love and support through these years. You taught me how to appreciate life and nature. You make it all worthwhile!

Thank you all, my friends, for still appearing to tolerate my never-ending interest in cars and icehockey to balance my interests in medicine.

October 15, 2005

Samy Mrena

ABBREVIATIONS

APC	= antigen-presenting cell
ATP	= adenosine triphosphate
BB	= biobreeding
BSA	= bovine serum albumin
BLG	= β-lactoglobulin
CI	= confidence interval
CF-ICA	= complement-fixing islet cell antibodies
CNS	= central nervous system
CRS	= congenital rubella syndrome
CTLA4	= cytotoxic T-lymphocyte antigen 4
DAISY	= Diabetes Autoimmunity Study in the Young
DIPP	= Type 1 Diabetes Prediction and Prevention Project
DiMe	= Childhood Diabetes in Finland Study
DPT-1	= Diabetes Prevention Trial – Type 1 Diabetes
DTH	= delayed-type hypersensitivity
EBV	= Epstein-Barr virus
ENDIT	= European Nicotinamide Diabetes Intervention Trial
EV	= enteroviruses
HIV	= human immuno-deficiency virus
IgG	= immunoglobulin G
FasL	= the specific ligand for Fas *in vivo*
FPIR	= first-phase insulin response
GADA	= antibodies to the 65 kD isoform of glutamic acid decarboxylase
GLUT2,4	= glucose transporter proteins
HLA	= human leukocyte antigen
HOMA-IR	= homeostasis model assessment of insulin resistance
IA-2	= islet antigen 2
IA-2A	= antibodies to the IA-2 protein
IAA	= insulin autoantibodies
ICA	= islet cell antibodies
ICAM-1	= intercellular adhesion molecule 1
T1D	= type 1 diabetes
IFN-γ	= interferon γ
Ig	= immunoglobulin
IL-2	= interleukin 2
IRS	= insulin receptor substrate
IVGTT	= intravenous glucose tolerance test
JDF	= Juvenile Diabetes Foundation
JDFU	= Juvenile Diabetes Foundation units
K_g	= glucose disappearance rate
MHC	= major histocompatibility complex
MMR	= mumps-measles-rubella
NOD	= non-obese diabetic

OGTT = oral glucose tolerance test
OR = odds ratio
PAA = proinsulin autoantibodies
PI3K = phosphatidylinositol 3-kinase
PRI = prognostic risk index receiver-operating characteristics (analysis)
ROC = receiver-operating characteristics (analysis)
RU = relative units
SD = standard deviation
SEM = standard error of mean
SUMO4 = small ubiquitin-like modifier 4
TBST = Tris-buffered saline with Tween 20
T1D = type 1 diabetes
TRAIL = TNF-related apoptosis-inducing ligand
TRIGR = Trial to Reduce T1D in the Genetically at Risk

LIST OF ORIGINAL PAPERS:

This thesis is based on the following papers, which are referred to in the text by their corresponding Roman numerals:

I. Mrena S, Savola K, Kulmala P, Åkerblom HK, Knip M, and the Childhood Diabetes in Finland Study Group. Staging of preclinical Type I diabetes in siblings of affected children. Pediatrics 1999; 104: 925-930.

II. Mrena S, Savola K, Kulmala P, Åkerblom HK, Knip M, and the Childhood Diabetes in Finland Study Group. Natural course of preclinical type 1 diabetes in siblings of affected children. Acta Paediatrica 2003; 92: 1403-1410.

III. Mrena S, Savola K, Kulmala P, Reijonen H, Ilonen J, Åkerblom HK, Knip M, and the Childhood Diabetes in Finland Study Group. Genetic modification of risk assessment based on staging of preclinical type 1 diabetes in siblings of affected children. J Clin Endocrinol Metab 2003;88: 2682-2689.

IV. Mrena S, Virtanen SM, Laippala P, Kulmala P, Hannila M-L, Åkerblom HK, Knip M, and the Childhood Diabetes in Finland Study Group. Models for predicting type 1 diabetes in siblings of affected children. Submitted for publication.

This thesis also includes some previously unpublished data.

CONTENTS

2 INTRODUCTION

Under normal physiological conditions, the entry of glucose into beta cells triggers the secretion of insulin, which is carried in the blood to the peripheral tissues, where it binds to insulin receptors, resulting in the uptake of glucose by cells and its metabolism into energy or storage as glycogen. This mechanism is described in Figure 1 (1). Type 1 diabetes (T1D) is a chronic disease, mainly affecting children and adolescents, that is characterized by hyperglycemia and ketosis resulting from a lack of the physiological insulin secretion described above, due to the destruction of beta cells in the pancreatic islets of Langerhans. When clinical symptoms of hyperglycemia caused by T1D (polydipsia, polyuria, weight loss and fatigue) appear, it is estimated that over 80-90% of the beta cells have already been destroyed. Once beta-cell damage has progressed to the clinical manifestation of T1D, the affected subjects require life-long therapy based on subcutaneous injections of insulin. The other, more common form of diabetes is type 2, which is classically a disease of adults and the elderly. It usually develops slowly and is associated with obesity and hypertension, and it is initially characterized by hyperglycemia and peripheral insulin resistance. Our knowledge of the pathogenesis of T1D, and especially of the preclinical period, has increased substantially during the last 30 years, and it is now perceived as an immune-mediated disease with a long preclinical period characterized by gradual beta-cell loss. In addition to cell-mediated autoimmunity, islet cell antibodies (ICA), insulin autoantibodies (IAA), antibodies to glutamic acid decarboxylase antibodies (GADA) and to the protein tyrosine phosphatase-related islet antigen 2 molecule (IA2-A), and several other antibodies are associated with progression to clinical T1D. Increasing knowledge and intensive research into the pathogenesis of T1D have raised hopes of finding an effective treatment for halting progressive beta-cell damage in individuals *en route* to overt disease.

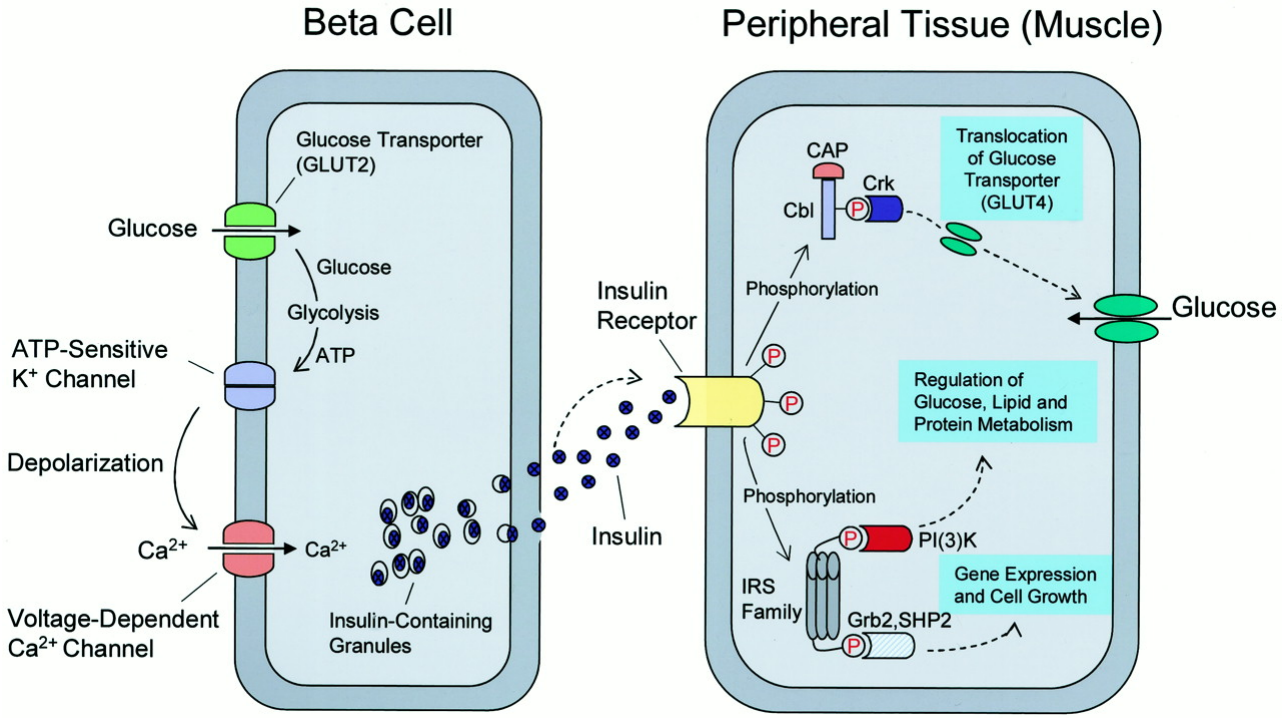

Figure 1. Insulin release and action.

Glucose enters beta cells via the glucose transporter (GLUT2) proteins and ATP is generated by glycolysis. This results in closure of the ATP-sensitive K^+ channels, depolarization of the plasma membrane, and opening of voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} leads to the release of insulin, which is carried in the bloodstream to cells throughout the body, where it binds to insulin receptors. This results in autophosphorylation of insulin receptors and phosphorylation of tyrosines on a variety of cellular proteins, including members of the insulin receptor substrate (*IRS*) family and the Cbl-CAP substrate-adapter protein complex. The phosphorylated proteins provide docking sites for SH2 domains of several proteins (*e.g.* phosphatidylinositol 3-kinase [*PI3K*], Grb2 and SHP2, and Crk) that activate different signaling pathways (*dashed lines*). This leads to translocation of the glucose transporter (GLUT4) and uptake of glucose by the cell, alterations in glucose, lipid and protein metabolism and changes in gene expression and cell growth (1).

3 REVIEW OF THE LITERATURE

3.1 Epidemiology

The incidence of T1D, and especially its considerable variation between various parts of the world, have been targets of intense research. Extensive registers and bodies of data have allowed us in recent years to view the incidence of T1D worldwide, and it has become evident that there is major geographical variation in the overall age-adjusted incidence rates of T1D in children under the age of 15 over a 5-year period from 1990 to 1994, ranging from 0.1/100,000 per year in Zunyi, China, and Caracas, Venezuela, to 36.8/100,000 per year in Sardinia and 36.5/100,000 per year in Finland. This represents a 540-fold variation in the incidence among the 100 populations observed worldwide, greater than that observed for any other non-communicable chronic disease (2; 3). On the other hand, the previously assumed "north-equatorial gradient" in the incidence of T1D does not seem to be as strong as previously suspected. Although the populations with very high incidence rates were mostly of European origin, populations with a relatively high rates were also found in tropical or subtropical regions such as Kuwait (4) and Puerto Rico (5). The worldwide variation in the incidence of T1D seems partly to reflect the distribution of various races and ethnic groups, implying that interpopulation differences in genetic susceptibility to T1D may contribute to the differences. Reports on national variations in disease incidence are rare, but clear variations have been reported in the Nordic countries, the United Kingdom, Italy and the United States (6), and there are also nearly 50-fold differences in T1D incidence within China, for example. In Italy the highest incidence rate (37/100,000/year) was observed in Sardinia, with three to six times lower incidence on the mainland, ranging from 5 per 100,000/year in Campania to 11 in Pavia in the age group 10-14 years (7; 8). A similar situation has been reported in Canada, with a relatively high incidence of 35.9/100 000 in Avallon, Newfoundland but rates ranging from 9.3 to 24.5/ 100 000 elsewhere (2; 3; 9).

The assumption for many years was that there is a considerable increase in the incidence rate throughout childhood, with a classic peak in early puberty (10; 11), occurring 1-2 years earlier in girls than in boys, and a subsequent decrease, followed by yet another rise later in life (12). The maximal growth spurt, characterized by a typical increase in endogenous insulin requirements and a decrease in peripheral insulin sensitivity, has been implicated in this pattern. The incidence in Finland, however, is reported to be high and quite stable from the age of 3 up to 14 years, and no pubertal peak is detectable any longer (13; 14). The highest age-specific risk of T1D in the Baltic region, including Finland, has been observed around 11–13 years of age. The highest incidence in males of all populations is recorded in the 10–14 year age group, whereas that in females is seen in the 5-9 year age group (14).

Although there is usually a female predominance among patients with autoimmune diseases, there is typically a higher incidence of T1D in boys than in girls in high-incidence areas, e.g. among Finnish children (15). Conversely, it was assumed previously that there is a female excess in low-incidence areas, but a relatively recent study has indicated that there is

no longer a statistically significant male excess in the incidence rate in Finland nor a female excess in low-incidence countries (2). There is still a noticably higher male excess in the high-incidence area of Sardinia, however, which has been linked to the HLA-DR3 allele. The linkage between the Xp chromosome and the HLA-DR3 allele has led to a suspicion that a gene located on the X-chromosome may play a role in the pathogenesis of T1D (8). Most studies indicate that the male excess becomes more apparent among patients diagnosed after puberty than in those diagnosed before or during puberty (16; 17).

The issue of a temporal increase in the incidence rate in industrialized countries has been a subject of ample debate, and numerous conflicting results have been reported. There is, however, evidence of a definite linear increase in T1D among children less than 15 years of age in most European countries, especially in Northern and Central Europe, and also in the Western Pacific (3; 18-20). This worldwide increase seems to be most conspicuous in countries with a low initial incidence rate (2; 3; 21; 22). In Finland, the incidence of T1D has increased 4.5-fold over the last 50 years, starting from a figure of 12/100,000 in 1953 and reaching 54/100,000 in 2003 (23). Tuomilehto et al. demonstrated that the incidence increased by more than 60% during the 20-year period 1965-1984, which corresponds to an annual increase of roughly 2.4% (15). This trend was seen in both sexes and in three age groups (0-4, 5-9 and 10-14 years), and is similar to those observed earlier in Sweden (24). There was also a noticable increase in the incidence of the disease in Norway throughout the period 1956-1982 (19), both in the age group 0-14 years (25) and in those aged 15-29 years (26), but recent results indicate that the incidence remained quite stable in all age groups of children under the age of 15 years during the years 1989-1998, although significant regional variation was observed (27). The incidence of T1D in Sweden shifted to a younger age group at diagnosis during a 16-year period, explaining the earlier observation of an increase in the incidence of childhood diabetes (24; 28), and other reports have also found a stable incidence rate and a noticeable shift towards a younger age at diagnosis. These observations support the “spring harvest hypothesis”, which suggests that the rise in childhood T1D may reflect increased exposure to isolated initiating factors in early childhood, as one might assume that the number of children with a genetic predisposition has remained stable (22). A recent comparison between Finland and Russian Karelia showed that there was an almost 6-fold difference in the incidence rate of T1D among children under the age of 15 over a 10-year period from 1990-1999, whereas there was no difference in the frequency of HLA risk genotypes in the background population (31). These observations suggest that the conspicuous difference in incidence must be due to factors related to standards of living and other lifestyle factors.

There are also some indications that, when populations with a low baseline incidence rate move to areas with a higher incidence rate, there is a rapid temporal increase in the incidence of T1D (32), and vice versa, e.g. T1D incidence rates have been reported to decrease among Sardinians living in Northern Italy, where the disease incidence is 1/3 of that in Sardinia (33). Another survey, however, indicates that children of Sardinian lineage have an increased risk of developing T1D for at least one generation after migration to Northern Italy, especially if both parents are from Sardinia (34). Similar trends were observed in the initially low-incidence population of Asian origin living in Great Britain. These findings further support the idea that genetic and environmental factors together play

a crucial role in the development of T1D. Other theories such as the "hygiene hypothesis" emphasize the impact of constantly increasing hygiene, especially in industrialized countries, which causes reduced stimulation of the immune system by early infections, potentially leading to a reprogramming of the immune system, favoring autoimmune diseases in some individuals and atopic diseases such as asthma, hay fever and eczema in others (35).

There is considerable evidence to support seasonal variation in the incidence of T1D (36-40). It has been observed that there is a peak in the incidence of new cases in the cold winter months, whereas lower incidence rates are more typical of the warm summer months. It also seems that children who will develop T1D are more often born in the summer months (41). This variation has been interpreted as suggestive of an infectious etiology, especially the involvement of viruses (42; 43). Other results, on the other hand, suggest that if a viral infection were the cause of beta-cell damage, the behavior of such a pathogen would be quite atypical of viruses. Diseases caused by viruses, such as mumps, measles and coxsackie B, which have been proposed as triggers of T1D, usually cause short-term epidemics in susceptible populations (44), whereas high-incidence countries seem to have a relatively stable or steadily rising incidence rate. In addition, the existence of a long prodromal period before the diagnosis of T1D implies that seasonal variation in clinical manifestation would reflect the role of infections as precipitating factors rather than as initiators or potentiators of beta-cell destruction. There have also been reports of variation in seasonality in relation to HLA susceptibility markers, as a more conspicuous seasonal variation has been observed among subjects carrying the DR4 allele than among those with DR3 (45; 46).

3.2 Pathogenesis of T1D

T1D was presumed earlier to be a rapidly developing disease, resembling those caused by acute viral infections, but with increasing knowledge as a consequence of extensive research in this field, we have learned that clinical symptoms are preceded by a long chronic preclinical period of more than 10 years in some cases characterized by progressive beta-cell destruction, as illustrated in Figure 2 (17; 47). Sadly, the direct cause that triggers the disease is still unknown, although dietary factors and viral infections are suspected to be involved in addition to genetic predisposition. Hyperglycemia and ketosis, symptoms characteristic of T1D, actually emerge late in the disease process, after most of the beta cells have already been destroyed, making the preclinical period an extremely fascinating topic of research into the disease process. If the mystery of the pathogenesis of T1D were to be revealed completely, this would also open up new possibilities, not only for preventive, but also for curative measures (17).

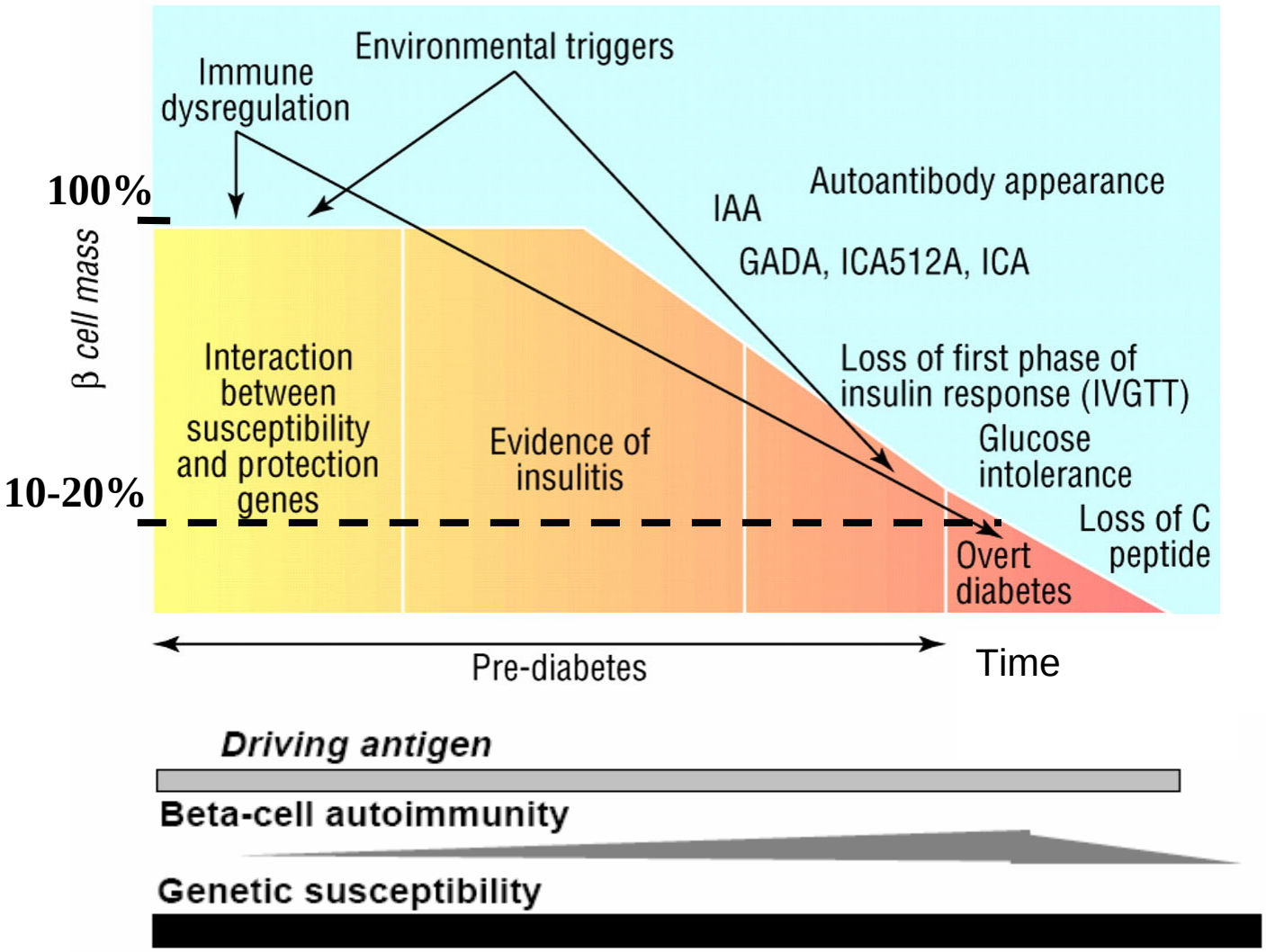

Figure 2. Gradual progression of beta-cell damage from initiation to clinical diabetes (80-90% of beta cells destroyed) [modified from the original (17; 47)].

3.2.1 Genetics

Genetic factors play an essential role in type 2 diabetes and the disease tends to run in families, whereas most cases of T1D are sporadic, as illustrated by the fact that its prevalence among first-degree relatives is only approximately 10% at the time of diagnosis . Family studies provided the first evidence in favor of a relation between genetic factors and T1D susceptibility, proving that the disease was more common among close relatives of affected patients than in the general population (49). The prevalence of T1D in fathers of diabetic children is reported to be 3.4-4.4% and that in mothers 1.8-2.0% (50-52). One study demonstrated that as many as 7.3% of diabetic children have grandparents with T1D (53). The disease risk in a sibling of an affected child has been estimated to be 2.5-10%, depending on the population (50; 54-56). The latest figures for siblings and the general population in Finland show a cumulative incidence of 4.1% for siblings of children with type 1 diabetes by the age of 20 and only about 0.5-0.6% in the general population by the age of 35 (57; 58). The lifetime risk of T1D in the Finnish population has been estimated to be close to 1% (59). Studies in twins have indicated that 70–75% of the T1D risk may be related to genetic effects and 25–30% to environmental factors (60). This appears to be a considerable overestimate of the role of genetic factors, however, given that genetic disease susceptibility can explain a very modest proportion of the manifold increase in the incidence rate seen in most developed countries since World War II. An estimated 23 to 50% of monozygotic twins of patients with T1D also develop the disease (61; 62). More than half of the affected sib pairs have two identical human leukocyte antigen (HLA) haplotypes, 40% share one haplotype and only 7% have a non-identical HLA haplotype, as compared with the expected distribution of 25%, 50% and 25% (63). The risk of developing T1D is approximately 12-24% in HLA-identical siblings, 4-8% in haploidentical siblings and less than 1% in non-identical siblings (56; 64-66). The most important determinants of genetic susceptibility to T1D are located in the major histocompatibility complex (MHC) or the HLA gene area on the short arm of chromosome 6. This gene region, also called IDDM1, accounts for 35-60% of the familial clustering of T1D (67-69), while the contribution of IDDM2, the insulin gene polymorphism on the short arm of chromosome 11, is less than 10% (67; 70). The insulin gene is a plausible susceptibility locus, since insulin or insulin precursors act as autoantigens in T1D. Ueda et al. recently confirmed earlier results reported less than 2 years ago that the gene region encoding the cytotoxic T-lymphocyte antigen 4 (CTLA4 or IDDM12) on the long arm of chromosome 2 comprises polymorphisms associated with an increased risk of common autoimmune disorders such as Grave's disease, autoimmune thyroiditis and T1D (71; 72). An additional genetic polymorphism predisposing subjects to T1D has been identified recently on the short arm of chromosome 1, in the PTNPN22 gene encoding the lymphoid protein tyrosine phosphatase, a suppressor of T-cell activation (73). Guo et al. reported in 2004 that a single nucleotide polymorphism (A163G) in a newly identified gene encoding the small ubiquitin-like modifier 4 (SUMO4) was associated with T1D (74). This gene is located in the IDDM5 region on the long arm of chromosome 6. Additional work has implied that the mutant SUMO4 protein could result in stronger stimulated cellular immune responses, which might explain the association with T1D. An overview of the type 1 diabetes susceptibility loci is seen in Figure 3a (75).

There are three classes of HLA genes, distinguished according to the structure and function of their protein products (Figure 3b) (76). The HLA class I region contains three functional, classical class I gene loci, HLA-A, HLA-B and HLA-C, which are highly polymorphic and are expressed by all nucleated cells. In addition, there are several other functional class I loci, including the non-classical class I genes HLA-E, HLA-F and HLA-G, which are less polymorphic and have restricted expression. The HLA class I genes encode the heavy α-chain of the cell-surface class I molecule, which, along with the light chain comprising β_2-microglobulin, is responsible for presenting antigens to $CD8^+$ T-cells.

The class II HLA genes, also known as immune response genes, HLA-DR, HLA-DQ and HLA-DP, are located at the centromeric end of the complex and encode the α and β-chains that form heterodimeric cell surface proteins expressed on the surfaces of antigen-presenting cells (dendritic cells, macrophages and B lymphocytes). These class II molecules bind foreign antigens non-specifically after their processing and present them to $CD4^+$ T-helper lymphocytes. The T-cells are both peptide-specific and HLA-restricted, so that they recognize the peptide only when it is bound to a certain class II molecule. The class III HLA region comprises genes encoding certain complement components (C2, C4 and properdin factor Bf), tumor necrosis factors α and β, heat shock proteins and the 21-hydroxylase enzyme. The HLA genes are highly polymorphic, which means that each gene has many alleles, leading to extensive variation among individuals, and it is this polymorphism that determines the specificity of the immune response (77; 78).

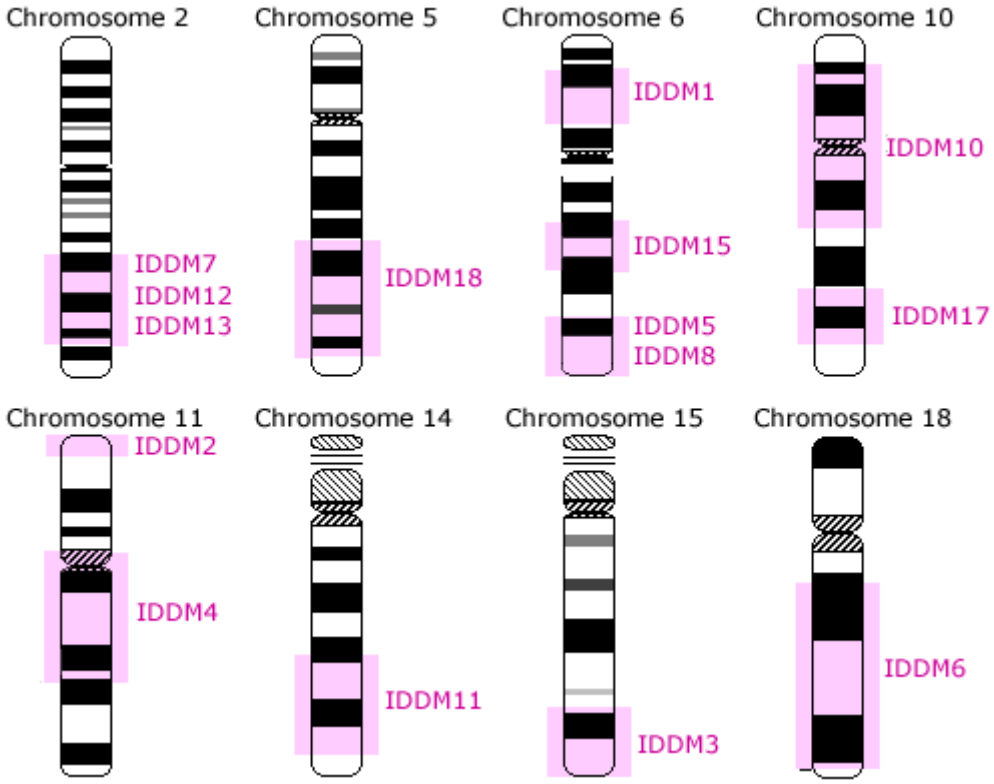

Figure 3a. An overview of type 1 diabetes suceptibility loci (75).

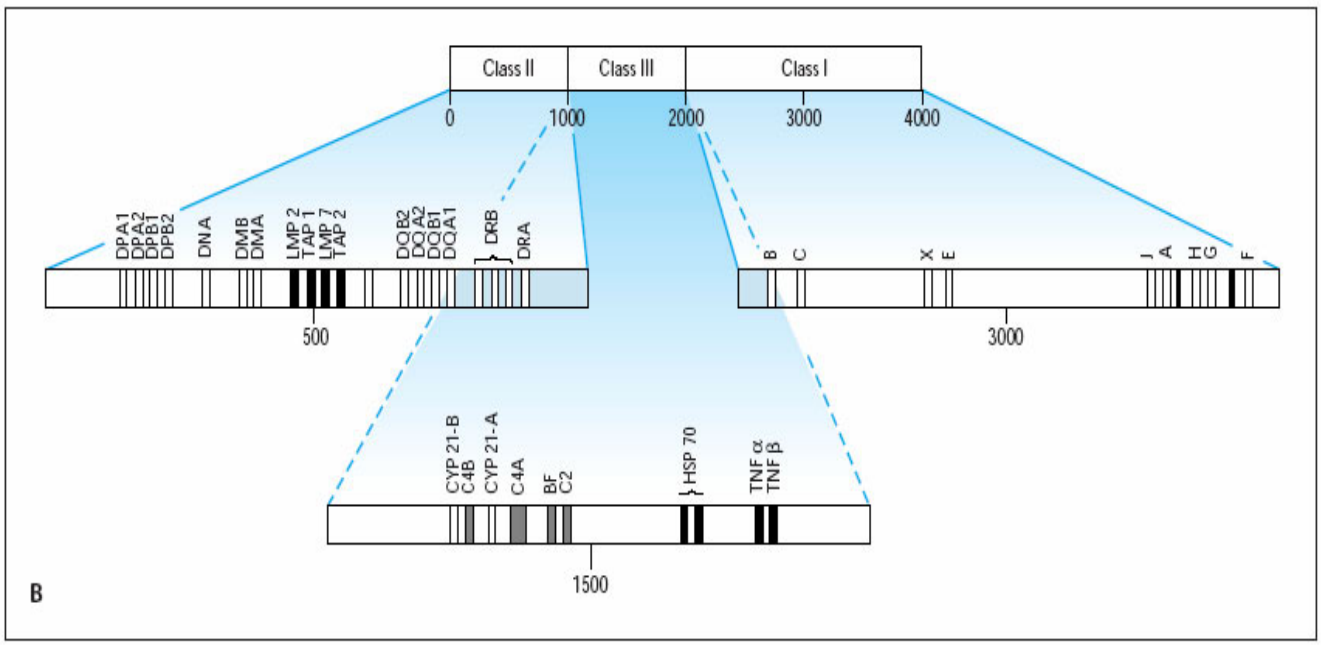

Figure 3b. The HLA region on the human chromosome 6p21 (76) .

There is a close association between the class II HLA-DR3 and DR4 alleles and T1D (65; 79), while the HLA-DR2 allele seems to be protective (80). HLA-DR3 and/or DR4 are found in more than 90% of Caucasian patients affected by T1D, as compared with a frequency of 50-65% among non-diabetic subjects (65; 81; 82). DR3/4 heterozygotes seem to have a particularly high risk of developing T1D, since 30-50% of patients carry this combination but only 1-6% of the background population, whereas the protective DR2 allele is present in only about 3% of affected subjects but 25-30% of the general population (65; 81-84). DR3/4 heterozygous siblings of affected children have also been shown to have multiple T1D-associated autoantibodies, a marker of rapid, aggressive beta-cell destruction, more frequently than siblings with other genotypes. The predictive value of the HLA-DR3/DR4 heterozygous genotype alone, without disease-associated antibody markers, has been reported to be 12%-19% among siblings (85; 86). HLA-DQB1 genes seem to confer the strongest diabetes susceptibility among the HLA family (87; 88). The HLA-DQ molecules associated with an increased risk of the disease or protection against it are listed in Table 1(89). The effect is modified by certain DQA1 and DRB1 alleles, however,as these are all closely coupled and inherited as coherent entities (haplotypes). A HLA-DQB1based risk estimate for Finnish children that defines the presence of the HLA-DQB1*02 and DQB1*0302 alleles associated with risk and of the protective DQB1*0301, DQB1*0602, and DQB1*0603 alleles is presented in Table 2 (89). A simplified HLA-DQ risk estimation model for the Finnish population has been presented by Ilonen et al.: high risk (DQB1*02/0302), moderate risk (DQB1*0302/x, where x stands for 0302 or a non-defined allele), low risk (DQB1*0301/0302, DQB1*02/0301, DQB1*02/x, DQB1*0302/0602–3, where x stands for 02 or a non-defined allele), and decreased risk (DQB1*x/x, DQB1*0301/x, DQB1*02/0602–3, DQB1*0301/0602–3, where x stands for a non-defined allele) . When a large Finnish nuclear family cohort (n=622) was genotyped for DRB1-DQA1-DQB1, the DRB1*0401-DQB1*0302 haplotype was found to be the most prevalent disease susceptibility haplotype. The results are shown in Table 3 (90). The Childhood Diabetes in Finland study showed a prevalence of 35% for at least one autoantibody and a frequency of 29% for two or more autoantibodies by the age of 6 years in siblings with the high risk HLA-DQB1*02/*0302 genotype (91). A surprising finding in a recent study that included three European populations showed that the highest risk in Finland was conferred by the DQB1*02/*0304 genotype and that the *0304/*0604 genotype was present only in affected cases, although at a low frequency, whereas consistent with previous results, the highest disease risk among the DQB1 genotypes in the Hungarian and Greek populations was conferred by the *02/*0302 genotype (92).

Haplotypes encoding risk-associated molecules	
DQA1*0301-DQB1*0302	DR4-DQ8
DQA1*0501-DQB1*0201	DR3-DQ2
DQA1*0301-DQB1*0201	DR4-DQ2 (Mediterranean), DR7-DQ2 (Black), DR3-DQ2/DR4-DQ8
Haplotypes encoding protective molecules	
DQA1*0101-DQB1*0602	DR15(2)-DQ6
DQA1*0501-DQB1*0301	DR5-DQ7
DQA1*0301-DQB1*0301	DR4-DQ7

Table 1. HLA-DQ molecules associated with increased risk of type 1 diabetes or protection against it [modified from the original (89)].

DQB1*Genotype	Children with T1D		Newborn infants		
	n	%	n	%	OR
02/0302	161	(28.8)	303	(2.9)	**13.63**
0302	191	(34.1)	1039	(9.9)	**4.73**
0302/0603	17	(3.0)	213	(2.0)	**1.52**
0301/0302	23	(4.1)	293	(2.8)	**1.50**
02	102	(18.2)	1384	(13.1)	**1.47**
02/0301	9	(1.6)	321	(3.0)	**0.52**
302/0602	6	(1.1)	324	(3.1)	**0.34**
Others	27	(4.8)	1704	(16.2)	**0.26**
02/0603	4	(0.7)	273	(2.6)	**0.27**
0301	10	(1.8)	1049	(10.0)	**0.16**
02/0602	2	(0.4)	406	(3.9)	**0.09**
0301/0603	1	(0.2)	198	(1.9)	**0.09**
0602	4	(0.7)	1421	(13.5)	**0.05**
0602 or 0603	3	(0.5)	1264	(12.0)	**0.04**
0301/0602	0	(0.0)	349	(3.3)	**0.00**
Total:	**560**		**10541**		

Table 2. HLA-DQB1 genotypes in Finnish children with type 1 diabetes and healthy newborn infants [modified from the original (89)]. **(OR = Odds ratio)**

HLA DRB1-DQA1-DQB1 haplotypes	Patients n	%	Controls (AFBAC) n	%	Odds ratio (95%CI)		*p*-value
DRB1*0405-DQB1*0302	5	(0.4)	0	-	11.04	(1.33–91.87)	0.03
DRB1*0401-DQB1*0302	351	(28.2)	81	(6.5)	5.64	(4.37–7.29)	$<10^{-6}$
DRB1*04-DQA1*03-DQB1*0304	10	(0.8)	2	(0.2)	5.03	(1.40–18.08)	0.02
(DR3)-DQA1*05-DQB1*02	273	(21.9)	95	(7.6)	3.40	(2.65–4.36)	$<10^{-6}$
DRB1*0404-DQB1*0302	118	(9.5)	41	(3.3)	3.07	(2.14–4.41)	$<10^{-6}$
DRB1*0402-DQB1*0302	1	(0.1)	0	-	3.00	(0.27–33.16)	
(DR13)-DQB1*0604	49	(3.9)	42	(3.4)	1.17	(0.77–1.78)	
(DR9)-DQA1*03-DQB1*0303	55	(4.4)	52	(4.2)	1.06	(0.72–1.56)	
DRB1*04-DQA1*03-DQB1*0301	29	(2.3)	31	(2.5)	0.93	(0.56–1.55)	
(DR7)-DQA1*02-DQB1*02	44	(3.5)	51	(4.1)	0.86	(0.57–1.29)	
(DR8)-DQB1*04	98	(7.9)	125	(10.0)	0.77	(0.58–1.01)	
(DR1)-DQB1*0501	131	(10.5)	225	(18.1)	0.53	(0.42–0.67)	$<10^{-6}$
(DR16)-DQB1*0502	5	(0.4)	11	(0.9)	0.45	(0.17–1.21)	
(DR4)-DQA1*03-DQB1*02	0	-	1	(0.1)	0.33	(0.03–3.68)	
(DR11/12/13)-DQA1*05-DQB1*0301	34	(2.7)	114	(9.2)	0.28	(0.19–0.41)	$<10^{-6}$
(DR13)-DQB1*0603	22	(1.8)	109	(8.8)	0.19	(0.12–0.30)	$<10^{-6}$
DRB1*0403-DQB1*0302	1	(0.1)	7	(0.6)	0.14	(0.03–0.67)	0.03
(DR15)-DQB1*0602	17	(1.4)	200	(16.1)	0.07	(0.04–0.12)	$<10^{-6}$
(DR14)-DQB1*0503	1	(0.1)	26	(2.1)	0.04	(0.01–0.16)	$2x10^{-7}$
(DR7)-DQA1*02-DQB1*0303	0	-	31	(2.5)	0.02	(0.00–0.11)	$<10^{-8}$

Table 3. Distribution of HLA DRB1-DQA1-DQB1 haplotypes in 622 Finnish children with type 1 diabetes and in affected family-based artificial controls (AFBAC) [modified from the original (90)].

3.2.2 Autoantigens and autoantibodies in T1D

The frequency of autoantibodies in the general population is low, e.g. ICA, IAA, and GADA are detected in only about 0.5-4% of non-diabetic children (93; 94). ICA have been shown to be present in 0.5-4% of non-diabetic subjects, in 3-8.1% of unaffected relatives of patients with T1D and in as many as 70-90% of patients with recent-onset T1D (95-100). About 4% of Finnish schoolchildren tested positive for at least one diabetes-associated autoantibody (59), whereas 13.8% of children with HLA-conferred susceptibility to T1D recruited from the general Finnish population had one or more autoantibodies in at least one sample by the age of 5 years. By that age 4.4% tested positive for two or more autoantibodies in at least one sample. Persistent positivity for two or more autoantibodies appears to reflect progressive destructive beta-cell autoimmunity, whereas positivity for a single autoantibody may represent harmless non-progressive or even regressive beta-cell autoimmunity (101). The presence of autoantibodies in cord blood was not predictive of the subsequent development of islet autoimmunity, but was rather the result of maternal transmission (102; 103). Autoantibodies are recommended as the first-line screening approach in siblings, as the risk of progression to T1D increases with the number of autoantibody reactivities detected (104). The addition of genetic markers increased the positive predictive values of all the autoantibodies substantially, but resulted in reduced sensitivity among the siblings of affected children. High-risk DQB1 genotypes seem to predispose children more strongly to the emergence of each T1D-related autoantibody reactivity and multiple autoantibodies than the moderate-risk genotypes (101).

3.2.2.1 Islet cell antibodies

ICA, first detected in patients with T1D more than 30 years ago (105), are antibodies of the IgG type, mostly IgG1. These react with antigens located in the cytoplasm of all endocrine cells of the pancreatic islets. (106; 107). Complement fixing ICA (CF-ICA) were described by Bottazzo et al. in 1980 as being detectable in the sera of about 50% of patients with newly diagnosed T1D, and seemed to correlate more closely with presentation with T1D than conventional ICA (108). The predominant opinion at present is that CF-ICA represent high-titre ICA (107; 109). The presence of ICA is detected not only in subjects who progress to clinical T1D, but also among non-progressors and in the general population (110). In an Australian study, low-titer ICAs (<20 JDFU) were detected in 6.4% of schoolchildren and high-titer ICAs (>20JDFU) in 0.9%, the frequency of this marker of islet autoimmunity being much higher than the number of children expected to develop diabetes (111). Four studies on ICA-positivity in the general population showed that around 0.5%-25% of ICA-positive children develope T1D (95; 98; 112; 113). The majority of the future patients with T1D are to be found in the ICA-positive group, however, where the risk of progression to overt diabetes is directly proportional to high ICA titers and young age (114). ICA are often present for months or years in individuals who present with T1D later (56), and the majority of progressing siblings (73-88%) test positive for ICA at diagnosis (115-118). The prevalence of ICA often falls considerably after the clinical manifestation of T1D (119). The persistence of ICA is presumed to reflect ongoing destructive processes in the beta cells (101), so that once all the beta cells in the pancreas have been destroyed, ICA are assumed to disappear in the absence of any antigenic stimulation (120). In a Japanese study,

children with abrupt-onset T1D had high ICA levels initially, but these decreased rapidly after the first year, whereas children with slow-onset T1D were ICA-positive with low titres for a relatively long period (121). In some ICA-positive relatives the antibodies have been reported to appear and disappear over time, although later surveys have shown this to be rare (59). This fluctuation may be a result of inter-assay variability rather than reflecting remission of islet cell immunity (122; 123). The distribution of ICA-positivity in first-degree relatives of patients with T1D in Finland (100) is shown in Table 4.

Characteristics	**Total**	**ICA-**	**ICA+ (≥*2.5 JDFU*)**	**ICA+ (≥*18JDFU*)**	**Age, years mean(± SEM)**
Female/ male ratio	1,415 (56%) /1,107 (44%)	1,317 (57%) /1,001 (43%)	98 (48%) /106 (24%) [a]	50 (48%) /55 (52%	20.4(±0.3)
Relation to the proband					
Parents	1,012 (40%)	940 (41%)	72 (35%)	32 (30%)	34.8(±0.1)
Siblings	1,510 (60%)	1,378 (59%)	132 (65%)	73 (70%) [b]	10.8(±0.2)
Fathers	390 (15%)	361 (16%)	29 (14%)	12 (11%)	35.4(±0.2)
Mothers	622 (25%)	579 (25%)	43 (21%)	20 (19%)	34.4(±0.2)
Brothers	717 (28%)	640 (28%)	77 (38%)	43 (41%)	10.5(±0.2)
Sisters	793 (31%)	738 (32%)	55 (27%)	30 (29%) [c]	11.0(±0.2)
Total:	**2,522**	**2,318**	**204**	**105**	

[a] $p < 0.05$.
[b] $p < 0.05$ when compared with parents.
[c] $p < 0.05$ when compared with brothers.

Table 4. ICA-positivity in relatives of children with type 1 diabetes in Finland (SEM = standard error of mean) [modified from the original (100)].

No indications of an association between ICA and HLA class I or class II antigens were observed in some earlier reports on patients with T1D (124-126), but other, more recent studies have suggested the opposite. The prevalence of ICA was higher in heterozygous DR3/DR4 patients than in patients without DR3 or DR4, and high-titer ICA were more frequently found together with the HLA DQA1*0301-DQB1*0302 haplotype at the diagnosis of T1D in patients younger than 10 years of age (127; 128).

ICA-positive heterozygous DR3/DR4 siblings had a 70% cumulative incidence of progression to T1D after an 8-year follow-up, whereas the cumulative incidence in ICA-negative heterogygotes was only 5% (85). The HLA DQA1*0301-DQB1*0302 haplotype is also reported to be associated with high ICA titers in siblings, but these associations were only observed in relation to specific genotypes (129). ICA were more frequent and had significantly higher titers in siblings of affected children with the high-risk DQB1 genotype than in siblings with the low or decreased risk genotypes (130). High levels of ICA seem to be related to HLA identity, the DR4 and DQB1*0302 alleles and the susceptible DQB1 genotypes (131). In another survey the frequency of ICA positivity was higher in those siblings who were HLA-identical to the affected sibling (9.9%) than among those who were haploidentical (5.3%) or non-identical (2.4 %) (132).

3.2.2.2 Insulin autoantibodies

It was first recognized in 1983 that antibodies against endogenous insulin were detectable in subjects with newly diagnosed T1D before treatment with exogenous insulin (133). IAA were initially found in 18% of patients with recent-onset T1D, while the current experience is that they are present in about 40-50% of such cases (114; 134). IAA are in most cases the first autoantibodies to appear in young children with emerging signs of beta-cell autoimmunity, implying that insulin may be the primary autoantigen in most cases of childhood T1D (115; 135-137). The prevalence of IAA in the general population has been reported to range from 0.9% to 3.3% (94; 113; 138; 139) and is reported to be higher among siblings of affected children, varying from 1.4 to 6.9% (86; 118; 140-143). There is a definite inverse correlation between IAA prevalence and levels and age at the diagnosis of T1D, IAA being more frequent and present at higher titers in children developing diabetes before the age of 5 years (144). IAA may also correlate with the rate at which beta–cell destruction proceeds, the titers being highest in those progressing to diabetes most rapidly (145). IAA are quite often detected in non-diabetic subjects with ICA, whereupon their simultaneous presence confers a substantially greater risk of progression to T1D than does the presence of either antibody alone (134). There is more fluctuation over time in IAA levels than in ICA levels, but the reason for this is unknown (123; 146). The presence of IAA does not always predict diabetes, but may reflect an inherited propensity to autoimmunity. Fluctuations in IAA positivity have been observed, whereas fluctuations in other autantibodies are rare (59; 146).

Controversial results have been presented regarding the relation between IAA and HLA alleles. IAA were not related to the presence of HLA-DR3 or DR4 in twins (146), and in an American study the levels of IAA did not differ with respect to HLA-DR antigens in newly diagnosed children with T1D (147). On the other hand, the frequency of IAA was reported

to be increased in affected subjects carrying the HLA-DR4 haplotype (131; 148; 149), whereas T1D patients with the HLA-DR3 haplotype had very low IAA levels (150). The HLA-DR4 allele seems to contribute to the level of anti-insulin autoimmunity, and DR4-associated diabetes susceptibility is thought to be secondary to this influence, in contrast to DR3 susceptibility (148). Like ICA, IAA are also associated with the HLA DQA1*0301-DQB1*0302 haplotype at the clinical diagnosis of T1D in patients younger than 10 years of age (93; 127; 141), and similar associations have been observed in relation to specific genotypes in siblings of patients with T1D (129). IAA have also been found to be more frequent among siblings of patients with T1D with the high-risk DQB1 genotype (DQB1*02/*0302 genotype /DQB1*0302 allele) than in those with low risk genotypes (130; 131). DR3/4 hetreozygosity also seems to be linked to high IAA frequencies in siblings (131). The role of insulin as an autoantigen in T1D is unequivocal: it is beta-cell specific and it is expressed on the surface of the beta cell, but whether this immune response is primary or secondary to beta-cell damage remains a open question. Proinsulin, the precursor of insulin, has been implicated as a possible autoantigen in T1D, and there has been some debate as to whether proinsulin autoantibodies (PAA) may be even more closely associated with T1D than IAA (151). No significant difference was observed in the prevalence of PAA and IAA among 151 patients with newly diagnosed T1D, or among 114 age-matched non-diabetic first-degree relatives. A similar observation was made in a study of 179 patients with newly diagnosed diabetes and 1028 schoolchildren, where the reduced background prevalence of IAA versus PAA in the school children resulted in an increased specificity of IAA and suggested that IAA perform better than PAA in predicting the risk of diabetes (152).

3.2.2.3 Glutamic acid decarboxylase antibodies

Autoantibodies to a 64 kD islet-cell protein were detected in patients with newly diagnosed T1D in the early 1980's (153). These antibodies were observed in prediabetic subjects years before clinical presentation with T1D, and it was shown later that patients with stiff-man syndrome, a rare neurological syndrome affecting the γ-aminobutyric acid nervous system, had antibodies recognizing cells in the cerebellum and in the pancreatic islets of Langerhans (154). This 64-kD antigen was subsequently identified biochemically as the enzyme glutamate decarboxylase (GAD), which catalyzes the formation of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) from glutamine (155). GAD may play a role in the inhibition of glucagon and somatostatin secretion in the islets and in the regulation of insulin release. Two forms of GAD with different molecular weights exist (GAD_{67}, GAD_{65}), but human islets express only the smaller form, GAD_{65}. This is not specific to beta cells, however (156). GAD_{65} antibodies have been detected in 20-90% of cases studied before and at the diagnosis of T1D (152; 157), about 5-13% of first-degree relatives and up to 3% in the general population (152). The diagnostic sensitivity of GADA for T1D seems to be higher in adult subjects than in children (116; 158). Among siblings positive for GADA, 58-69% subsequently present with clinical T1D (86; 140). There is a significant association between ICA and GADA, but it is weaker than that between ICA and IA-2A (116; 157; 159). GADA levels are higher in postpubertal subjects and females affected by T1D (159; 160). Beta-cell destruction seems to be slower in high-risk subjects who are GADA-

positive than in those testing negative for GADA (161). Humoral immunity to GAD often remains elevated in cases where most of the beta cells have been destroyed, even years after diagnosis, possibly because GAD_{65} is also found in other tissues, e.g. in the central nervous system (CNS) (117; 159; 162). GADA were more frequent and had significantly higher titers among siblings of children with T1D with the high-risk DQB1 genotype (157), while their frequency has been reported to be 0.5-4% among non-diabetic siblings with genotypes conferring low or decreased risk (130). It has also been suggested that individuals carrying the HLA DR3-DQ2 haplotype are more prone to develop GADA (152; 163), although other findings suggest that type 1 diabetes susceptibility alleles do not control the development of GADA (164).

3.2.2.4 IA-2 and IA-2β antibodies

Antibodies against a 50kD fragment derived from trypsin digestion of the 64kD islet protein, as described in 1982, have been observed to react with GADA, whereas 37kD and 40kD fragments were not reported to interact (153; 165). Patients with T1D were observed to have antibodies against all of these proteolytic fragments. Later studies have shown the 40kD antigen to be identical to the intracellular portion of the islet cell antigen 512 (ICA512), a transmembrane protein that is a member of the protein tyrosine phosphatase (PTP) family (166). ICA512 is now more commonly known as the islet cell antigen 2 (IA-2) molecule (167). Notkins et al identified 21 proteins in pancreatic islet cells that are related to the PTP family (168), among which IA-2β, also known as phogrin, was shown to be the precursor of the 37kD tryptic fragment (169). Humoral autoimmunity to IA-2 and IA-2β is mainly directed at the cytoplasmic portions of these proteins. Four antigenic domains have been identified in the IA-2 and IA-2β molecules, including autoantibodies specific to the juxtamembrane (JM) region, the PTP-like domain of IA-2 and the PTP-like domain of IA-2β and antibodies that are cross-reactive between the PTP-like domains of IA-2 and IA-2β (170). A recent study has compared epitope and isotype-specific IA-2 antibody responses and demonstrated that siblings who progressed to clinical diabetes more often had juxtamembrane epitope-specific IA-2 antibodiesand less often isotype-specific IgE-IA-2 antibodies than those who did not progress (170). The non-progressors also had higher integrated titers of IgE-IA-2 antibodies. Approximately 50-86% of patients with newly diagnosed T1D have antibodies to IA-2, while the prevalence in the general population is less than 2% (152; 169; 171-173). IA-2A usually emerge as the last of the autoantibodies, or one of the last, during the preclinical disease process (174; 175), and 58% to 69% of siblings positive for IA-2A develop signs of clinical T1D over the next 5-10 years (86; 140). The prevalence of IA-2A is similar in both sexes and in children and adolescents, a point on which these antibodies differ from GADA, which are associated with female gender and older age, and from ICA and IAA, which are associated with young age (114; 145; 159; 160; 172). A non-diabetic sibling with multiple T1D-associated autoantibodies including IA-2A carries an approximately 50-80% risk of developing diabetes within the next 5 years, compared with a risk of around 15% in the absence of IA-2A (141; 176). By contrast, IA-2A positivity in the general population of schoolchildren seems to confer a 6% risk of developing signs of overt T1D within 10 years (94). The prevalence of IA-2A is highest in those under the age of 5 years, and in patients with HLA DR4, the strongest single allele

predisposing subjects to T1D (in contrast to GADA, which are associated with DR3 in affected patients), and the HLA haplotype DQA1*0301-DQB1*0302 (104; 116; 152; 177; 178). ICA titers and IA-2A levels are relatively closely related, suggesting that IA-2 is an essential antigen for ICA, while IA-2A and GADA seem to have a weak inverse correlation. IA-2A were more frequent among siblings of children with T1D having the high-risk DQB1 genotype than among those with low risk genotypes (130), and it has been suggested that while GADA may be an indicator of general autoimmunity in patients with T1D, IA-2A may be a more specific marker of beta-cell destruction (172). The presence of IA-2A is also associated with a more rapid progression to clinical T1D in first-degree relatives of affected children (104; 174). Like GAD, both IA-2 and IA-2β are also expressed in the CNS as well as in the islets. Though they are obviously major autoantigens in T1D, their functional roles have remained undefined (169). IA-2A have a high positive predictive value but a low sensitivity compared with ICA, for example, implying that screening programs relying on the presence of IA-2A would only identify a small high-risk group while excluding a subtantial proportion of the subjects at risk (152).

3.2.3 Insulitis and the mechanisms of beta-cell destruction

T1D is the result of T-cell mediated autoimmune destruction of the insulin-producing beta cells in the pancreatic islets with the preservation of alpha and delta cells and the cells secreting pancreatic polypeptide (1; 179). Two alternative destructive pathways are shown in Figure 4. Examinations of the pancreatic tissue from patients who have died shortly after being diagnosed with T1D, have revealed that there is pronounced inflammatory infiltration by $CD8^+$ and $CD4^+$ cells, B lymphocytes, macrophages and natural killer cells, commonly referred to as insulitis (114). The expression of HLA class I molecules on islet cells is increased, and class II molecules may be overexpressed on beta cells, macrophages and the endothelium. Expression of intercellular adhesion molecule 1 (ICAM-1) on the vascular endothelium has been observed to be increased, a feature favoring the adhesion and accumulation of mononuclear cells in the islets (114; 180). Intriguingly, recent studies have reported that there is a benign autoimmune response towards the islets in the early neonatal phase, and it has been proposed that T1D may develop in those individuals who fail to organize a well controlled protective autoimmune response to damaged pancreatic tissue at the neonatal stage. Consequently, T1D-prone individuals do not benefit from any physiological protective mechanism which counters neonatal beta–cell death by promoting processes of apoptotic beta-cell clearance and repair of the damaged islets, and are devoid of the postnatal local T-cell activation that subsequently results in the recruitment of islet antigen-specific T cells into the pool of peripheral regulatory T cells (181).

Observations that diabetes can be transferred from non-obese diabetic (NOD) mice or biobreeding (BB) rats to non-diabetic animals by T lymphocytes support the concept that T cells play an important role in the pathogenesis of autoimmune diabetes. The T-cell responses seem to be similar in humans, mice and rats. In mice the $CD4^+$ Th1 cells secrete mainly interleukin 2 (IL-2) and interferon γ (IFN-γ) and support macrophage activation, delayed-type hypersensitivity (DTH) responses and immunoglobulin (Ig) isotype switching

to IgG2a. The $CD4^+$ Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and/or IL-13. Several factors, including the dose of antigen, the type of antigen presenting cell (APC) and the HLA class II haplotype, influence the differentiation of naive $CD4^+$ T cells into specific Th subsets. The cytokines themselves are the best characterized factors influencing the differentiation into Th subsets. IFN-γ, for example, inhibits the differentiation and effector functions of Th2 cells, and IL-12 markedly delays the differentiation of Th1 cells, whereas IL-4, IL-10 and IL-13 have been reported to inhibit Th1 proliferation (182). Th1 cells provoke the disease when transferred to neonatal NOD mice, while Th2 cells only invade the islets, without inducing autoimmune diabetes (183). These observations suggest that the balance between Th1-associated destructive insulitis and Th2-associated non-destructive peri-insulitis plays a crucial role in the pathogenesis of autoimmune diabetes. This immunoregulatory balance is still unclear, however, and needs to be further investigated. The Th1 cells induce a predominantly cell-mediated immune response, while Th2 cells direct immunological responses towards humoral immunity. The counterregulatory and destructive and non-destructive insulitis mechanisms observed in mice are probably also operative in man (182; 184).

In a hypothesis based on the central role of $CD8^+$ T cells as presented in Figure 4A, autoantigens that are processed and presented as peptides in a complex with HLA class I molecules on the surface of beta cells are recognized by antigen-specific $CD8^+$ cytotoxic T lymphocytes (1). These $CD8^+$-cells can kill target cells by causing apoptosis via direct contact with surface membrane-bound ligands such as FasL, TNF-related apoptosis-inducing ligand (TRAIL) and membrane-bound TNFα (185). In addition, target cell killing can transpire without cell-to-cell contact, via the secretion of humoral substances such as perforin molecules, which are inserted into the membrane of the target cell as tubular entities through which the proteases granzyme A and B can pass, activating apoptosis nucleases in the cell (1; 185). The Fas-FasL apoptosis pathway seems to play a critical role in the destruction of beta cells. Fas is a type 1 transmembrane glycoprotein (cell-surface receptor protein) in the TNF/NGF superfamily, while FasL (the specific ligand for Fas *in vivo*) is a type 2 transmembrane glycoprotein in the TNF superfamily(186). In NOD lpr/lpr mice, which are deficient in Fas expression because of an incapacitating mutation in the Fas gene, spontaneous development of diabetes did not occur. Furthermore, the transfer of a particular NOD-derived, islet-reactive $CD8^+$ T-cell clone into young, irradiated NOD animals led to diabetes development several days later, but a parallel transfer into NOD lpr/lpr recipients did not provoke the disease (187). Another model proposes that the immune response may be initiated by a viral protein that shares an amino acid sequence (molecular mimicry) with a beta-cell protein (e.g. the non-structural coxsackie virus protein 2C and GAD), which may result in the appearance of antiviral cytotoxic $CD8^+$ lymphocytes that react with self-proteins of the beta cells (188; 189). This promotes the local production of cytokines and other inflammatory mediators that induce the expression of adhesion molecules in the vascular endothelium of the islets. The activation of endothelial cells allows increased adhesion and extravasation of the circulating leukocytes and the presentation of beta-cell antigens from the damaged beta cells by infiltrating macrophages and lymphocytes (114; 190).

There are some studies that support the “bystander“ hypothesis demonstrated in Figure 4B,

in which the destruction of beta cells is due to T-cell-mediated ($CD4^+$) apoptosis, starting with the destruction of some beta cells by an exogenous culprit (e.g. a virus, nutrient, or chemical of some kind) and the subsequent release of beta-cell proteins (191; 192). These proteins will be taken up by antigen-presenting cells (macrophages) and presented on the cell surface by the HLA class II molecules. This together with co-stimulatory molecular interactions such as that between B7-1(CD80) or B7-2 (CD86) and the APC and CD28 receptors on T cells activates the antigen-presenting cells to produce and secrete monokines (IL-1, TNFα), and the activated T cells release IFN-γ (1; 193). The magnitude of beta-cell damage is dependent on the velocity of the feed-back circuit between the antigen-presenting cells and the T-helper lymphocytes, i.e. on the efficiency of antigen presentation/recognition, the amount of cytokine production and the capacity of beta-cell defense mechanisms. Because of their low superoxide dismutase content, beta cells are extremely vulnerable to damage by IL-1. In the course of beta-cell destruction some of the cell proteins may be modified by free radicals, strengthening the immune process further (187; 192). IL-1 secreted by macrophages and monocytes is cytotoxic to islet beta cells, this process being dependent on both the concentration of IL-1 and the metabolic activity of the beta cells, because resting cells are resistant to cytotoxic action (192; 194). IFN-γ potentiates the effects of IL-1 on beta cells, and experimental data indicate that IL-1-induced beta-cell destruction is mediated by toxic free radicals such as oxygen (O_2^-) and nitric oxide (NO) produced by macrophages, endothelial cells or beta cells themselves (195; 196). Studies on human pancreatic islets have revealed that although combinations of IL-1, TNFα and IFN-γ suppress islet insulin release and induce NO generation, human islets are more resistant to the effects of NO than rodent islets. It was also observed that even in the case of the effects of NO being inhibited, the cytokine effects remained, demonstrating that there are also other mediators inflicting damage on beta cells apart from NO, or that macrophages or endothelial cells are the main source of NO in human tissue (197). IFN-γ is also a potent promoter of beta-cell destruction. It is produced not only by $CD4^+$ Th1 cells, but also by $CD8^+$ cells, which are often abundant in islets with insulitis (198). IFN-γ has been shown to cause damage to islets regardless of NO (197). It has been claimed that the production of IFN-α is more relevant to human islets than IFN-γ, because IFN-α is produced by many cells which are infected by viruses, and since histological changes in the pancreatic islets occur even before the lymphocytes capable of releasing IFN-γ appear. This IFN-α expression induced by viruses is usually a transient phenomenon, but it remains unexplained why IFN-α was detected in the islets of a patient who had been diagnosed with T1D several months earlier (199).

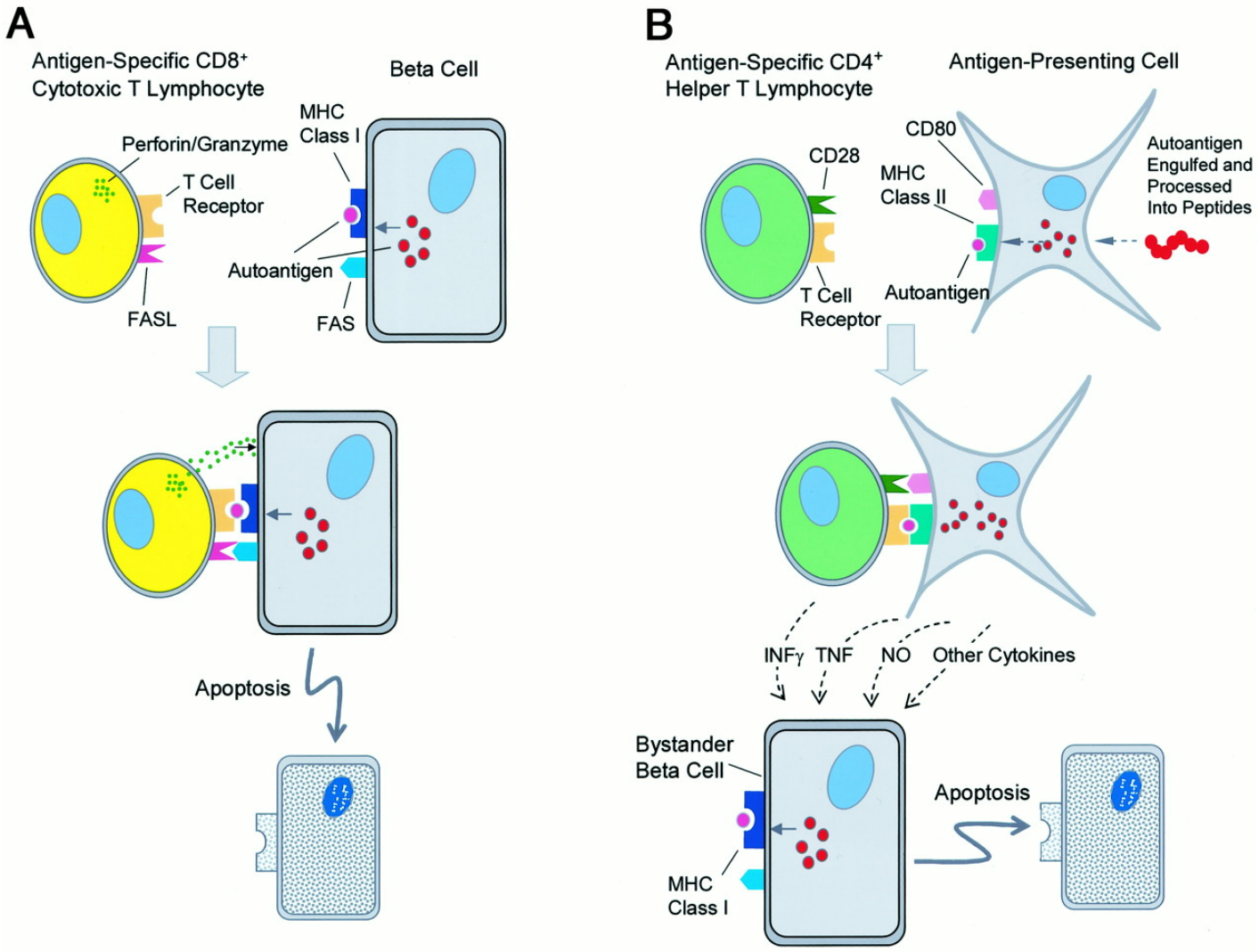

Figure 4. Mechanisms of immune-mediated beta–cell killing.

(*A*) Direct killing of beta cells. Autoantigens that are processed and presented as peptides in a complex with HLA class I molecules on the surface of beta cells are recognized by antigen-specific $CD8^+$ cytotoxic T lymphocytes. This results in the excitement of a number of co-stimulatory molecules (*e.g.* FAS/FASL). A cascade of signal transduction events ensues, resulting in beta-cell death by apoptosis through one or more effector pathways (*e.g.* FAS/FASL, perforin/granzyme).
(B) Indirect (bystander) killing of beta cells. Autoantigens that are engulfed, processed and presented as peptides in a complex with HLA class II molecules on the surface of APCs (*e.g.* macrophages or dendritic cells) are recognized by antigen-specific $CD4^+$ helper T lymphocytes. This excites co-stimulatory molecules (*e.g.* CD28/CD80) and triggers the release of a variety of cytokines (*e.g.* interferon γ (IFN-γ), tumor necrosis factor α (TNFα), and NO from both $CD4^+$ T cells and APCs, resulting in apoptosis of nearby beta cells (1).

3.2.4 Environmental factors

It is likely that the T1D disease process is initiated by a non-genetic, probably environmental factor or factors operating in a genetically susceptible subject to trigger a destructive immune process. These exogenous factors possibly operate over a limited period in early childhood to induce the destructive immune process (200). It is even possible that the process may already begin *in utero*. This is followed by a long prodromal period before the clinical onset of T1D. The strongest evidence for the role of environmental factors is the fact that the concordance rate for T1D in monozygotic twins is only about 30-50% (62; 200; 201).

3.2.4.1 Viral infections

Viral infections have been implicated in the initiation of T1D for a long time (42). A list of potentially pathogenic viruses is shown in Table 5 (202) Acute virus infections can induce a transient autoimmune response, and chronic virus infections such as HIV and hepatitis B and C can cause persistent autoimmune responses and even severe tissue damage. The seasonality of T1D and the increased frequency of viral antibodies in subjects with T1D, the isolation of certain viruses from the pancreas of persons who had died soon after the diagnosis of T1D and a number of animal studies provide evidence for the association of viral infections with T1D (203; 204). There is also evidence of epidemics of T1D in isolated areas, further suggesting a link with viral infections (205).

An association between viruses and T1D was first proposed in 1899, when a Dr. Harris reported that his patient developed diabetes after a mumps infection (206). There seems to be a delayed effect of mumps on the development of T1D, because a lag period of 2-4 years has been observed between mumps epidemics and the subsequent rise in T1D incidence (204; 207). There is also some documentation of manifestation of T1D after mumps vaccinations (207), although other studies have shown no relation between the prevalence of diabetes-associated autotoantibodies and the mumps-measles-rubella (MMR) vaccination (208). In Finland there was a transient plateau in the steadily rising incidence of T1D after MMR vaccinations were introduced in 1983 (209).

Children with the congenital rubella syndrome (CRS) have a high risk of diabetes. An autoimmune etiology has been implicated, as islet surface cell antibodies and IAA have been detected in 20% of non-diabetic individuals and in 50–80% of patients with diabetes after a congenital rubella infection (189). As many as 12–20% of individuals infected with rubella *in utero* will develop diabetes within the next 5–20 years (208; 210-212). In addition to the congenital rubella syndrome, isolated cases of T1D have been reported after rubella infection in adults (213). CRS has been reported to be associated with the same HLA-DR haplotypes as T1D (214; 215), and the rubella virus has been shown to be able to cause diabetes in both rabbits and hamsters (210; 216). A recent report has questioned whether CRS-associated diabetes is autoimmune, as was earlier been suspected, since no increased frequency of T1D-associated autoantibodies could be observed in a series of subjects with confirmed CRS (217).

Cytomegalovirus (CMV) has also been implicated in the etiology of T1D (218). Thirty-five percent of patients with newly diagnosed T1D showed serological evidence of a recent CMV infection, and the association between ICA and CMV infection can even cause beta-cell damage, including degranulation (189). Prospective studies have shown that CMV is not a major cause of T1D, however (219; 220)

Mammalian genomes contain many retroviral sequences which are transmitted to the next generation through DNA. Most of these are non-infectious. The expression of a beta-cell specific retroviral antigen p73 in NOD mice is associated with initiation of the pathogenic immune process (221). Since it has been observed that IAA and the retroviral antigen p73 cross-react in patients with T1D and in their relatives, retroviruses have been implicated as playing a possible pathogenic role in T1D (203; 222). These observations have remained unconfirmed, however.

Epstein-Barr virus (EBV) has been associated with the clinical manifestation of T1D (189). It has been demonstrated that children affected by T1D have lower levels of IgG against an EBV capsid protein than the general population, suggesting that patients with T1D have a defective immune response to this virus (223). There are EBV sequences that are structurally related to the HLA-DQ8 β-chain, and antibodies to this EBV epitope have been observed in a number of patients with T1D (224). Although EBV may have precipitated T1D in a minority of affected patients, it is probably not a trigger in the majority of patients (189).

It is possible that enteroviruses (EV) may play a major role in the pathogenesis of T1D (225). Enteroviruses are small, non-enveloped positive-strand RNA viruses belonging to the picornavirus family (226). The first reports connecting enterovirus infections with T1D were published more than 30 years ago by Gamble and Taylor, showing that coxsackie virus B antibodies are more frequent in patients with T1D than in control subjects (227). There is a conspicuous connection between epidemics of EV and subsequent rises in the incidence of T1D (228; 229). Several studies have demonstrated increased frequencies of enterovirus RNA in children with T1D (33%) compared with non-diabetic controls (4%) (226). The prospective Diabetes Autoimmunity Study in the Young (DAISY) in the United States and the German BABYDIAB study failed to find any association between enteroviruses and T1D (230; 231), but the extensive prospective Type 1 Diabetes Prediction and Prevention (DIPP) Project in Finland has revealed an unequivocal temporal association between enterovirus infections and the appearance of the first diabetes-associated autoantibodies (227). An increased frequency of enterovirus RNA has also been observed in the peripheral circulation of patients with newly diagnosed T1D (232). Enteroviral IgG and IgM levels have been reported to be increased during pregnancy (225) or at delivery (233) in mothers whose offspring later progress to T1D diabetes as compared with control women. A few studies have suggested that in some cases the diabetic disease process could already have been initiated by maternal enterovirus infections during pregnancy (234). Prospective studies have shown signs of an excess of enterovirus infections years before clinical presentation (225; 235; 236). It is also known that several enterovirus strains are capable of damaging human beta cells *in vitro* (237; 238).

Strong suspicion of association with type 1 diabetes

Enteroviruses
-*Coxsackie A strains* (epidemiological investigations)
-*Coxsackie B strains* – All six types are associated with autoimmunity, especially B4 (extensive epidemiology). Induces diabetes in mice.
-*Echoviruses* (epidemiological investigations).

Rubella
Follows intrauterine infection in offspring. Diabetes may appear after a long time period. Positivity for islet cell surface antibodies has been observed. Vaccination entails an attenuated risk.

Viruses that might have a modest role in pathogenesis of type 1 diabetes

Cytomegalovirus
A cross-reactive epitope with GAD 65.

Epstein-Barr virus
Associated with autoimmune diseases, including diabetes.

Mumps
Many reports, but the role is still not confirmed. Vaccination entails an attenuated risk.

Retrovirus
Evidence is controversial in humans.

Rotavirus
Reports of islet autoimmunity in children after rotavirus infection.

Table 5. Viruses implicated in the pathogenesis of type 1 diabetes [modified from the original (202)].

3.2.4.2 Dietary factors

Nutritional factors have been suspected of being involved in the development of T1D, but research into this issue is limited and the results often contradictory.

Certain proteins, especially cow's milk proteins, have been reported to induce autoimmune diabetes in the BB rat (239; 240). Increased levels of IgA antibodies to cow's milk and β-lactoglobulin (BLG) have been observed in children with newly diagnosed T1D (241; 242), and higher IgG antibodies to BLG and bovine serum albumin (BSA) have been reported in diabetic children (241; 243). An inverse correlation has been observed between the duration of breast feeding and the risk of developing T1D (239; 244; 245). A recent double-blinded, randomized pilot nutritional intervention trial involving feeding on either casein hydrolysate or conventional cow's milk-based formula until the age of 6-8 months demonstrated that the cumulative incidence of autoantibodies by the age of 5 years was somewhat smaller in the casein hydrolysate group, hinting at a possiblity for manipulating spontaneous beta-cell autoimmunity by dietary intervention in infancy (246). Positive correlations have been reported between the average consumption of cow's milk and the incidence of T1D in various countries (247), and it has been observed in Finland that a high daily consumption of native cow's milk (≥3 glasses of milk) beyond infancy is associated with an increased risk of seroconversion to autoantibody positivity and progression to overt T1D among initially unaffected siblings of children with T1D (248). Maternal cow's milk consumption seems to have no effect on the later life of the child, as elimination of cow's milk from the maternal diet during the last trimester of pregnancy had no effect on the emergence of diabetes-associated autoantibodies in the offspring in a Swedish study (249).

Streptozocin, which is chemically related to nitrosamines, has been shown to induce diabetes in experimental animals (250), and similar compounds may be present in the diet or may be produced from nitrites and nitrates in the gastrointestinal tract (251). There is a possible association between the risk of developing T1D and the intake of nitrates and nitrites, as long-term frequent exposure to nitrosamines is potentially toxic to beta cells (239). An association between the intake of nitrates and nitrites and diabetes has also been observed in Finland (252).

Many food products that are rich in carbohydrates are also rich in wheat gliadin, which is a protein observed to induce autoimmune diabetes in BB rats (239; 253). Alternatively, it is also possible that the carbohydrate-rich food induces beta-cell stress, which makes these cells more susceptible to damage by various cytokines at least in experimental models (192; 194; 254). Soy is also a subject of controversy, since there are animal experiments that implicate it as being diabetogenic, while others have observed that soy suppresses the development of diabetes (255).

The 'Accelerator Hypothesis', which aims at explaining the increase in the incidence of T1D in childhood, and also the acceleration in its development, Imaintains that children who are heavier as toddlers run a higher risk of T1D later in childhood The increase in insulin resistance as a consequence of weight gain in children up-regulates metabolic activity in the beta cells, and is thereby also likely to increase their immunogenicity (256;

257). A 10% increment in relative weight relative to controls was associated with a 50 to 60% increase in the risk of T1D before the age of three in Finnish children and a 20 to 40% increase after that age (258).

3.2.4.3 Additional factors

Other exogenous factors have also been inplicated as being associated with T1D. *Plasmodium falciparum* is suspected to have a protective role in relation to T1D, mediated by genetic selection. Areas with past high malaria morbidity seem to be associated with a lower risk of T1D, and it may also be an indicator of other parasitic infection that could be protective against T1D. Vaccinations have also been associated with an increased risk of developing T1D (209; 259), although a recent Danish study could not find any evidence of a link with childhood vaccinations (217). Vitamin D has also been associated with protection from the development of autoimmune diseases in animal models, although further studies are needed to investigate its role (260; 261). The link between psychological stress and unfavorable diabetes control is widely accepted, and there has been some debate on the role of stressful events in the development of T1D. Numerous large, well-controlled surveys have failed to find any unequivocal evidence that T1D is caused by stressful life events (262).

3.3 Prediction of type 1 diabetes

Genetic and immunological markers, and metabolic indicators such as glucose tolerance and first-phase insulin response to intravenous glucose, provide information that facilitates the identification of individuals at risk of progression to clinical T1D. Predictions are still far from accurate at the individual level, but increasing knowledge accumulated over the past 20 years regarding the natural course of preclinical T1D has gradually improved our methods of risk assessment. It is crucial to develop an optimal predictive strategy for the identification of potential progressors to T1D in order to enable successful T1D prevention as soon as effective preventive modalities have been established.

3.3.1 Relatives of patients with T1D

The presence of ICA has formed the basis for predicting T1D in first-degree relatives for a long time. ICA positivity in unaffected first-degree relatives of children with T1D is associated with a 30–50% cumulative risk of progressing to clinical diabetes within 5–10 years, and this risk increases with increasing ICA titers, as siblings positive for ICA alone have a cumulative risk amounting to 50-60% of progressing to T1D within 10 years (95-97; 104; 140; 141). IAA alone are not very useful in this respect, but in combination with ICA they are more predictive of the disease than ICA alone (97; 134; 263; 264). GADA are common in combination with ICA, but rare in their absence (159; 265). It has been claimed that GADA do not significantly increase the risk of progression to T1D conferred by ICA and IAA in combination (104; 159), however, the combination of GADA with IA-2A seems to be as effective as ICA in screening for autoimmunity in children with newly diagnosed

diabetes (140; 141; 266-268). High levels of GADA have been associated with a low risk of progression to T1D, which is consistent with the assumption that distinct subsets of ICA and GADA with variable prognostic significance can be identified (269). It seems, however, that as many as 42-55% of GADA-positive first-degree relatives of children with T1D develop signs of clinical diabetes within 5-8 years (140; 141). High ICA titers (>80JDFU) have been inversely associated with age, and persistently high ICA titers are particularly predictive of T1D (104; 142). IA-2A have been reported as being highly specific predictors of the development of T1D, as they seem to appear at a relatively late phase of the preclinical disease (169; 172; 173). A combination of high-titer IA-2A and IAA has also been found to confer a high risk of progression to T1D among first-degree relatives (270), while GADA did not increase the risk any further. IA-2A have also been associated with rapidly progressive T1D presenting at a young age (104; 116). The DiMe study demonstrated that the higher the levels of ICA, IAA and IA-2A, the higher the risk for T1D, whereas GADA titers did not add to the risk (140). The presence of multiple autoantibodies is highly predictive in family members of affected patients as compared with single antibody positivity, the risk of T1D rising from less than 10% when ICA are detected alone to more than 80% when at least three antibodies are observed (104; 116; 141; 271). It has also been suggested, however, that screening of relatives of patients with T1D for GADA, IA-2A and IAA provides sufficient information, so that screening for all four disease-associated autoantibodies (ICA,GADA, IA-2A and IAA) is unnecessary, as the detection of two or more autoantibodies has been reported to have a positive predictive value exceeding 90% in some series (141). Age also seems to be a factor modifying the risk of T1D in ICA-positive relatives, with the highest risk found in children below the age of 10 years (97).

The intravenous glucose tolerance test (IVGTT, see Methods) is an efficient method for determining the degree of beta-cell dysfunction, and a reduced first-phase insulin response (FPIR see, Methods) has been shown to be highly predictive of progression to T1D in ICA-positive first-degree relatives (272). Prediction of the time to diagnosis by estimating beta-cell loss on the basis of FPIR does not seem to be feasible, however (273; 274). The combination of two positive ICA results and two reduced FPIRs has been shown to be a reliable tool for identifying future progressors to T1D among children and adolescents (275-277), but although the loss of FPIR is associated with a high risk of overt diabetes, this risk is considerably modulated by other risk markers such as the levels of ICA and IAA (278). The reduction in FPIR is a late phenomenon in the T1D disease process, and accordingly there is only a limited time for possible intervention after its observation. A French study has shown the sensitivity of two or more low insulin responses to intravenous glucose to be 60% and the specificity 96% in family members of children with T1D, while the positive predictive value was 43% (279).

It is 5-10 times more probable that a monozygotic twin will present with T1D than any other type of sibling, although siblings in general have a greater risk of progression than the general population (127; 132; 280). In an American study there was a risk of progression to clinical T1D of 20% for an initially non-diabetic monozygotic twin within 10 years of diagnosis in the index case and 29% within 25 years, while British data provided slightly lower frequencies, with a risk of 19% within 10 years of diagnosis in the index case and 27% within 25 years (281).

The risk of HLA-identical siblings developing T1D has been observed to be 10-30% by the age of 20-30 years (56; 282; 283), and it has been reported that the DR or DQ genotype in the family may confer an even greater risk than the degree of HLA identity (85; 284). Though the predictive value of genetic factors alone is relatively low, the combination of ICA screening with genetic factors improves the prediction of T1D in first-degree relatives, since more than 30% of ICA-positive HLA-identical siblings were observed to progress to T1D within 5 years compared with 7% of those who were ICA positive but haploidentical or non-identical (132). In France, ICA-positive, DR3/4 heterozygous siblings had a cumulative risk of 70% of developing T1D within 8 years (85). By contrast, the DQB1*0602 allele is protective in first-degree relatives testing positive for ICA (285), although the protective effect is overcome by high antibody levels (142).

3.3.2 The general population

As mentioned earlier, close to 90% of all patients with T1D have no family history of the disease at diagnosis, and accordingly it is apparent that successful intervention is needed at the population level to achieve a substantial reduction in the incidence of T1D (13; 48; 286). As our knowledge of risk assessment in first-degree relatives of children with T1D is increasing, especially in siblings, the challenge now is to try to identify future diabetic patients in the general population. When assessing the predictive value of ICA positivity among children with no familial history of T1D in relation to the risk of progression to diabetes within 10 years, it became evident that ICA are only 2-3 times more prevalent in siblings than in the general population (287). This makes ICA positivity a quite weak marker for future T1D in the general population, since T1D is more than 10 times more frequent in siblings than in the background population. Also, the reported proportions of initially ICA-positive subjects becoming ICA-negative during follow-up have been relatively high in the general population, varying from 14% to 78% in different studies (287-291). Another survey has indicated, however, that ICA-positive schoolchildren with levels of 10 JDFU or more have a risk of progressing to T1D that is similar to that seen among first-degree relatives testing positive for ICA (113). Only a small proportion of children in the general population with signs of an immune response to islet cells will progress to T1D (287). The following ICA frequencies have been observed in various countries: 4.1% in Finland, 3.0% in Sweden, 2.8% in England, 1.5% in France and 1.7% in the State of Washington, USA (39; 98; 115; 282; 287), suggesting that there is a correlation between the frequency of ICA in the background population and the incidence of T1D. There are two studies that have shown a positive association between ICA and the frequency of high risk HLA-DQB1 genotypes in the population (39; 280). On the other hand, DQB1 alleles conferring decreased disease susceptibility do not provide protection from humoral beta-cell autoimmunity, deterioration of beta-cell function or progression to overt T1D in initially unaffected schoolchildren (292). The appearance of autoantibodies over the first 2 years of life in 1005 children with increased *HLA DQB1*-conferred susceptibility to T1D is illustrated in Figure 5 (17). Early screening for HLA-conferred susceptibility to T1D in the general population followed by consecutive screening of the genetically susceptible individuals for diabetes-predictive autoantibodies might provide a

feasible strategy for the identification of high-risk subjects in the population. This is the approach taken in the Finnish DIPP Study (293). In a recent survey of 11,840 schoolchildren in Germany who were tested for all four diabetes-associated autoantibodies, 821 (6.9%) children were positive for a single autoantibody, whereas 83 (0.7%) had multiple autoantibody specificities. If the primary screening were performed by testing GADA/IA-2A/IAA, 94% of probands with single antibodies and all those with multiple antibodies would have been identified. The combination of GADA/IA-2A identified 97.6% of the probands at risk, GADA/IAA 98.8% and IA-2A/IAA as many as 85.5% (294). In another recent study of 4505 healthy schoolchildren, six subjects developed diabetes, all from among the 12 individuals with multiple disease-associated autoantibodies, representing a positive predictive value of 50% (95% CI 25–75%) and a sensitivity of 100% (58–100%) (295). Another study, however, showed that screening for ICA and IAA failed to identify a proportion of the genetically susceptible children who subsequently developed islet antibodies. Testing for GADA and IA-2A would not have avoided this. Maximizing the sensitivity of detecting the risk of type T1D therefore requires repeated screening for islet antibodies throughout childhood (296). Assessing the risk of developing T1D by screening genetically predisposed cohorts for disease-associated autoantibody markers provides the best possible risk estimation for the time being, but it is still far from being a cast-iron model. We do not yet know unambiguously at what age screening should be started, exactly how children should be screened and how often the screening should be repeated (297). It will be necessary in the near future, however, hopefully with a preventive modality available, to have a functional risk assessment model available to target the most appropriate subjects for intervention.

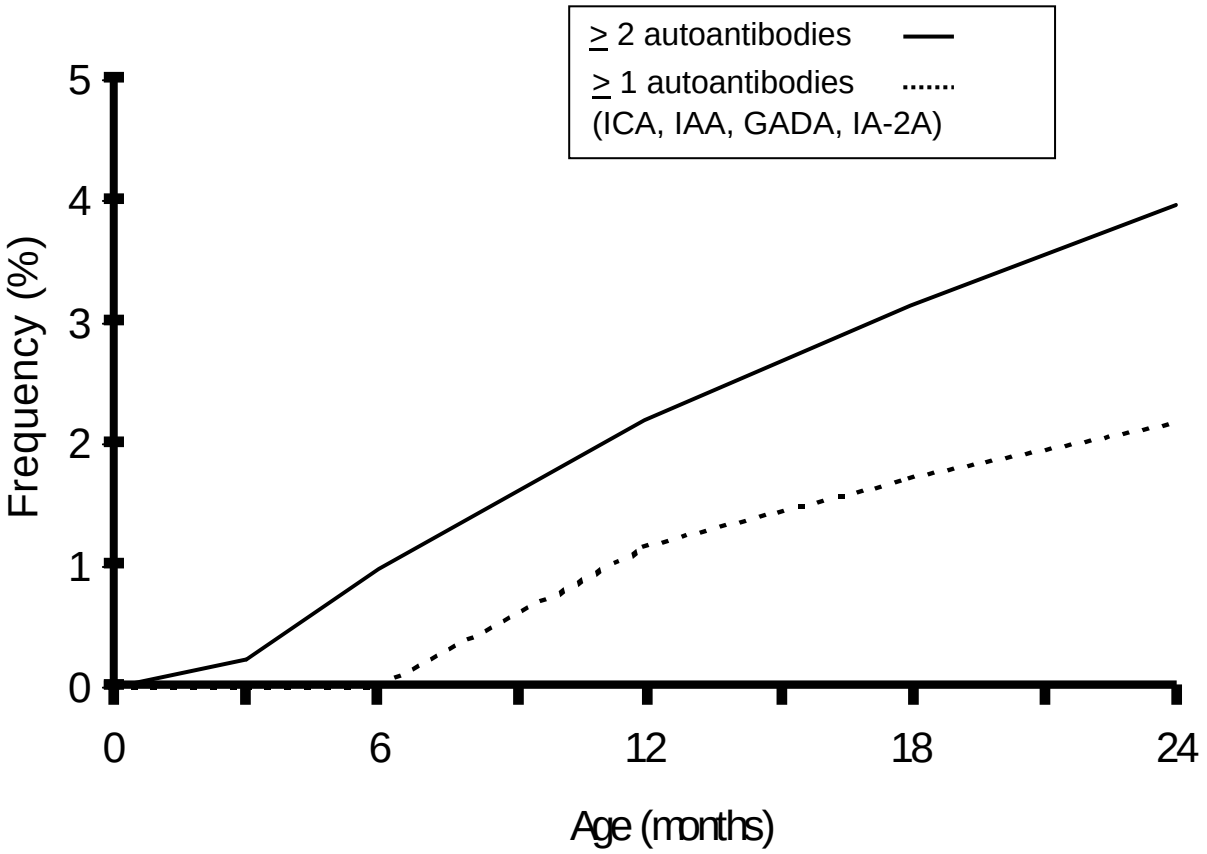

Figure 5. The appearance of beta-cell autoimmunity in the general population in Finland during the first 2 years of life in 1005 DIPP children with increased *HLA DQB1*-conferred susceptibility to T1D [modified from the original (17)].

3.4 Prevention of type 1 diabetes

Increased understanding of the long prodromal period preceding the clinical symptoms of T1D provides a window for trials aimed at preventing or delaying manifestation of the clinical disease. There are three types of prevention: primary, secondary and tertiary. The main goal of primary prevention is to reduce the incidence of T1D by reducing the risk of the disease through the identification and modification of environmental factors that mediate beta-cell destruction. This intervention type focuses on individuals without any signs of beta-cell damage. The purpose of secondary prevention is to reduce the incidence by interrupting or slowing down the process of beta-cell destruction. The target for this kind of prevention is subjects with signs of ongoing beta-cell damage. Finally, tertiary prevention may be initiated after the manifestation of clinical T1D to preserve and facilitate the residual beta-cell function or prevent the development of secondary complications (286; 298-300).

Many factors have to be considered when choosing subjects for prevention trials. Sex is an important factor, if the intervention modality is based on the use of a drug with potential teratogenic effects, in which case women of fertile age should be excluded. Young antibody-positive subjects have been observed to have a greater risk of progressing to T1D (278), but when studying young cohorts with aggressive beta-cell destruction it should be taken into account that weak delaying effects may be missed. All prevention trials in progress, with the exception of the DIPP Study, include first-degree relatives, since their risk of progression to T1D is more than 10 times higher than that in the general population (56). The major clinical target for preventive measures should nevertheless be the general population, since almost 90% of patients with newly diagnosed T1D have no affected family member. It may be possible to identify subjects from the general population with a risk similar to that observed in family members by screening for genetic susceptibility followed by monitoring for the appearance of diabetes-predictive autoantibodies (286).

The international Trial to Reduce T1D in the Genetically at Risk (TRIGR) project is a double-blind, randomised trial aimed at assessing whether weaning of infants with an increased risk of T1D to a highly hydrolyzed formula is capable of reducing the cumulative incidence of diabetes-associated autoantibodies and/or clinical T1D by the age of 6 years and the cumulative incidence of overt T1D by the age of 10 years. In the Finnish pilot study 242 newborn infants who had a first-degree relative with T1D and carried risk-associated HLA-DQB1 alleles [HLA-DQB1*0302 and /or *0201 and negative for the protective alleles (*0602-03, *0301)] received either casein hydrolysate or conventional cow's milk-based formula up to the age of 6–8 months, after exclusive breastfeeding. The data on the emergence of autoantibodies by the age of 4.6 years provided the first evidence ever in man that it may be possible to manipulate beta-cell autoimmunity in a safe way, by dietary intervention in infancy. Based on these encouraging experiences, the trial proper was initiated in 2002 to provide a final answer to the question of whether weaning to a highly hydrolyzed formula protects infants from the emergence of beta-cell autoimmunity and from progression to clinical diabetes.

The idea of using nicotinamide in prevention trials with prediabetic subjects was based on the observation that it prevents or reduces the disease incidence in animal models of

autoimmune diabetes, and that it prolongs remission and preserves beta-cell function in human T1D (301; 302). Nicotinamide could potentially protect the beta cell by inhibiting poly(ADP)ribose polymerase (PARP) and by repleting intracellular stores of nicotinamide adenine dinucleotide (NAD), which prevents cellular damage. Nicotinamide has also been reported to inhibit cytokine-induced NO production in the islets and cytokine-induced HLA class II expression on cultured cells. The European Nicotinamide Diabetes Intervention Trial (ENDIT) screened more than 40,000 relatives, randomizing 552 to either nicotinamide or a placebo. Unfortunately the 5-year follow-up showed that there was no significant difference in the rate of progression to clinical diabetes between the two groups. Accordingly the European Nicotinamide Diabetes Intervention Trial (ENDIT) failed to confirm any protective effect of nicotinamide on the development of human T1D.

Parenteral or oral insulin treatment is based on the hypothesis that antigen-specific therapy, e.g. low-dose insulin treatment, may influence beta-cell specific autoimmunity by activating suppressor or regulatory T cells and by shifting the balance from a Th1-mediated immune response to a Th2-biased one. Evidence supporting this concept has been obtained from animal studies, where oral or parenteral insulin therapy prevented diabetes, reduced the severity of insulitis and was associated with an increased Th2 response (303; 304). The Schwabing Insulin Prophylaxis Trial was a randomized, controlled pilot study designed to examine whether insulin therapy can delay or prevent the clinical manifestation of T1D in high-risk first-degree relatives of patients with T1D. The eligible first-degree relatives screened ICA-positive and had a reduced IVGTT and a normal OGTT upon inclusion. The German data suggested that insulin prophylaxis may delay the onset of overt diabetes in high-risk relatives (305). The American Diabetes Prevention Trial - Type 1 Diabetes (DPT-1) initiated in 1996 included two approaches: parenteral administration of insulin to first and second-degree relatives with a T1D risk of more than 50%, and oral administration of insulin to those with a risk of 25-50% . The parenteral and oral insulin did not delay or prevent T1D, although a borderline significant protective effect of oral insulin was observed in a subset of the oral administration subjects with high initial IAA levels (306; 307). In the ongoing intervention part of the Finnish DIPP study, genetically susceptible children identified from the general population who test positive for at least two T1D-associated antibodies in two consecutive samples are being invited to take part in a randomized controlled trial evaluating the possible preventive effect of intranasally administered insulin. In the DIPP project as a whole more than 90,000 consecutive newborn infants from the general population have been screened for HLA-conferred susceptibility to T1D at birth, and more than 9,000 children carrying high and moderate-risk HLA-DQB1 genotypes continue to be followed up at 3–12 month intervals (308). The DIPP intervention differs from previous prevention trials in two aspects: (i) the target group represents the general population; and (ii) the treatment is started as soon as possible after the appearance of at least two diabetes-associated autoantibodies.

3.5 Prospects for treatment of type 1 diabetes

Normoglycemic control is not easily achieved in patients without any endogenous insulin secretion. Accordingly, the only means of keeping such patients in good metabolic control without typical organ complications of T1D involves replacement of the pancreatic beta cells either with an artificial pancreas or with glucose-responsive insulin-producing tissue that is preferentially resistant to any subsequent immune attack.

Islet transplantation aims at physical replacement of the damaged beta cells in the pancreas of patients with T1D. This idea was originally introduced in the early 1980's (309) Islet cell transplants can now be performed with greater chances of success than just a few years ago (310), but even so, it needs continuous treatment with immunosuppressive drugs to protect the islets from recurrent autoimmune responses and allorejection. This may cause problems such as mouth ulcers, diarrhea and acne with time and entail longer-term risks including malignancy and serious infections, elevation of serum cholesterol and blood pressure, and in isolated cases a decline in renal function and acute retinal bleeding (311).

There have also been attempts to construct glucose-sensing, insulin-secreting non-islet cells (surrogate beta cells) as replacement tissue. Surrogate cells would optimally remain invisible to autoimmune reactivity (312) . Adult stem cell-derived, in vitro-generated islets may one day be an alternative to cadaver islets for treating diabetic patients. Embryonic stem cells are pluripotent cell lines derived from the inner cell mass of blastocyst-stage embryos, and their differentiation in culture may reproduce the characteristics of early embryonic development (313). New data suggest that embryonic stem cells may have a potential for differentiating into endocrine pancreas cells (314).

4 AIMS OF THE RESEARCH

The objectives of this work were:

1. to assess whether it is clinically relevant to classify individuals with signs of beta-cell autoimmunity into various stages of preclinical T1D;

2. to assess whether genetic modification of this classification enhances its potential;

3. to evaluate the progression of preclinical T1D in siblings of affected children; and

4. to establish a predictive model for T1D integrating sociodemographic, genetic, immunological and metabolic markers and to test its utility for the prediction of T1D in siblings of affected children.

5 SUBJECTS AND METHODS

5.1 Subjects

The population was derived from the nationwide "Childhood Diabetes in Finland" (DiMe) Study, in which children with T1D under the age of 15 years and their families, were invited to participate between September 1986 and April 1989. The aim was to evaluate the genetic, immunological and environmental factors leading to the development of T1D (315). The follow-up was prospective and observation of the siblings was initiated shortly after the proband was diagnosed as having T1D. Blood samples were taken at intervals of 3-6 months during the first 2 years and 6-12 months during the following 2 years. If the sibling was found to test negative for ICA and IAA on all occasions over the first 4 years, antibody surveillance was discontinued. Siblings positive for ICA and/or IAA on at least one occasion over the initial 4 years were subsequently observed at an interval of 12 months or less. Such siblings were also invited for sequential intravenous glucose tolerance tests (IVGTTs) at an interval of 6-12 months starting from the time when antibodies were detected for the first time. All the siblings were observed for progression to T1D up to the end of 2002. Observation of the siblings progressing to T1D ended at diagnosis, which was based on clinical symptoms and an increased random blood glucose concentration (>10 mmol/l), elevated fasting glucose concentration (>6.7mmol/l), or random blood glucose on two occasions in the absence of symptoms (316).

The 801 index cases (440 males, 54.9%) in the DiMe study had a mean age of 8.4 years at the time of diagnosis (range 0.8-14.9 years), and the mean age of the 83 cases (35 males, 42.2%) who underwent at least one IVGTT was 9.7 years (range 2.1-19.7 years) at the time of the diagnosis of the index case and 11.2 years (range 3.2-20.0 years) at the time of the first IVGTT. Siblings testing negative for all autoantibodies were assumed in the subsequent classifications to have a normal FPIR. Further data are introduced in Table 6. Autoantibody and genetic susceptibility data from the DiMe Study have been published previously (131; 140; 161; 172; 317).

Table 6. Descriptive data.

		N	Males (%)	Mean age at initial sampling (years, range)	Data included
Substudy I	A	758	351 (46.3%)	9.9 (0.8-19.7)	Autoantibody
Substudy I	B	712	323 (44.5%)	9.9 (0.8-19.7)	Autoantibody and metabolic
Substudy II	A	715	325 (45.5%)	9.9 (1.3-20.4)[a]	Autoantibody
Substudy II	B	641	285 (44.5%)	10.0 (1.3-20.4)[b]	Autoantibody and metabolic
Substudies III,IV	A	701	324 (46.2%)	9.9 (0.8-19.7)	Autoantibody
Substudy III	B	659	298 (45.2%)	9.9 (0.8-19.7)	Autoantibody and metabolic

Mean age at the final sampling (years, range):
[a] 13.2 (1.5-24.1)
[b] 13.2 (3.0-24.1)

5.2 Methods

5.2.1 Islet cell antibodies

The presence of ICA was determined by a standard immunofluorescence assay performed on sections of frozen human pancreas from a blood group O donor (105) using fluorescein-conjugated anti-human IgG (Sigma, St. Louis, MO, USA). End-point dilution titers were identified and the results were expressed in Juvenile Diabetes Foundation units (JDFU) relative to an international reference standard (318). The detection limit was 2.5 JDFU. The sensitivity of the ICA assay was 100% and the specificity 98% in the most relevant international standardization round (319).

5.2.2 Insulin autoantibodies

IAA were analyzed by a modification (12) of the liquid phase radioimmunoassay described by Palmer et al. (133). The samples were treated with acid charcoal to remove insulin prior to the assay. 80 µl of serum was incubated for 20h with mono^{125}I(TyrA14)-human insulin (Novo Research Institute, Bagsvaerd, Denmark) and the free and bound insulin fractions were separated using polyethylene glycol. The results were expressed in nU/ml, where 1 nU/ml corresponds to a specific binding of 0.01%. The interassay coefficient of variation was less than 8%. If the specific insulin binding exceeded 54 nU/ml (representing the 99th percentile in 105 non-diabetic subjects), the subject was considered IAA-positive. The sensitivity of the IAA assay was 78% and the specificity 100% in the first proficiency testing program.

5.2.3 Glutamic acid decarboxylase (GAD65) antibodies

An immunoprecipitation radioligand assay was used to detect GADA (161; 320). The labeled GAD65 antigen was obtained from recombinant human islet GAD65 cDNA that had been transcribed and translated according to the manufacturer's instructions (Promega, Madison, WI, USA) in the presence of [^{35}S]methionine (Amersham, Amersham, Bucks, UK). Sera (2 µl) were incubated overnight at +4 ^{0}C with approximately 30,000 cpm human GAD65 in a total volume of 50 µl Tris-buffered saline with Tween (TBST). To isolate the immunocomplexes, 25 µl Protein A-Sepharose®CL-4B (Pharmacia, Uppsala, Sweden) in a total volume of 100 µlTBST was added. A scintillation counter was used to count the amount of immunocomplexes precipitated. All the samples were analyzed in quadruplicate with and without competition from excess amounts of unlabeled recombinant GAD 65 (1µg/well) produced in an expression system using baby hamster kidney cells and purified with Triton X-114 (161). The results were expressed in relative units (RU), representing the specific binding as a percentage of that obtained with a positive standard serum. One relative GADA unit = 100 × [{cpm (unknown sample) - cpm (unknown sample incubated with an excess of unlabeled GAD65)} / {cpm(positive standard serum) - cpm(positive

standard serum incubated with an excess of unlabeled GAD65)}]. The limit for GADA positivity was set at 6.5 RU, which represents the 99th percentile in a series of 372 healthy control subjects (321). The disease sensitivity of the GADA assay was 79% and the specificity 97% based on the 1995 Multiple Autoantibody Workshop (322)

5.2.4 IA-2 antibodies

A radiobinding assay modified from that described by Bonifacio et al. (171) was used to analyze IA-2A (172). The recombinant plasmid pSP64poly(A) encoding the intracellular portion of the full-length IA-2 protein, including amino acids 605-979 (provided by E. Bonifacio, Milan, Italy), was transformed in Esterichia coli JM109 cells and purified by standard techniques. The purified plasmid was then transcribed and translated in the TNT Coupled Reticulocyte Lysate System (Promega) in the presence of [^{35}S]methionine according to the manufacturer's instructions, to produce the radioactive IA-2 protein. Sera (2 µl) were incubated overnight at +4 ^{0}C in 96 deep well plates with 10,000 cpm of labeled IA-2 protein diluted in 50 µl of 50 mM Tris and 150 mM NaCl (pH 7.4) containing 0.1% Tween 20 (TBST). To isolate the immune complexes, 5 µl Protein-A Sepharose® CL-4B (Pharmacia) in a total volume of 50 µl TBST was added on the following day. After thorough washing, the radioactivity of the samples was measured with a liquid scintillation counter (1450 Microbeta® Trilux, Wallac, Turku Finland) after adding 10 µl of scintillation fluid (OptiPhase Supermix, Wallac). Each plate contained a dilution series of a pool of two local positive sera diluted in a pool of two local negative sera. A standard curve was constructed on each plate using the results of the dilution series (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:512 and the pool of the two negative sera), the dilutions being assigned arbitrary values of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.1 relative units (RU), respectively. A subject was considered IA-2A positive, if the serum antibody levels exceeded 0.429 RU, which represents the 99th percentile in 374 healthy Finnish children and adolescents. The disease sensitivity of this assay was 62% and the specificity 97% based on 140 samples included in the 1995 Multiple Autoantibody Workshop (322).

5.2.5 Intravenous glucose tolerance test

The siblings participating in an IVGTT were given a glucose dose of 0.5 g/kg in 3 min ($\pm$ 15 s) after fasting overnight for 10-16 h. Blood samples were taken before the glucose infusion and at 1, 3, 6, 10, 20, 30, 40, 50 and 60 min thereafter. Serum insulin concentrations were measured radioimmunologically (323) and blood glucose levels by the glucose oxidase method (324). The sum of the insulin concentrations at 1 and 3 min was defined as the FPIR to glucose. To evaluate the degree of glucose tolerance, the glucose disappearance rate (K_g) was assessed and expressed as the percentage decrease in blood glucose per minute (%/min). FPIR levels below 45 mU/L, which represents the 3rd percentile of FPIR values in healthy control subjects (272), after adjustment for the insulin assay used based on an exchange of serum insulin samples between Oulu and Boston, and K_g values below 1.30%/min were considered to be abnormal.

5.2.6 Homeostasis model assessment of insulin resistance (HOMA-IR)

The HOMA-IR index was calculated to estimate insulin sensitivity. The index was based on the formula HOMA-IR = fasting glucose (mmol/L) x fasting insulin (mU/L) / 22.5, as described previously (325; 326). Insulin resistance was related to insulin secretion by calculating the HOMA-IR/FPIR ratio.

5.2.7 Genotyping

HLA-A, B, C and DR typing was performed by conventional HLA serology as described by Tuomilehto-Wolf et al. (327). The HLA-conferred disease susceptibility was graded in four categories: no risk, HLA nonDR3/nonDR4; low risk, HLA DR 3/nonDR4; moderate risk, HLA DR4/nonDR3, and high risk, HLA DR3/DR4.

HLA-DQB1 typing was performed by a previously described method based on time-resolved fluorescence (328). Four sequence-specific oligonucleotide probes were used to identify the following DQB1 alleles known to be associated with either susceptibility to or protection from T1D in the Finnish population: DQB1*0302, DQB1*02, DQB1*0602 or *0603, and DQB1*0301 (317). The genotype was classified into one of four categories: high-risk genotype (DQB1*02/0302), moderate-risk genotypes (DQB1*0302/x, where x stands for 0302 or a non-defined allele), low-risk genotypes (DQB1*0301/0302, DQB1*02/0301, DQB1*02/x, DQB1*0302/0602-3, where x stands for 02 or a non-defined allele), and genotypes conferring decreased risk (DQB1*x/x, DQB1*0301/x, DQB1*02/0602-3, DQB1*0301/0602-3, where x stands for a non-defined allele).

5.2.8 Data handling and statistical analyses

Cross-tabulation and chi-square (x^2) statistics were used to analyze distributions and frequencies. The parametric one-way analysis of variance was employed when normally distributed variables were compared between groups, and the non-parametric Kruskall-Wallis analysis of variance when analyzing variables with a skewed distribution. The t-test was employed for comparisons between two groups in the case of normally distributed variables and the Mann-Whitney U-test in the case of variables with skewed distributions. Correlation analyses were performed with a non-parametric test (Spearman) due to the skewed distribution of the variables tested. The 95% confidence intervals (CI) were calculated by the exact method. Cox regression analysis was used to identify factors associated with the risk of progression to overt T1D, whereas multiple linear regression analysis was employed for the estimation of variables potentially related to the age at diagnosis. The data for the analysis of the multivariate model in the case of the total series of 701 siblings initially included the following potential predictors: age at first sampling, sex, HLA-conferred disease susceptibility (two or four categories), degree of HLA identity with the index case, autoantibody positivity and titers (ICA, IAA, GADA and IA-2A), age at diagnosis and sex of the index case, number of children in the family, and number of first-degree relatives affected by T1D. In the smaller series comprising 77 autoantibody-

positive siblings who had undergone an IVGTT, the FPIR, K_g, HOMA-IR and HOMA-IR/FPIR ratio (natural logarithm-transformed due to skewed distribution) were also included in the analyses. The Cox regression analyses were performed with the STATA statistical software package, version 8.0 (STATA corporation, College Station, TX, USA), and the other statistical tests with the SPSS 11 software (SPSS, Inc., Chicago, IL, USA).

6 RESULTS

6.1 Classifications (I, II, III)

The siblings were classified into four stages of preclinical T1D (no prediabetes, early, advanced or late prediabetes) according to two sets of criteria (Table 7). **Classification 1** was based on the number of antibodies detectable in the first sample available. In most of the 758 siblings this sample was obtained within 3 weeks of the diagnosis of T1D in the index case. The first group (no prediabetes) included the siblings who tested negative for all four antibodies analyzed, ICA, IAA, GADA or IA-2A, the second group (early prediabetes) those who had only one of the antibody specificities, the third group (advanced prediabetes) those with two antibody specificities, and the fourth group (late prediabetes) those with at least three.

Classification 2 was based on a combination of autoantibodies and FPIR. The siblings with no antibodies were still placed in the first group (no prediabetes), those with one antibody specificity detectable but a normal FPIR comprised the second group (early prediabetes), those with two or more antibodies but still with a normal FPIR the third group (advanced prediabetes), and those with an abnormal FPIR and at least one antibody specificity the fourth group (late prediabetes). Initially antibody-positive siblings with no available FPIR were excluded from this classification, leaving 712 siblings in the analysis.

Similar criteria were used when the siblings were classified at the end of the follow-up in substudy II.

Classification 1.

No prediabetes	AB = 0
Early prediabetes	AB = 1
Advanced prediabetes	AB = 2
Late prediabetes	AB $\geq$ 3

Classification 2.

No prediabetes	AB = 0, normal FPIR
Early prediabetes	AB = 1, normal FPIR
Advanced prediabetes	AB $\geq$ 2, normal FPIR
Late prediabetes	AB $\geq$ 1, decreased FPIR

Table 7. Classifications used for risk assessment in siblings of children with type 1 diabetes. (AB= autoantibodies, FPIR= first-phase insulin response).

6.2 Prediction of of type 1 diabetes in siblings of affected children (I, II, III, IV)

Among the 758 siblings, 35 (4.6%) progressed to clinical T1D during prospective observation for an average of 9 years (range 0.02-10.3 years), i.e. by the end of 1997, leaving 723 (95.4%) unaffected. Only six sibs in the group with no signs of prediabetes initially (0.9%; CI 0.3-2.0%) developed T1D, as compared with three (6.1%; CI 1.3-16.9%) in the early prediabetes category, three (23.1%; CI 5.0-53.8%) in the advanced prediabetes category and 23 (65.7%; CI 47.8-80.9%) among those with late prediabetes.

Six of the 661 subjects among the 712 with available metabolic data who were classified as having no prediabetes (0.9%; CI 0.3-2.0%) progressed to T1D, one of those with early prediabetes (6.7%; CI 0.2-32.0%), six (26.1%; CI 10.2-48.4%) with advanced prediabetes and 12 (92.3%; CI 64.0-99.8%) with late prediabetes. Thus there were altogether 25 (3.5%) siblings who manifested clinical signs of T1D during the observation period, i.e. by the end of 1997.

Among the 712 siblings with available genetic data, the OR for HLA-identical siblings presenting with any sign of prediabetes relative to haploidentical or non-identical siblings was 1.9 (CI 1.2-3.1) and that for presenting with late prediabetes was 4.1 (CI 2.0-8.5). The HLA-identical siblings with late prediabetes (n=18) had an OR of 775 for clinical diabetes by comparison with the haploidentical or non-identical siblings without any signs of prediabetes (n=468). Siblings who were heterozygous for DR 3/4 had a five-fold risk (CI 2.3-10.9) of presenting with any stage of prediabetes and a 25-fold risk (CI 5.3-11.8) of presenting with late prediabetes as compared with siblings having the non-DR3/non-DR4 combination. The DR3/4-heterozygous siblings with late prediabetes initially (n=10) had an OR of 1809 for clinical diabetes as compared with those having the non-DR3/non-DR4 combination and no initial signs of prediabetes (n=201). Similar results were also obtained when comparing the DQB1 genotypes, in that siblings with the high risk DQB1 genotype had an OR of 90.5 (CI 11.7-703) for presenting with any signs of prediabetes and an OR of 43.0 (CI 5.3-348) for presenting with late prediabetes as compared with siblings carrying genotypes conferring decreased risk. The risk of progression to overt T1D in the siblings with the high risk genotype and late prediabetes (n=9) was 1773 relative to the siblings with genotypes associated with decreased genetic predisposition and without signs of beta-cell autoimmunity (Table 8).

The HLA-identical siblings among the 659 with available genetic and metabolic data had a 2.2-fold risk (CI 1.2-4.1) of presenting with any signs of prediabetes as compared with the haploidentical or non-identical siblings and an OR of 4.4 (CI 1.4-14.1) for presenting with late prediabetes. The HLA-identical siblings with late prediabetes (n=7) had a 930-fold risk of clinical T1D relative to the haploidentical or non-identical siblings with no signs of prediabetes (n=468), and the DR3/4-heterozygous siblings had 7.6-fold risk (CI 2.7-21.1) of presenting with any signs of prediabetes and a 23-fold risk (CI 2.7-205) of presenting with late prediabetes relative to the non-DR3/non-DR4 combination. The risk of progression to T1D was also increased to 804 among the DR3/4-heterozygous siblings with late prediabetes (n=5) as compared with those with non-DR3/non-DR4 combinations and no initial signs of prediabetes. Similar risk ratios were observed when looking at DQB1

genotypes. Siblings carrying the high risk DQ genotype had a 5.7-fold risk (CI 2.3-14.4) of presenting with any signs of beta-cell autoimmunity and an 18-fold risk (CI 2.0-169) of manifesting late prediabetes relative to those with genotypes conferring decreased risk. The risk of progression to overt T1D was 591-fold for siblings with the high-risk genotype and late prediabetes relative to those with genotypes conferring decreased risk and with no initial signs of prediabetes (Table 9).

The risk of developing T1D in the total series of 701 siblings, followed up to the end of 2002, was associated with age at first sampling, HLA DR-conferred disease susceptibility, number of initially detectable diabetes-associated autoantibodies and number of affected family members. Among the 77 autoantibody-positive siblings with metabolic data available, the age of the sibling, HLA DR-conferred disease susceptibility, number of disease-associated autoantibodies, FPIR and HOMA-R/FPIR ratio proved to be significant predictors of progression to T1D.

Table 8. Odds ratios (OR) for clinical diabetes in relation to the combination of genetic risk (HGR= high genetic risk, DGR= decreased genetic risk) and stage of preclinical diabetes based on autoantibodies.

	OR (CI)		n	Type 1 diabetes
HLA IDENTITY:				
No prediabetes + DGR	1		468	3
Early prediabetes +HGR	25.8	(2.3-285)	7	1
Advanced prediabetes + HGR	155	(25.8-930)	8	4
Late prediabetes + HGR	775	(122-4162)	18	15
HLA DR:				
No prediabetes + DGR	1		201	0
Early prediabetes + HGR	-		2	0
Advanced prediabetes + HGR	100	(4.5-2236)	3	1
Late prediabetes + HGR	1809	(105-31310)	10	9
HLA DQB1:				
No prediabetes + DGR	1		197	0
Early prediabetes + HGR	-		2	0
Advanced prediabetes + HGR	131	(9.2-1873)	5	2
Late prediabetes + HGR	1773	(102-30609)	9	9

HLA IDENTITY: HGR= HLA-identical, DGR= HLA-haploidentical or non-identical
HLA-DR: HGR= DR 3/4, DGR= non-DR3/non-DR4
HLA DQB1: HGR= high risk DQB1 genotype, DGR= DQB1 genotypes conferring decreased risk

Table 9. Odds ratios (OR) for clinical diabetes in relation to the combination of genetic risk (HGR= high genetic risk, DGR= decreased genetic risk) and stage of preclinical diabetes based on autoantibodies and FPIR.

	OR (CI)		n	Type 1 Diabetes
HLA IDENTITY:				
No prediabetes + DGR	1		468	3
Early prediabetes +HGR	155	(7.8-3099)	2	1
Advanced prediabetes + HGR	155	(28.9-832)	10	5
Late prediabetes + HGR	930	(84.2-10274)	7	6
HLA-DR:				
No prediabetes + DGR	1		201	0
Early prediabetes + HGR	-		1	0
Advanced prediabetes + HGR	603	(30.1-12077)	4	3
Late prediabetes + HGR	804	(42.4-15258)	5	4
HLA DQB1:				
No prediabetes + DGR	1		197	0
Early prediabetes + HGR	-		1	0
Advanced prediabetes + HGR	985	(53.6-18091)	6	5
Late prediabetes + HGR	591	(29.5-11837)	4	3

HLA Identity: HGR= HLA-identical, DGR= HLA-haploidentical or non-identical
HLA-DR: HGR= DR 3/4, DGR= non-DR3/non-DR4
HLA DQB1: HGR= high risk DQB1 genotype, DGR= DQB1 genotypes conferring decreased risk

6.3 Natural course of the prediabetic period (II, III)

Among the 715 siblings with at least two samples available for **classification 1**, 620 (86.7%) had no prediabetes, 47 (6.6%) early prediabetes, 13 (1.8%) advanced prediabetes and 35 (4.9%) late prediabetes according to the first sample, i.e. a total of 95 (13.3%) siblings had signs of prediabetes. Among the siblings with no initial signs of prediabetes, 585 (94.4%) remained unaffected throughout the follow-up, while 35 (5.6%) tested antibody-positive in the last available sample. Out of these 26 (4.2%) had progressed to early prediabetes, one (0.2%) to advanced prediabetes and one (0.2%) to late prediabetes, while seven (1.1%) developed signs of clinical diabetes. Among the 47 siblings with initial signs of early prediabetes, 32 (68.1%) had reverted to no prediabetes by the end of the observation period, nine (19.1%) had remained in the early prediabetes phase, and three (6.4%) had progressed to late prediabetes, while three (6.4%) had presented with clinical T1D. Correspondingly, one of the 13 siblings initially classified as having advanced prediabetes, (7.7%) had seroconverted to antibody negativity by the end of the follow-up, seven (53.8%) had retained their stage, one (7.7%) regressed to early prediabetes, and four (30.8%) had progressed to clinical T1D, while of the 35 siblings initially having late prediabetes, 10 (28.6%) remained at that stage, one (2.9%) regressed to advanced prediabetes and 24 (68.6%) progressed to clinical T1D (Fig. 6). Altogether 26 (27.4%; CI 18.7-37.5%) out of the 95 antibody-positive siblings at the initial sampling remained in the same category of prediabetes at the final sampling, while 35 (36.8%; CI 27.2-47.4%) experienced regression of their prediabetic stage and 34 (35.8%; CI 26.2-46.3%) progressed (Fig. 6).

On the other hand, among the 641 siblings with at least two samples available for **classification 2**, 600 (93.6%) had no prediabetes, only six (0.9%) had early prediabetes, 22 (3.4%) had advanced prediabetes and 13 (2.0%) had late prediabetes according to the first sample. Of the siblings with no signs of prediabetes initially, 584 (97.3%) remained so during the follow-up, while 16 (2.5%) seroconverted to antibody positivity, nine (1.5%) progressing to early prediabetes, two (0.3%) to advanced prediabetes and five (0.8%) developing signs of clinical diabetes. Among the six siblings with early prediabetes initially, three (50.0%) regressed to no prediabetes, two (33.3%) remained with early prediabetes and one (16.7%) progressed to advanced prediabetes, so that none in this group presented with signs of clinical T1D. Of the 22 siblings classified as having advanced prediabetes initially, 12 (54.5%) remained at that stage, two (9.1%) progressed to late prediabetes and eight (36.4%) developed overt diabetes during the follow-up, while one of the 13 siblings with late prediabetes initially (7.7%) regressed to advanced prediabetes and the remaining 12 (92.3%) all progressed to clinical T1D. Altogether 14 (34.1%; CI 20.1-50.6%) out of the 41 siblings with signs of prediabetes at the initial sampling retained the same stage at the final sampling, four (9.8%; CI 2.7-23.1%) had regressed and as many as 23 (56.1%; CI 39.7-71.5%) had progressed, a larger proportion than in **classification 1** (difference 20.3%; CI 2.3-38.3%; $p = 0.04$), while the regression rate was also higher using **classification 2** (difference 27.1%; 13.8-40.4%; $p = 0.003$) (Fig. 7).

According to **classification 1** based on autoantibodies, no change occurred in the stage of prediabetes in the preclinical period in half of the 38 siblings who progressed to clinical disease. All but six of the progressors passed through the stage of late prediabetes. Among these six, four experienced the stage of advanced prediabetes before diagnosis, one had early prediabetes, testing positive for IAA only, and one had no signs of prediabetes in her first and only sample, taken 6.7 years before she presented with overt T1D. In the vast majority of the progressors who passed through the stage of late prediabetes (27/32; 84%) this was the stage that immediately preceded diagnosis, but there were five individuals in whom the number of autoantibodies decreased again before diagnosis. One of these remained positive for ICA, becoming negative for the other three antibody specificities, another became negative for all antibodies except IA-2A, while three initially triple-positive subjects became negative for one antibody (one for GADA, one for ICA and one for IAA), thus passing through the stage of advanced prediabetes before clinical disease.

Thirteen out of the 25 progressors experienced a change in their stage of prediabetes in the preclinical period according to **classification 2**, the combination of antibodies and FPIR. Nineteen siblings passed through the stage of late prediabetes, and in 16 cases this was the stage immediately preceding the diagnosis of clinical T1D. Three progressors had a reduced FPIR initially, but it subsequently increased in the preclinical period and exceeded the third percentile, so that they may be said to have regressed from late prediabetes to advanced prediabetes. Among the six progressors who did not experience late prediabetes before the diagnosis of clinical T1D, four had advanced prediabetes and one early prediabetes, while no signs of prediabetes had been observed in one progressor on initial sampling 6.7 years before the diagnosis. No further samples were available for that girl. Only two progressors passed through early prediabetes, with a median duration of 3.2 years (range 1.0-5.5 years), 16 experienced advanced prediabetes, with a duration ranging from 0.5 to 7.7 years (median 2.7 years), whereas the median duration of late prediabetes was 1.2 years (range 0.2-4.0 years) in the 19 progressors who went through that stage. There were no significant differences in duration between the stages.

Comparison of the 31 siblings who initially tested positive for one or more antibodies and subsequently presented with clinical T1D with the 64 antibody-positive siblings who have so far remained non-diabetic showed that those who developed overt disease were younger, had a higher number of autoantibodies and higher levels of ICA, GADA and IA-2A in their first sample and had a lower FPIR in their first IVGTT. There was substantial overlapping between the two groups for all the characteristics studied, however.

When assessing whether HLA-conferred disease susceptibility is related to the natural course of preclinical T1D, we observed that the higher the genetic risk, the more likely it was that a sibling among the 701 with genetic data available would progress from a milder stage to a more severe one. This held true for the degree of HLA identity, since about two thirds of the HLA-identical siblings progressed in terms of **classification 1**, whereas less than 20% of the HLA haploidentical or non-identical ones did so. About 40% of the siblings who were positive for DR3 and/or DR4 progressed, while only 6% of those carrying the non-DR3/non-DR4 combination did so. Close to half of the siblings with DQB1 risk genotypes progressed in their prediabetic stage, whereas about 10% of those with DQB1

genotypes conferring decreased risk progressed. In contrast, a sibling with decreased HLA-conferred disease susceptibility was more likely to regress or remain stable in terms of prediabetic stage. Similar trends were also seen when the siblings were classified according to the combination of autoantibodies and FPIR in relation to the degree of HLA identity, the DR phenotype or the DQB1 genotype, but the results remained non-significant.

There was a close association between the HLA-conferred genetic risk and the severity of preclinical T1D. Among the 659 siblings with available genetic data, 10% of the HLA-identical cases had late prediabetes initially, whereas only 3% of the haploidentical and non-identical siblings were at that stage. When using the criteria in **classification 2**, 4% of the HLA-identical siblings had late prediabetes, while the corresponding proportion among the HLA haploidentical and non-identical siblings was 1%. Similarly, when looking at the DR phenotypes, HLA DR3 and/or DR4 alleles conferred a greater risk of developing late preclinical T1D than the non-DR3/non-DR4 phenotype. According to the second set of criteria the difference was less obvious, although the HLA DR3 and/or DR4 alleles were still associated with an increased frequency of preclinical T1D. The risk of developing late prediabetes was also assessed in relation to the DQB1 genotype. When comparing siblings having high, moderate, and low risk genotypes with those having DQB1 genotypes conferring decreased risk it was again evident that strong genetic susceptibility is associated with an increased risk of developing signs of late prediabetes. This was also true when using the second set of criteria.

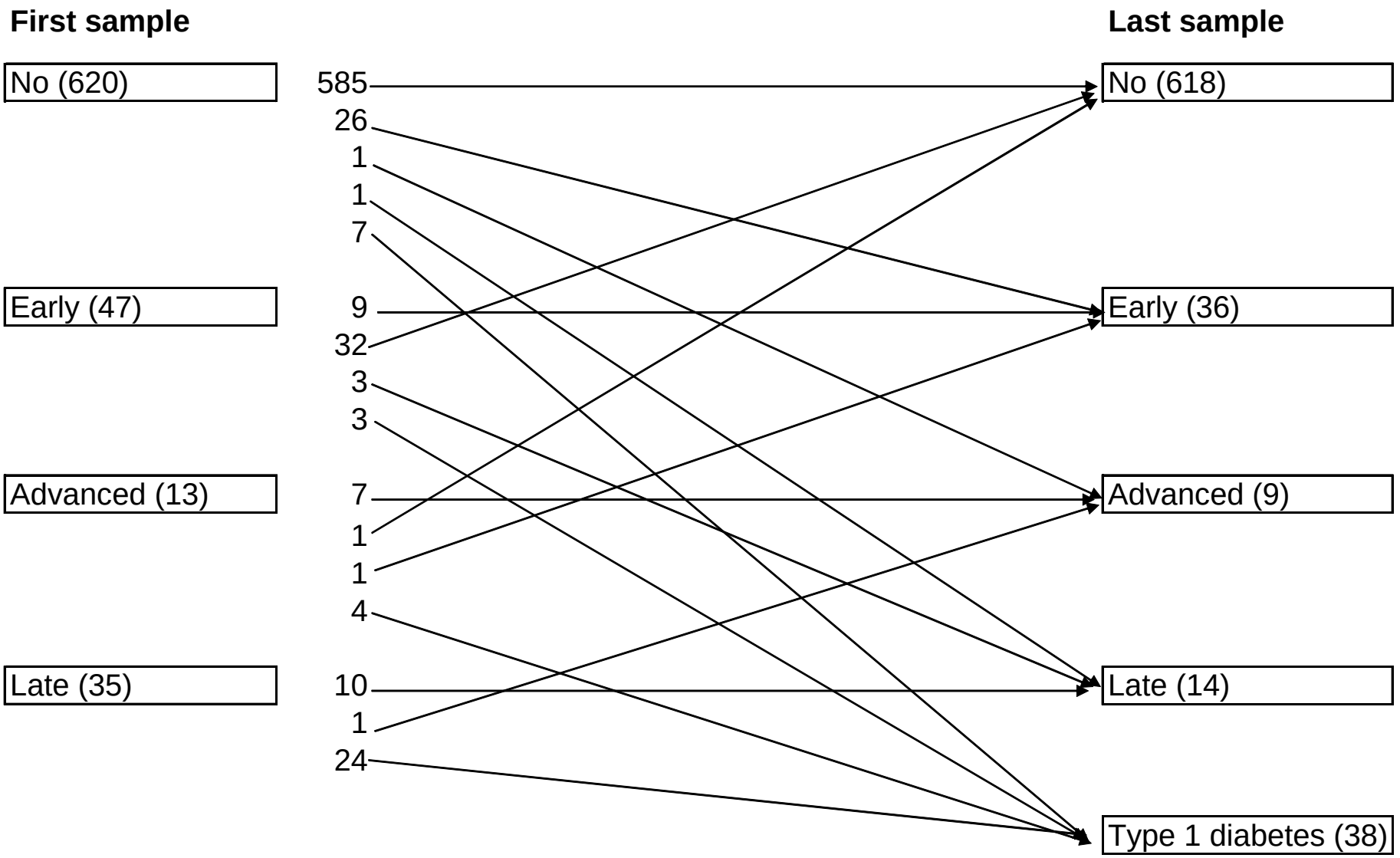

Figure 6. Course of preclinical type 1 diabetes in 715 siblings of affected children over a median follow-up period of 3.6 (0.01-9.8) years. Staging based on **classification 1**.

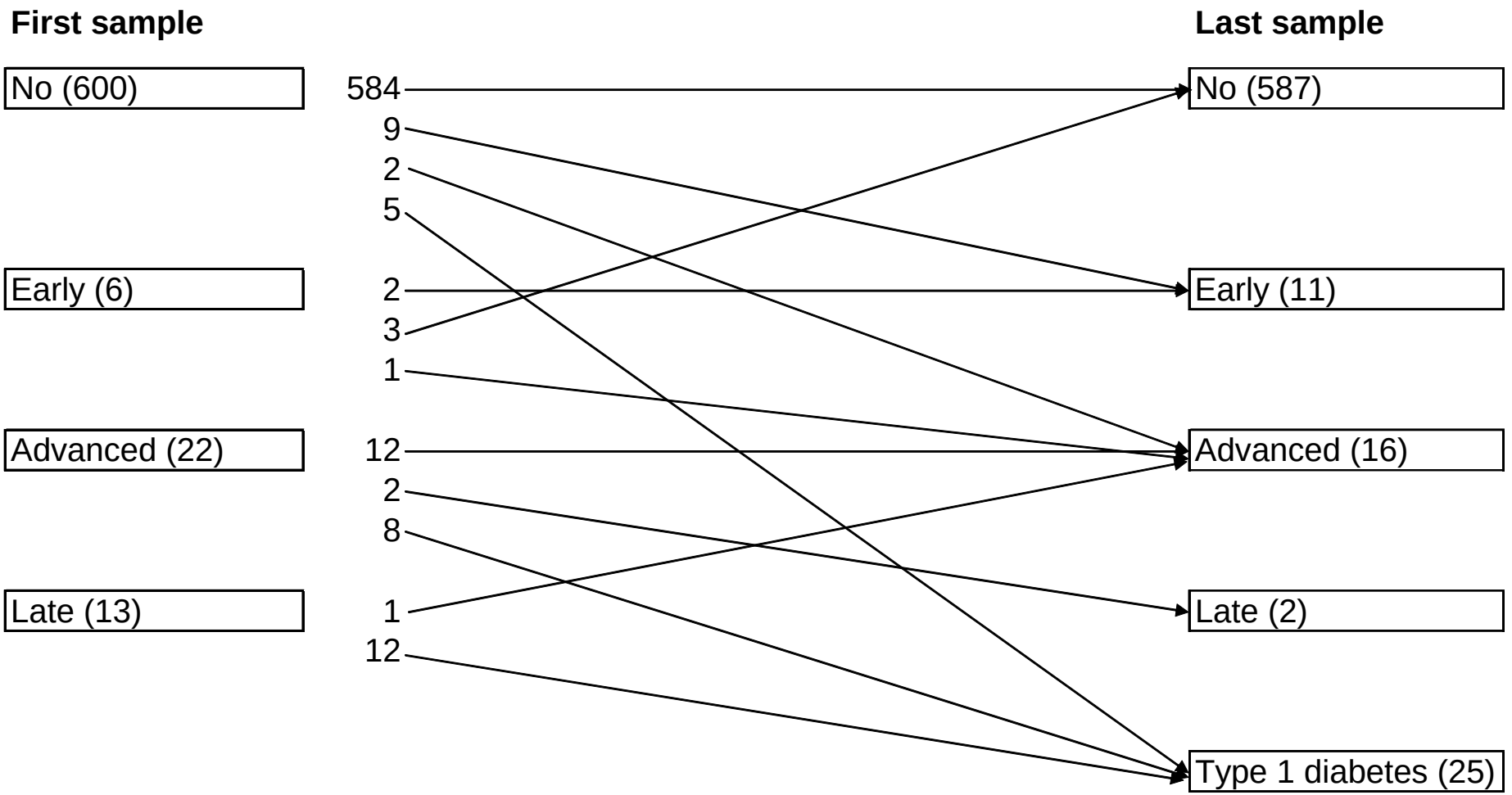

Figure 7. Course of preclinical type 1 diabetes in 641 siblings of affected children over a median follow-up period of 3.5 (0.08-9.8) years. Staging based on **classification 2**.

6.4 Duration of the prediabetic period (time to diagnosis) (I, II, III)

There were significant differences in the time to diagnosis between the various categories of prediabetes among the 25 individuals who presented with clinical signs of T1D. A significantly shorter time to diagnosis was observed in the subjects with late prediabetes than in those with no signs of prediabetes. The median duration of early prediabetes was 1.3 years (range 0.1-5.6 years) in the nine siblings who experienced this stage, that of advanced prediabetes was 0.7 years (range 0.02-4.2 years) in the 18 subjects who were at this stage at some point, and that of late prediabetes 2.7 years (range 0.01-9.0 years) in the 32 siblings who passed through this stage. Thus the advanced prediabetes stage was significantly shorter in duration than the late prediabetes stage ($p = 0.005$).

Siblings with late prediabetes according to **classification 1** who had the DQB1*0302/x genotype had a significantly shorter time to diagnosis (1.2 vs. 4.3 years; $p=0.01$) than those who were heterozygous for DQB1*02/0302, but no other differences in time to diagnosis were observed between the siblings with an increased HLA-defined genetic risk and those with decreased genetic susceptibility.

6.5 Multivariate risk assessment (IV)

Based on the Cox regression model, we calculated an individual prognostic risk index (PRI) for each subject and then performed a receiver-operating characteristics (ROC) analysis to define a cut-off index leading to the best separation between progressors and non-progressors. The cut-off index based on the total series proved to be 0.25, resulting in a sensitivity of 78.7%, a specificity of 95.7% and a positive predictive value (PPV) of 56.9% for T1D. There were altogether 65 out of 701 (9.3%) siblings with a positive prognostic risk index, as many as 37 of whom presented with clinical T1D. Comparison of the siblings who did not progress to overt T1D in spite of a PRI exceeding 0.25 with those progressing to overt T1D, in order to define possible factors providing protection from disease progression, showed that the non-progressors had a lower total number of autoantibodies and lower titers of ICA, GADA and IA-2A at initial sampling and were less often HLA-identical to the index case (Table 10).

The remaining 636 siblings (90.7%) had a PRI below 0.25, and only 10 of them (1.6%) developed clinical T1D. Comparison of the latter with thse siblings who remained unaffected, in order to assess factors predisposing these "protected" children to overt T1D, showed that the progressors had higher GADA and IA-2A titers and more autoantibodies detectable at initial sampling than the siblings who remained unaffected (Table 10).

It also became evident that none of the 636 siblings with a PRI less than 0.25 had more than two autoantibodies detectable and 587 (92.3%) had no autoantibodies, whereas 32 out of the 65 siblings (49.2%) with an index exceeding 0.25 tested positive for more than two antibodies. In addition, the siblings with a PRI less than 0.25 were initially significantly older [mean age 10.3 (4.4; SD) years *vs.* 7.1 (4.1) years; $P<0.001$]. Among those who presented with T1D, the siblings with a PRI exceeding 0.25 had a shorter duration of the

preclinical period than those with an index less than 0.25 [mean 4.9 (4.0) years *vs.* 8.8 (3.3) years; *P*=0.007]. The PRI was inversely related to the duration of the prediabetic period (r_s=-0.40; p=0.006).

6.6 Prediction of age at diagnosis (IV)

The age at disease presentation was most effectively predicted with a linear regression model including age, IA-2A levels and number of autoantibodies detectable at initial sampling soon after the clinical diagnosis of the index case. This model explained 76% of the variation in age at diagnosis among the siblings. When comparing the predicted age at diagnosis in the siblings with the observed age , 18 (48.6%) of the observed ages among the 37 progressors were within the confidence interval of the prediction. The youngest (4.9 years) and oldest (24.8 years) predicted ages were relatively well in line with the youngest (1.5 years) and oldest (24.8 years) observed ages (Table 11A).

The second model for the prediction of age at diagnosis, applied to 77 siblings for whom metabolic data were available, was based on the age of the sibling, the initial IA-2A titer, the risk conferred by the DR-phenotype and the initial FPIR value. This model explained 83% of the variation in age at diagnosis. When comparing the predicted ages at diagnosis in the 25 progressors with the observed ages, all but one of the observed ages were within the confidence interval of the estimated age. The sibling with the youngest predicted age at diagnosis (4.9 years) had an observed age of 5.5 years, which was close to the youngest observed age (5.4 years), while the oldest (22.4 years) predicted age was well in accordance with the oldest observed age (24.8 years) (Table 11B).

Table 10. Comparison of PRI in progressors and non-progressors, median (range). (PRI= prognostic risk index)

	PRI<0.25			**PRI>0.25**		
	Progressors (n=10)	**Non-progressors** (n=626)	**Statistics**	**Progressors** (n=37)	**Non-progressors** (n=28)	**Statistics**
Age at first sampling, mean (SD)	7.4 (4.1)	6.6 (4.0)	p=0.34	0.7 (3.3)	10.3 (4.4)	p=0.67
Number of autoantibodies (AAB) at first sampling	3 (0-4)	0 (0-3)	**p<0.001**	1 (0-1)	0 (0-1)	**p<0.001**
ICA, JDF-units	40 (0-640)	0 (0-160)	**p<0.001**	0 (0-5)	0 (0-40)	p=0.22
IAA, nU/ml	37 (0-1238)	29.5 (0-103)	p=0.17	20.0 (36-206)	19.2 (0-83)	p=0.62
GADA, RU	22.2 (0.6-395)	0.7 (0.0-294)	**p=0.004**	5.4 (0.0-154)	0.4 (0.0-288)	**p<0.001**
IA-2A, RU	10.7 (0.1-159)	0.17 (0.09-115)	**p<0.001**	0.2 (0.3-2.1)	0.19 (0.04-3.7)	**p=0.035**
FPIR, mU/ml	55.5 (5.0-137)	70.5 (47.0-140)	p=0.059	73.3 (47.5-173)	94.0 (44.0-286)	p=0.16
K_g, %/min	1.52 (0.66-2.6)	1.42 (1.2-4.1)	p=0.91	1.54 (1.2-1.7)	1.67 (1.11-3.75)	p=0.21
HOMA-index	1.64 (0.15-5.7)	1.94 (1.1-3.7)	p=0.26	3.0 (0.99-12)	2.01 (0.17-12)	p=0.09
HOMA-IR/FPIR ratio	0.03 (0.01-0.11)	0.03 (0.01-0.05)	p=0.42	0.05 (0.14-0.16)	0.02 (0.001-0.16)	p=0.54
Number of first-degree relatives	1 (1-2)	1 (1-1)	p=0.15	1 (1-1)	1 (1-2)	p=0.73
HLAidentity (identical/ haploidentical and non-identical)	22/15	9/19	**p=0.029**	3/7	143/483	p=0.59
HLA DR	13/21/2/1 [a]	5/20/1/2 [a]	p=0.38	8/2 [b]	320/306 [b]	p=0.07
[a](DR3,4/DR4,x/DR3,y/other DR)				[b](DR3,4 and DR4,x/DR3,y and other DR)		

Table 11. Model for the prediction of age at diagnosis in 65 siblings with a PRI exceeding 0.25 in the total series (A) and 35 siblings in the smaller series of children with metabolic data available (B).

A. Age at diagnosis = 5.24 + 1.38 (age at sampling) – 0.039 [IA2A (RU)] - 0.65 (number of autoantibodies detected)

CASE	NUMBER OF AUTOANTIBODIES	PREDICTED AGE AT DIAGNOSIS (CI, years)	OBSERVED AGE AT DIAGNOSIS
703	3	8.46 (5.75-11.17)	3.83
705	3	4.91 (2.20-7.62)	1.49
1505	3	18.17 (15.46-20.88)	15.20
2703	3	20.23 (17.52-22.94)	16.72
4003	3	21.73 (19.02-24.44)	24.81
4308	2	7.76 (5.05-10.47)	**5.70**
6703	3	11.63 (8.92-14.34)	**12.92**
8406	3	6.53 (3.82-9.24)	**7.18**
8407	0	9.30 (6,59-12.01)	**8.84**
12805	3	8.61 (5.90-11.32)	**9.49**
14206	3	13.10 (10.39-15.81)	19.81
14512	3	14.73 (12.02-17.44)	**13.42**
14513	4	13.74 (11.03-16.45)	18.80
17406	0	9.01 (6.3-11.72)	15.49
25303	3	13.52 (10.81-16.23)	10.54
25304	2	7.14 (4.43-9.85)	**5.46**
25603	3	20.52 (17.81-23.23)	**20.91**
27203	2	9.23 (6.52-11.94)	5.44
30703	4	15.95 (13.24-18.66)	12.88
32403	3	15.68 (12.97-18.39)	**15.19**
37003	3	8.59 (5.88-11.30)	**10.43**
38705	4	13.41 (10.70-16.12)	17.32
40004	0	9.47 (6.76-12.18)	13.82
41904	3	6.87 (4.16-9.58)	3.04
42303	2	24.83 (22.12-27.54)	**24.34**
45205	4	9.27 (6.56-11.98)	17.30
45303	3	10.25 (7.54-12.96)	6.95
51605	2	7.69 (4.98-10.40)	**6.02**
57204	3	8.66 (5.95-11.37)	**10.06**
60304	0	12.14 (9.43-14.85)	9.25
60503	2	18.52 (15.81-21.23)	13.48
65103	3	12.14 (9.43-14.85)	**14.02**
68705	2	15.53 (12.82-18.24)	20.19
72004	4	7.62 (4.91-10.33)	**5.52**
75204	3	10.03 (7.32-12.74)	**9.77**
75205	0	7.75 (5.04-10.46)	**9.47**
87303	2	22.94 (20.23-25.65)	**22.06**

B. Age at diagnosis = -2.82 + 1.12 (age at sampling) – 0.021 [IA2A (RU)] + 0.066 [FPIR (mU/l)]+ 1.97 [DR genotype (0= nonDR3/nonDR4. 1= DR3/nonDR4. 2= DR4/nonDR3. 3= DR3/DR4)]

CASE	NUMBER OF AUTOANTIBODIES	PREDICTED AGE AT DIAGNOSIS (CI. years)	OBSERVED AGE AT DIAGNOSIS
1505	3	16.53 (11.99-21.07)	**15.20**
2703	3	18.26 (13.72-22.80)	**16.72**
4003	3	19.94 (15.40-24.48)	24.81
6703	3	12.73 (8.19-17.27)	**12.92**
8407	0	9.43 (4.89-13.97)	**8.84**
14206	3	15.33 (10.79-19.87)	**19.81**
14512	3	15.17 (10.63-19.71)	**13.42**
14513	4	16.96 (12.42-21.50)	**18.80**
17406	0	11.96 (7.42-16.50)	**15.49**
25304	2	4.87 (0.33-9.41)	**5.46**
25603	3	21.09 (16.55-25.63)	**20.91**
27203	2	6.28 (1.74-10.82)	**5.44**
30703	4	15.26 (10.72-19.80)	**12.88**
32403	3	16.79 (12.25-21.33)	**15.19**
37003	3	9.33 (4.79-13.87)	**10.43**
38705	4	15.63 (11.09-20.17)	**17.32**
45205	4	15.52 (10.98-20.06)	**17.30**
45303	3	6.89 (2.35-11.43)	**6.95**
51605	2	7.83 (3.29-12.37)	**6.02**
57204	3	11.98 (7.44-16.52)	**10.06**
65103	3	11.27 (6.73-15.81)	**14.02**
68705	2	17.36 (12.82-21.90)	**20.19**
72004	4	7.53 (2.99-12.07)	**5.52**
75204	3	12.66 (8.12-17.20)	**9.77**
87303	2	22.43 (17.89-26.97)	**22.06**

7 DISCUSSION

When a child presents with clinical symptoms of T1D, a chain reaction of events follows. The diagnosis is accompanied by anxiety and worries over the lifetime responsibilities facing the parents. The situation leads to a number of questions that are typical and repeat themselves continuously when the family meets with the doctor caring for their newly diagnosed child. One of the most frequent questions asked by families with more than one child is: "Will my other children also develop this disease?" Another frequent question is: "When would my other child with a high risk of developing T1D present with clinical symptoms?". The ever-increasing incidence in the general population and the possible future preventive and treatment modalities have placed a demand on identifying individuals at risk and predicting the natural course of their preclinical disease process.

7.1 Limitations of the present research

Finland offers unique possibilities for studying T1D, as the incidence rate is the highest in the world. The present results are based on a nationwide survey of siblings of children with T1D in Finland, providing an opportunity to observe the genetic, immunological and clinical characteristics of the disease in a large population. Even a study of this magnitude has its limitations, however. Of the total of 767 siblings, only 48 were diagnosed with T1D by the end of 2002, which places limitations on the statistical power of the results and on their interpretation when assessing factors influencing the development of the disease. Genetic data were not available for all the siblings, and the DQ typing in particular did not cover the total cohort. Only siblings testing positive for at least one autoantibody reactivity were invited for an IVGTT. Seventy-eight of the 97 initially antibody-positive siblings (80%) underwent an IVGTT, and accordingly metabolic data were available on these subjects. The statistical analyses were performed on the assumption that autoantibody-negative siblings had a normal FPIR, whereas the antibody-positive siblings with no metabolic data available were excluded from the analyses of the role of metabolic markers. As this was a nationwide investigation, samples were taken in different hospitals by a large number of staff members, and it has to be taken into consideration that this may have introduced increased variability into the results despite the use of standard methods. The method used for the analysis of IAA in the DiMe study is not as sensitive as the modern microassay, and this may have an impact on the assessment of the role of IAA as a risk factor. Predictive models will be important tools for the identification of siblings with a high T1D risk when curative or preventive modalities become available, but this stage still lies beyond the horizon.

7.2 Classification of preclinical diabetes and risk of progression to overt type 1 diabetes

To facilitate and simplify the assessment of the risk of T1D among siblings of affected children, the first stage in this research was to test two types of classification for easily identifying siblings with a high risk of T1D, and accordingly possible participants in future

prevention trials. There were some differences in the risk of progression in relation to the stage of prediabetes that depended on the type of classification used. In **classification 1**, based exclusively on the number of antibodies, the risk of developing T1D was 66% in those with late prediabetes, while **classification 2,** according to both antibodies and FPIR, resulted in a risk as high as 92% for siblings in this category. Similar, although smaller differences were also observed at the earlier stages of preclinical T1D. This illustrates the fact that the analysis of FPIR improves the predictive power of the staging strategy, and it is therefore highly recommendable to perform an IVGTT in the case of siblings testing positive for one or more autoantibodies. FPIR as such nevertheless remains an indirect indicator of the residual beta-cell mass, which usually declines towards clinical presentation with T1D (104; 276; 278; 329).

7.3 Natural history of preclinical diabetes

The observation that regression to no prediabetes from an initial stage of advanced or late prediabetes was an extremely rare phenomenon in our series and was seen in only one individual with advanced prediabetes initially supports the concept that preclinical diabetes can be graded based on the number of autoantibodies. Altogether, regression of any kind was infrequent among siblings with advanced or late prediabetes initially, as such a phenomenon was observed in less than 10% of these siblings. This finding emphasizes that advanced and late prediabetes do reflect destructive beta-cell autoimmunity that will most likely result eventually in subtotal beta-cell destruction and overt T1D. Within the time frame of the present study, close to 60% of the siblings with initially advanced or late prediabetes progressed to clinical T1D. Our data further support the view that early prediabetes often represents harmless beta-cell autoimmunity associated with a relatively low risk of progression to clinical T1D. This idea is supported by the observation that half or more of those with early prediabetes initially had regressed and had no signs of prediabetes at the end of the follow-up. This may be partly due to technical factors, probably reflected in the higher proportion of false positive samples among those found positive for a single antibody reactivity than among those testing positive for two or more disease-associated antibodies.

Comparing the two sets of criteria used for grading the prediabetic process, it seems that **classification 2**, based on a combination of antibody status and FPIR, is somewhat more predictive, since the proportion of siblings with a progressive process was significantly higher and the proportion of those with regression significantly lower. In fact, all but one of the 13 siblings with an initially reduced FPIR presented with clinical T1D during the prospective observation. One has to bear in mind, however, that there was a difference in the number of subjects included, since not all the antibody-positive siblings agreed to undergo an IVGTT. Accordingly, **classification 2**, which was based not only on the number of antibodies but also on the FPIR, covered fewer subjects than the staging based exclusively on the number of antibodies.

We observed significant differences in the time to clinical diagnosis in relation to the stage of preclinical diabetes, with the shortest average duration recorded in the siblings with late

prediabetes. It became evident, however, that there was wide variation in this time period within the same stage, e.g. among those with late prediabetes it varied from 0.02 to 7.7 years. This demonstrates that, although there are significant differences in the time to diagnosis among siblings at different stages of preclinical diabetes, the variation from subject to subject is extensive, making it difficult to predict the time point of diagnosis on an individual basis. We also aimed to assess whether there is any HLA-associated genetic marker that predicts faster or retarded progression to clinical T1D. It has prevously been reported that the HLA-A24 allele is associated with rapid progression to T1D in ICA-positive relatives (330). The DR3/DQB1*02 haplotype has been observed to be associated with a slowly progressive prediabetic process (45), but we observed almost no relationship between time to diagnosis and the risk genotype. Accordingly, HLA-defined disease susceptibility did not provide any clear-cut explanation for the conspicuous inter-individual variation in time to diagnosis. When looking only at the siblings with late prediabetes, we observed a shorter time to diagnosis among those with the DQB1*0302/x genotype than among those who were heterozygous for DQB1*02/0302. This suggests that the DQB1*0302 allele is linked to a particularly aggressive autoimmune process.

It was demonstrated in the first paper that the analysis of four T1D-associated autoantibodies facilitates the estimation of T1D risk in unaffected siblings. Although the classification into stages of preclinical diabetes based on a combination of the number of antibodies and FPIR is an effective tool for grading the disease risk in members of the families of affected children, it is obvious that it will not be possible to identify all siblings who will progress to clinical disease at the time of diagnosis in the first affected child, since some will have no signs of preclinical diabetes at that point. In the first survey six (0.9%) out of the 661 children with no signs of preclinical diabetes at the time of diagnosis of the index case in the family subsequently presented with clinical T1DThe majority of these seroconverted to antibody positivity later in the prediabetic process, however. Staging of preclinical T1D may prove an important tool in the near future for the identification of siblings who should be treated with effective preventive modalities as soon as such treatment becomes available.

The second paper set out to define characteristics that could identify individuals with a progressive process from among those with signs of prediabetes initially. Those with a progressive process were observed to be characterized by younger age, a higher number of detectable antibodies and higher autoantibody levels than those initially prediabetic siblings who remained stable or regressed. Those with a progressive process also had a reduced FPIR. Unfortunately, there was no single characteristic that provided complete discrimination between the progressing siblings and the remaining ones without any overlap. We found no infallible means of differentiating on an individual basis between those with a progressive process and those with stable or regressive β-cell autoimmunity.

When observing the individual course of the prediabetic process in those siblings who presented with clinical T1D, one can observe that about half of the progressors did not change their prediabetic status at any time before diagnosis. In the remaining half the pattern was highly variable, with a logical progression from no prediabetes or early prediabetes through advanced and late prediabetes to clinical disease in some cases but substantial fluctuations in others, e.g. starting from early prediabetes and passing through

the stages of no prediabetes, late prediabetes, advanced prediabetes and again early prediabetes to end up with overt T1D. These findings suggest that the progression to clinical diabetes runs an individual path, and that the process is not necessarily continuously progressive but may regress at some point to proceed again later. Such an individual pattern of progression supports the "multiple hit" model, according to which an unsynchronized series of exogenous factors determine the path and pace of beta-cell destruction (254). The early identification of characteristics that differentiate between those initially antibody-positive siblings who develop clinical T1D and those who remain unaffected turned out to be complicated. Although the two groups differed significantly in age and in the initial number of autoantibodies and their levels, there was still considerable overlap between them in relation to all the features analyzed.

Our observations suggest that 40-60% of siblings with signs of prediabetes at the time of diagnosis of the index case progress further during a prospective observation period with a median duration of more than 3 years, while the remaining siblings retain their prediabetic stage or regress. Regression is common among those with early prediabetes initially, but rare among those with advanced or late prediabetes. Progressing siblings are characterized by young age, a strong humoral immune response to beta-cell antigens other than insulin and a reduced FPIR initially. No single characteristic is capable of distinguishing unequivocally between those siblings who have a progressive process and are most likely to develop overt T1D and those with stable or regressive beta-cell autoimmunity.

7.4 Risk of progression to clinical type 1 diabetes in relation to autoantibodies and HLA-conferred disease susceptibility

In general, autoantibodies alone are more sensitive for the prediction of future diabetes in siblings than are antibodies combined with genetic susceptibility (331). In the general population of Finland, the risk of developing T1D is 11-fold among subjects with the DQ high-risk genotype as compared with low-risk persons, and the same risk is 3.5-fold in siblings of affected children relative to siblings with decreased genetic susceptibility (317). In our third paper, late prediabetes, i.e. positivity for at least three diabetes-associated autoantibodies according to **classification 1**, gave risks as high as 775 when combined with HLA identity, 1809 when combined with DR3/4 heterozygosity and 1773 when combined with the DQB1*02/0302 genotype as compared with siblings having decreased HLA-defined disease susceptibility and no signs of prediabetes. The combination of late prediabetes with genetic risk markers similarly resulted in high relative risks according to **classification 2**. Some of these risk ratios were higher than the figures of 209 associated with late prediabetes in **classification 1,** defined by autoantibodies alone, or of 1310 conferred by the stage of late prediabetes based on **classification 2,** as reported in our first paper. These risk ratios were not significantly higher than the previous ones, however. On the other hand, all the siblings with a high DQ-conferred risk and late prediabetes went on to clinical disease, whereas less than 60% of the other siblings presented with T1D. In the siblings with a reversible autoimmune process (early prediabetes), genetic susceptibility seems to add to the predictive power of autoantibody positivity, but when a child develops

autoantibodies of a more irreversible character (advanced or late prediabetes), genetic susceptibility adds little to the risk conferred by autoantibodies alone.

We also observed a significantly larger proportion of siblings with late prediabetes among those with a strong HLA-defined disease predisposition than among those with decreased susceptibility, while there was a larger proportion of siblings with no signs of prediabetes among those with genotypes conferring decreased risk. These observations suggest that strong HLA-conferred disease susceptibility predisposes the individual to more advanced stages of prediabetes, while siblings with low risk genotypes are more likely to carry signs of early or no prediabetes. This indicates that HLA-conferred disease susceptibility has an impact on the likelihood of an autoimmune process being triggered. High-risk genotypes are more likely to be associated with a non-reversible immunological process, represented by advanced or late prediabetes.

HLA-defined predisposition also seems to be predictive of progression or regression from the initial prediabetic stage. The stronger the genetic risk, the more likely it is that a sibling will progress in terms of the prediabetic stage. Inversely, the lower the genetic risk the more likely it is that the sibling will regress. This indicates that siblings with strong susceptibility genotypes not only have a higher risk of developing autoantibodies, but that their prediabetic autoimmune process more often represents a non-reversible process. The opposite was observed for siblings with HLA genotypes conferring decreased diabetes susceptibility, in whom the emergence of autoantibodies is infrequent, and if a prediabetic process is initiated it is more likely to be reversible.

7.5 Predictive models of type 1 diabetes

In the final paper we set out to design predictive models for T1D that integratw sociodemographic, genetic, immunological and metabolic markers and to test their utility for the prediction of T1D in siblings of affected children. This approach is unique in the sense that most earlier surveys presenting predictive models have been based on relatively selected populations (270; 278; 332). It is nevertheless important and clinically relevant to assess predictive strategies in an unselected population of siblings. According to a previous study, the combination of risk markers such as a multiplex family history (an additional sibling with T1D, or an additional parent with T1D) and genetic susceptibility conferred by *IDDM1* (HLA) in a multivariate model allowed the identification of multiple antibody–positive children who did not have HLA risk genotypes, including two offspring with protective HLA genotypes who developed multiple autoantibodies and diabetes (333). Our work generated a novel approach for predicting T1D with a multivariate model that included the HOMA-IR/FPIR ratio. The two-step predictive strategy devised in this survey seems to offer a feasible means of identifying those siblings of children with newly diagnosed diabetes who will most probably progress to clinical diabetes and for predicting their likely age at diagnosis. This kind of information may be useful when the parents of a child with recently diagnosed diabetes are to be informed about the risk of clinical disease in the other children within the family. Our risk assessment is based on analysis of the HLA class II genotype and all four predictive autoantibody reactivities soon after T1D has been diagnosed in the index case. The results suggest that a short IVGTT providing fasting

glucose and insulin concentrations as well as an estimate of the early insulin response to intravenous glucose can provide additional data that improve the accuracy of both the risk and time estimates. The HOMA-IR index also appeared to be a useful predictive marker, as a high value in relation to insulin secretion was observed to be associated with an increased risk of progression to clinical T1D. These refined predictive models may be used to identify individuals who would most conspicuously benefit from preventive measures aimed at arresting or retarding the prediabetic disease process.

When considering clinical aspects of risk assessment in siblings of children with T1D, we must conclude from our experience that the most efficient way seems to be the screening of siblings close to the time of diagnosis of the index case. Based on knowledge acquired from this and other studies, IVGTT should be performed on all antibody-positive siblings. A follow-up of autoantibody-positive siblings at 6 months intervals is advisable, while samples taken at intervals of up to 1 year seem to be adequate for autoantibody-negative siblings. If a sibling remains repeatedly negative for all of the autoantibodies tested, the follow-up can be discontinued.

8 CONCLUSIONS

1. The analysis of four T1D-associated autoantibodies facilitates estimation of the T1D risk in unaffected siblings of children with T1D. The staging of preclinical diabetes based on a combination of the number of antibodies and FPIR is an effective tool for grading the risk of T1D in family members of affected children. It is obvious, however, that it will not be possible to identify all siblings who will progress to clinical T1D at the time of diagnosis in the first affected child, since some progressors have no signs of preclinical diabetes at that point.

2. Genetic susceptibility adds to the risk assessment based on diabetes-associated autoantibodies when attempting to predict progression to clinical T1D. Increased HLA-defined disease susceptibility is associated with a more frequent emergence of autoantibodies and an irreversible prediabetic process in siblings of children with T1D. In the siblings with a reversible autoimmune process (early prediabetes) genetic susceptibility seems to add to the predictive power of autoantibody positivity, but when a child develops autoantibodies of a more irreversible character (advanced or late prediabetes), HLA-defined susceptibility adds little to the risk conferred by autoantibodies alone. HLA-conferred susceptibility seems to have an impact on the likelihood of an autoimmune process being triggered. High-risk genotypes are more likely to be associated with a non-reversible immunological process, represented by advanced or late prediabetes.

3. When looking only at siblings with late prediabetes, we observed a shorter time to diagnosis among those with the DQB1*0302/x genotype than among those who were heterozygous for DQB1*02/0302. This suggests that the DQB1*0302 allele is linked to a particularly aggressive autoimmune process.

4. About half (40-60%) of all siblings with signs of prediabetes at the time of diagnosis of the index case progress further during a prospective observation period with a median duration of more than 3 years, while the remaining siblings retain their prediabetic stage or regress. Regression is common among those with early prediabetes initially, but rare among those with advanced or late prediabetes. Progressing siblings are characterized by young age, a strong humoral immune response to beta-cell antigens other than insulin and a reduced FPIR initially. No single characteristic, however, is capable of distinguishing unequivocally between those siblings who have a progressive process and are most likely to develop overt T1D and those with stable or regressive beta-cell autoimmunity.

5. Regression of any kind was infrequent among siblings with advanced or late prediabetes initially, as such a phenomenon was observed in less than 10% of cases. This finding emphasizes that advanced and late prediabetes do reflect destructive beta-cell autoimmunity that will most likely result eventually in subtotal beta-cell destruction and overt T1D, whereas positivity for only one autoantibody (early prediabetes) seems to represent a more harmless autoimmune process. Within the time frame of the present work, close to 60% of the siblings with initially advanced or late prediabetes progressed to clinical T1D. These observations confirm previous findings indicating that a reduced FPIR often reflects

irreparable beta-cell damage.

6. The Cox regression model devised here seemed to offer a feasible strategy for the identification of siblings of children with newly diagnosed diabetes who will most probably progress to clinical diabetes. A PRI exceeding 0.25 seems to pinpoint individuals with the highest risk of progression to clinical T1D, thereby functioning as an effective tool for use in risk assessment. This kind of information may be useful when the parents of a child with recently diagnosed diabetes are to be informed about the risk of clinical disease in other children in the family.

7. The model for predicting age at diagnosis appeared to work well or satisfactorily among the true progressors but poorly among those who did not present with clinical T1D during the observation period.

8. The HOMA-IR index appears to be a useful predictive marker, as a high HOMA-IR/FPIR ratio was observed to be associated with an increased risk of progression to clinical T1D. Neither the glucose elimination rate during the IVGTT nor the fasting HOMA-IR index or HOMA-IR/FPIR ratio had any significant impact on age at diagnosis, however.

9. A short IVGTT, providing fasting glucose and insulin concentrations as well as an estimate of the early insulin response to intravenous glucose, can provide additional data that improve the accuracy of both the risk and time estimates.

9 REFERENCES

1. Notkins AL: Immunologic and genetic factors in type 1 diabetes. *J Biol Chem* 277:43545-43548, 2002
2. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J: Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care* 23:1516-1526, 2000
3. Onkamo P, Vaananen S, Karvonen M, Tuomilehto J: Worldwide increase in incidence of Type I diabetes--the analysis of the data on published incidence trends. *Diabetologia* 42:1395-1403, 1999
4. Shaltout AA, Moussa MA, Qabazard M, Abdella N, Karvonen M, Al-Khawari M, Al-Arouj M, Al-Nakhi A, Tuomilehto J, El-Gammal A: Further evidence for the rising incidence of childhood Type 1 diabetes in Kuwait. *Diabet Med* 19:522-525, 2002
5. Frazer de Llado TE, Gonzalez de Pijem L, Hawk B: Incidence of IDDM in children living in Puerto Rico. Puerto Rican IDDM Coalition. *Diabetes Care* 21:744-746, 1998
6. Karvonen M, Tuomilehto J, Libman I, LaPorte R: A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. *Diabetologia* 36:883-892, 1993
7. Cherubini V, Chiarelli F, Altobelli E, Verrotti A, Carle F: Regional variability in the epidemiology of childhood diabetes in Italy. *J Pediatr Endocrinol Metab* 10:471-478, 1997
8. Carle F, Gesuita R, Bruno G, Coppa GV, Falorni A, Lorini R, Martinucci ME, Pozzilli P, Prisco F, Songini M, Tenconi MT, Cherubini V: Diabetes incidence in 0- to 14-year age-group in Italy: a 10-year prospective study. *Diabetes Care* 27:2790-2796, 2004
9. Newhook LA, Curtis J, Hagerty D, Grant M, Paterson AD, Crummel C, Bridger T, Parfrey P: High incidence of childhood type 1 diabetes in the Avalon Peninsula, Newfoundland, Canada. *Diabetes Care* 27:885-888, 2004
10. Akerblom HK, Reunanen A: The epidemiology of insulin-dependent diabetes mellitus (IDDM) in Finland and in northern Europe. *Diabetes Care* 8 Suppl 1:10-16, 1985
11. Nystrom L, Dahlquist G, Ostman J, Wall S, Arnqvist H, Blohme G, Lithner F, Littorin B, Schersten B, Wibell L: Risk of developing insulin-dependent diabetes mellitus (IDDM) before 35 years of age: indications of climatological determinants for age at onset. *Int J Epidemiol* 21:352-358, 1992
12. Krolewski AS, Warram JH, Rand LI, Kahn CR: Epidemiologic approach to the etiology of type I diabetes mellitus and its complications. *N Engl J Med* 317:1390-1398, 1987
13. Tuomilehto J, Virtala E, Karvonen M, Lounamaa R, Pitkaniemi J, Reunanen A, Tuomilehto-Wolf E, Toivanen L: Increase in incidence of insulin-dependent diabetes mellitus among children in Finland. *Int J Epidemiol* 24:984-992, 1995
14. Padaiga Z, Tuomilehto J, Karvonen M, Podar T, Brigis G, Urbonaite B, Kohtamaki K, Lounamaa R, Tuomilehto-Wolf E, Reunanen A: Incidence trends in childhood onset IDDM in four countries around the Baltic sea during 1983-1992. *Diabetologia* 40:187-192, 1997
15. Tuomilehto J, Rewers M, Reunanen A, Lounamaa P, Lounamaa R, Tuomilehto-Wolf E, Akerblom HK: Increasing trend in type 1 (insulin-dependent) diabetes mellitus in childhood in Finland. Analysis of age, calendar time and birth cohort effects during 1965 to 1984. *Diabetologia* 34:282-287, 1991
16. Gale EA, Gillespie KM: Diabetes and gender. *Diabetologia* 44:3-15, 2001
17. Knip M: Etiopathogenetic Aspects of Type 1 Diabetes. In *Pediatric and Adolescent*

Medicine Chiarelli F, Dahl-Jørgensen, K., Kiess W, Ed. Basel, Karger, 2005, p. 1-27
18. Secular trends in incidence of childhood IDDM in 10 countries. Diabetes Epidemiology Research International Group. *Diabetes* 39:858-864, 1990
19. Variation and trends in incidence of childhood diabetes in Europe. EURODIAB ACE Study Group. *Lancet* 355:873-876, 2000
20. Pundziute-Lycka A, Urbonaite B, Ostrauskas R, Zalinkevicius R, Dahlquist GG: Incidence of type 1 diabetes in Lithuanians aged 0-39 years varies by the urban-rural setting, and the time change differs for men and women during 1991-2000. *Diabetes Care* 26:671-676, 2003
21. Casu A, Pascutto C, Bernardinelli L, Songini M: Type 1 diabetes among sardinian children is increasing: the Sardinian diabetes register for children aged 0-14 years (1989-1999). *Diabetes Care* 27:1623-1629, 2004
22. Gale EA: The rise of childhood type 1 diabetes in the 20th century. *Diabetes* 51:3353-3361, 2002
23. Somersalo O: Studies of childhood diabetes. I. Incidence in Finland. *Ann Paediatr Fenn* 1:239-249, 1954
24. Nystrom L, Dahlquist G, Rewers M, Wall S: The Swedish childhood diabetes study. An analysis of the temporal variation in diabetes incidence 1978-1987. *Int J Epidemiol* 19:141-146, 1990
25. Joner G, Sovik O: Increasing incidence of diabetes mellitus in Norwegian children 0-14 years of age 1973-1982. *Diabetologia* 32:79-83, 1989
26. Joner G, Sovik O: The incidence of type 1 (insulin-dependent) diabetes mellitus 15-29 years in Norway 1978-1982. *Diabetologia* 34:271-274, 1991
27. Joner G, Stene LC, Sovik O: Nationwide, prospective registration of type 1 diabetes in children aged <15 years in norway 1989-1998: no increase but significant regional variation in incidence. *Diabetes Care* 27:1618-1622, 2004
28. Pundziute-Lycka A, Dahlquist G, Nystrom L, Arnqvist H, Bjork E, Blohme G, Bolinder J, Eriksson JW, Sundkvist G, Ostman J: The incidence of Type I diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. *Diabetologia* 45:783-791, 2002
29. Weets I, De Leeuw IH, Du Caju MV, Rooman R, Keymeulen B, Mathieu C, Rottiers R, Daubresse JC, Rocour-Brumioul D, Pipeleers DG, Gorus FK: The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: evidence for earlier disease manifestation. *Diabetes Care* 25:840-846, 2002
30. Bruno G, Merletti F, Biggeri A, Cerutti F, Grosso N, De Salvia A, Vitali E, Pagano G: Increasing trend of type I diabetes in children and young adults in the province of Turin (Italy). Analysis of age, period and birth cohort effects from 1984 to 1996. *Diabetologia* 44:22-25, 2001
31. Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, Ilonen J, Knip M, Hyoty H: A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. *Ann Med* 37:67-72, 2005
32. Bodansky HJ, Staines A, Stephenson C, Haigh D, Cartwright R: Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population. *Bmj* 304:1020-1022, 1992
33. Bruno G, Merletti F, Pagano G: Incidence of insulin-dependent diabetes among Sardinian migrants to northern Italy. *Lancet* 347:193, 1996
34. Bruno G, Pagano G, Faggiano F, De Salvia A, Merletti F: Effect of Sardinian heritage

on risk and age at onset of type 1 diabetes: a demographic case-control study of Sardinian migrants. *Int J Epidemiol* 29:532-535, 2000
35. Gale EA: A missing link in the hygiene hypothesis? *Diabetologia* 45:588-594, 2002
36. Karvonen M, Tuomilehto J, Virtala E, Pitkaniemi J, Reunanen A, Tuomilehto-Wolf E, Akerblom HK: Seasonality in the clinical onset of insulin-dependent diabetes mellitus in Finnish children. Childhood Diabetes in Finland (DiMe) Study Group. *Am J Epidemiol* 143:167-176, 1996
37. Muntoni S, Stabilini L, Stabilini M, Mancosu G: Steadily high IDDM incidence over 4 years in Sardinia. *Diabetes Care* 18:1600-1601, 1995
38. Mooney JA, Helms PJ, Jolliffe IT, Smail P: Seasonality of type 1 diabetes mellitus in children and its modification by weekends and holidays: retrospective observational study. *Arch Dis Child* 89:970-973, 2004
39. Levy-Marchal C, Patterson C, Green A: Variation by age group and seasonality at diagnosis of childhood IDDM in Europe. The EURODIAB ACE Study Group. *Diabetologia* 38:823-830, 1995
40. Green A, Patterson CC: Trends in the incidence of childhood-onset diabetes in Europe 1989-1998. *Diabetologia* 44 Suppl 3:B3-8, 2001
41. Samuelsson U, Johansson C, Ludvigsson J: Month of birth and risk of developing insulin dependent diabetes in south east Sweden. *Arch Dis Child* 81:143-146, 1999
42. Gundersen E: Is diabetes of infectious origin? *J Infect Dis* 41:197-202, 1927
43. Afoke A, Ludvigsson J, Hed J, Lindblom B: Raised IgG and IgM in "epidemic" IDDM suggest that infections are responsible for the seasonality of type I diabetes. *Diabetes Res* 16:11-17, 1991
44. Karvonen M, Jantti V, Muntoni S, Stabilini M, Stabilini L, Tuomilehto J: Comparison of the seasonal pattern in the clinical onset of IDDM in Finland and Sardinia. *Diabetes Care* 21:1101-1109, 1998
45. Ludvigsson J, Samuelsson U, Beauforts C, Deschamps I, Dorchy H, Drash A, Francois R, Herz G, New M, Schober E: HLA-DR 3 is associated with a more slowly progressive form of type 1 (insulin-dependent) diabetes. *Diabetologia* 29:207-210, 1986
46. Weinberg CR, Dornan TL, Hansen JA, Raghu PK, Palmer JP: HLA-related heterogeneity in seasonal patterns of diagnosis in Type 1 (insulin-dependent) diabetes. *Diabetologia* 26:199-202, 1984
47. Devendra D, Liu E, Eisenbarth GS: Type 1 diabetes: recent developments. *Bmj* 328:750-754, 2004
48. Dahlquist G, Blom L, Holmgren G, Hagglof B, Larsson Y, Sterky G, Wall S: The epidemiology of diabetes in Swedish children 0-14 years--a six-year prospective study. *Diabetologia* 28:802-808, 1985
49. Degnbol B, Green A: Diabetes mellitus among first- and second-degree relatives of early onset diabetics. *Ann Hum Genet* 42:25-47, 1978
50. Malcova H, Sumnik Z, Drevinek P, Lebl J, Venhacova J, Vavrinec J, Cinek O: [Type I diabetes mellitus and associated autoimmune diseases in the first-degree relatives of diabetic children: questionnaire based study]. *Cas Lek Cesk* 143:625-629, 2004
51. Familial risk of type I diabetes in European children. The Eurodiab Ace Study Group and The Eurodiab Ace Substudy 2 Study Group. *Diabetologia* 41:1151-1156, 1998
52. Waldhor T, Schober E, Rami B, Tuomilehto J: The prevalence of IDDM in the first degree relatives of children newly diagnosed with IDDM in Austria--a population-based study. Austrian Diabetes Incidence Study Group. *Exp Clin Endocrinol Diabetes* 107:323-

327, 1999
53. Douek IF, Gillespie KM, Dix RJ, Bingley PJ, Gale EA: Three generations of autoimmune diabetes: an extended family study. *Diabetologia* 46:1313-1318, 2003
54. Lorenzen T, Pociot F, Hougaard P, Nerup J: Long-term risk of IDDM in first-degree relatives of patients with IDDM. *Diabetologia* 37:321-327, 1994
55. Bingley PJ, Bonifacio E, Gale EA: Can we really predict IDDM? *Diabetes* 42:213-220, 1993
56. Tarn AC, Thomas JM, Dean BM, Ingram D, Schwarz G, Bottazzo GF, Gale EA: Predicting insulin-dependent diabetes. *Lancet* 1:845-850, 1988
57. Harjutsalo V, Podar T, Tuomilehto J: Cumulative incidence of type 1 diabetes in 10,168 siblings of Finnish young-onset type 1 diabetic patients. *Diabetes* 54:563-569, 2005
58. Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J: Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52:1052-1055, 2003
59. Knip M: Natural course of preclinical type 1 diabetes. *Horm Res* 57 Suppl 1:6-11, 2002
60. Pitkaniemi J, Onkamo P, Tuomilehto J, Arjas E: Increasing incidence of Type 1 diabetes--role for genes? *BMC Genet* 5:5, 2004
61. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J, Kesaniemi YA: Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia* 35:1060-1067, 1992
62. Barnett AH, Eff C, Leslie RD, Pyke DA: Diabetes in identical twins. A study of 200 pairs. *Diabetologia* 20:87-93, 1981
63. Payami H, Thomson G, Motro U, Louis EJ, Hudes E: The affected sib method. IV. Sib trios. *Ann Hum Genet* 49 (Pt 4):303-314, 1985
64. Tillil H, Kobberling J: Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. *Diabetes* 36:93-99, 1987
65. Platz P, Jakobsen BK, Morling N, Ryder LP, Svejgaard A, Thomsen M, Christy M, Kromann H, Benn J, Nerup J, Green A, Hauge M: HLA-D and -DR antigens in genetic analysis of insulin dependent diabetes mellitus. *Diabetologia* 21:108-115, 1981
66. Wagener DK, Sacks JM, LaPorte RE, Macgregor JM: The Pittsburgh study of insulin-dependent diabetes mellitus. Risk for diabetes among relatives of IDDM. *Diabetes* 31:136-144, 1982
67. Knip M, Kukko M, Kulmala P, Veijola R, Simell O, Akerblom HK, Ilonen J: Humoral beta-cell autoimmunity in relation to HLA-defined disease susceptibility in preclinical and clinical type 1 diabetes. *Am J Med Genet* 115:48-54, 2002
68. Cavan D, Bain S, Barnett A: The genetics of type I (insulin dependent) diabetes mellitus. *J Med Genet* 29:441-446, 1992
69. Friday RP, Trucco M, Pietropaolo M: Genetics of Type 1 diabetes mellitus. *Diabetes Nutr Metab* 12:3-26, 1999
70. Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SC, Jenkins SC, Palmer SM, et al.: A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371:130-136, 1994
71. Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Tosi R, Pozzilli P, Todd JA: The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes

Registry. *Hum Mol Genet* 5:1075-1080, 1996
72. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA, Gough SC: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506-511, 2003
73. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 36:337-338, 2004
74. Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, Wang J, Dong Z, Brusko T, Atkinson M, Pozzilli P, Zeidler A, Raffel LJ, Jacob CO, Park Y, Serrano-Rios M, Larrad MT, Zhang Z, Garchon HJ, Bach JF, Rotter JI, She JX, Wang CY: A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat Genet* 36:837-841, 2004
75. Dean L MJ: *The Genetic Landscape of Diabetes*, 2005
76. Shiina T, Inoko H, Kulski JK: An update of the HLA genomic region, locus information and disease associations: 2004. *Tissue Antigens* 64:631-649, 2004
77. Redondo MJ, Fain PR, Eisenbarth GS: Genetics of type 1A diabetes. *Recent Prog Horm Res* 56:69-89, 2001
78. Sita N: The Human HLA System. *Journal of Indian Rheumatology Association* 11:79-83, 2003
79. Wolf E, Spencer KM, Cudworth AG: The genetic susceptibility to type 1 (insulin-dependent) diabetes: analysis of the HLA-DR association. *Diabetologia* 24:224-230, 1983
80. Ilonen J, Herva E, Tiilikainen A, Akerblom HK, Koivukangas T, Kouvalainen K: HLA-Dw3 as a marker of resistance against juvenile diabetes mellitus. *Tissue Antigens* 11:144-146, 1978
81. Svejgaard A, Ryder LP: HLA and insulin-dependent diabetes: an overview. *Genet Epidemiol* 6:1-14, 1989
82. Svejgaard A, Jakobsen BK, Platz P, Ryder LP, Nerup J, Christy M, Borch-Johnsen K, Parving HH, Deckert T, Molsted-Pedersen L, et al.: HLA associations in insulin-dependent diabetes: search for heterogeneity in different groups of patients from a homogeneous population. *Tissue Antigens* 28:237-244, 1986
83. Wassmuth R, Lernmark A: The genetics of susceptibility to diabetes. *Clin Immunol Immunopathol* 53:358-399, 1989
84. Deschamps I, Khalil I: The role of DQ alpha-beta heterodimers in genetic susceptibility to insulin-dependent diabetes. *Diabetes Metab Rev* 9:71-92, 1993
85. Deschamps I, Boitard C, Hors J, Busson M, Marcelli-Barge A, Mogenet A, Robert JJ: Life table analysis of the risk of type 1 (insulin-dependent) diabetes mellitus in siblings according to islet cell antibodies and HLA markers. An 8-year prospective study. *Diabetologia* 35:951-957, 1992
86. Yamamoto AM, Deschamps I, Garchon HJ, Roussely H, Moreau N, Beaurain G, Robert JJ, Bach JF: Young age and HLA markers enhance the risk of progression to type 1 diabetes

in antibody-positive siblings of diabetic children. *J Autoimmun* 11:643-650, 1998
87. Undlien DE, Friede T, Rammensee HG, Joner G, Dahl-Jorgensen K, Sovik O, Akselsen HE, Knutsen I, Ronningen KS, Thorsby E: HLA-encoded genetic predisposition in IDDM: DR4 subtypes may be associated with different degrees of protection. *Diabetes* 46:143-149, 1997
88. She JX: Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunol Today* 17:323-329, 1996
89. Ilonen J, Sjoroos M, Knip M, Veijola R, Simell O, Akerblom HK, Paschou P, Bozas E, Havarani B, Malamitsi-Puchner A, Thymelli J, Vazeou A, Bartsocas CS: Estimation of genetic risk for type 1 diabetes. *Am J Med Genet* 115:30-36, 2002
90. Hermann R, Turpeinen H, Laine AP, Veijola R, Knip M, Simell O, Sipila I, Akerblom HK, Ilonen J: HLA DR-DQ-encoded genetic determinants of childhood-onset type 1 diabetes in Finland: an analysis of 622 nuclear families. *Tissue Antigens* 62:162-169, 2003
91. Kimpimaki T, Kulmala P, Savola K, Vahasalo P, Reijonen H, Ilonen J, Akerblom HK, Knip M: Disease-associated autoantibodies as surrogate markers of type 1 diabetes in young children at increased genetic risk. Childhood Diabetes in Finland Study Group. *J Clin Endocrinol Metab* 85:1126-1132, 2000
92. Hermann R, Bartsocas CS, Soltesz G, Vazeou A, Paschou P, Bozas E, Malamitsi-Puchner A, Simell O, Knip M, Ilonen J: Genetic screening for individuals at high risk for type 1 diabetes in the general population using HLA Class II alleles as disease markers. A comparison between three European populations with variable rates of disease incidence. *Diabetes Metab Res Rev* 20:322-329, 2004
93. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsen AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark A: Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest* 95:1505-1511, 1995
94. Bingley PJ, Bonifacio E, Williams AJ, Genovese S, Bottazzo GF, Gale EA: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701-1710, 1997
95. Bruining GJ, Molenaar JL, Grobbee DE, Hofman A, Scheffer GJ, Bruining HA, de Bruyn AM, Valkenburg HA: Ten-year follow-up study of islet-cell antibodies and childhood diabetes mellitus. *Lancet* 1:1100-1103, 1989
96. Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 335:147-149, 1990
97. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, Schwartz S, Malone J, Shah S, Vadheim C, et al.: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N Engl J Med* 323:1167-1172, 1990
98. Karjalainen JK: Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. *Diabetes* 39:1144-1150, 1990
99. Vahasalo P, Knip M, Karjalainen J, Tuomilehto-Wolf E, Lounamaa R, Akerblom HK: Islet cell-specific autoantibodies in children with insulin-dependent diabetes mellitus and their siblings at clinical manifestation of the disease. Childhood Diabetes in Finland Study Group. *Eur J Endocrinol* 135:689-695, 1996
100. Eskola V, Vahasalo P, Akerblom HK, Knip M: Increased frequency of islet cell antibodies in unaffected brothers of children with type 1 diabetes. *Horm Res* 59:195-200, 2003

101. Kukko M, Kimpimaki T, Korhonen S, Kupila A, Simell S, Veijola R, Simell T, Ilonen J, Simell O, Knip M: Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk of type 1 diabetes recruited from the general population. *J Clin Endocrinol Metab* 90:2712-2717, 2005
102. Ludvigsson J, Wahlberg J: Diabetes-related autoantibodies in cord blood from children of healthy mothers have disappeared by the time the child is one year old. *Ann N Y Acad Sci* 958:289-292, 2002
103. Stanley HM, Norris JM, Barriga K, Hoffman M, Yu L, Miao D, Erlich HA, Eisenbarth GS, Rewers M: Is presence of islet autoantibodies at birth associated with development of persistent islet autoimmunity? The Diabetes Autoimmunity Study in the Young (DAISY). *Diabetes Care* 27:497-502, 2004
104. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EA: Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 43:1304-1310, 1994
105. Bottazzo GF, Florin-Christensen A, Doniach D: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2:1279-1283, 1974
106. Dean BM, Bottazzo GF, Cudworth AG: IgG subclass distribution in organ specific autoantibodies. The relationship to complement fixing ability. *Clin Exp Immunol* 52:61-66, 1983
107. Bruining GJ, Molenaar J, Tuk CW, Lindeman J, Bruining HA, Marner B: Clinical time-course and characteristics of islet cell cytoplasmatic antibodies in childhood diabetes. *Diabetologia* 26:24-29, 1984
108. Bottazzo GF, Dean BM, Gorsuch AN, Cudworth AG, Doniach D: Complement-fixing islet-cell antibodies in type-I diabetes: possible monitors of active beta-cell damage. *Lancet* 1:668-672, 1980
109. Kolb H, Dannehl K, Gruneklee D, Zielasek J, Bertrams J, Hubinger A, Gries FA: Prospective analysis of islet cell antibodies in children with type 1 (insulin-dependent) diabetes. *Diabetologia* 31:189-194, 1988
110. Johnston C, Millward BA, Hoskins P, Leslie RD, Bottazzo GF, Pyke DA: Islet-cell antibodies as predictors of the later development of type 1 (insulin-dependent) diabetes. A study in identical twins. *Diabetologia* 32:382-386, 1989
111. Colman PG, McNair P, King J, Caudwell J, Jankulovski C, Tait BD, Honeyman MC, Harrison LC: Screening for preclinical type 1 diabetes in a discrete population with an apparent increased disease incidence. *Pediatr Diabetes* 1:193-198, 2000
112. Levy-Marchal C, Dubois F, Noel M, Tichet J, Czernichow P: Immunogenetic determinants and prediction of IDDM in French schoolchildren. *Diabetes* 44:1029-1032, 1995
113. Schatz D, Krischer J, Horne G, Riley W, Spillar R, Silverstein J, Winter W, Muir A, Derovanesian D, Shah S, et al.: Islet cell antibodies predict insulin-dependent diabetes in United States school age children as powerfully as in unaffected relatives. *J Clin Invest* 93:2403-2407, 1994
114. Atkinson MA, Maclaren NK: The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 331:1428-1436, 1994
115. Landin-Olsson M, Palmer JP, Lernmark A, Blom L, Sundkvist G, Nystrom L, Dahlquist G: Predictive value of islet cell and insulin autoantibodies for type 1 (insulin-dependent) diabetes mellitus in a population-based study of newly-diagnosed diabetic and matched control children. *Diabetologia* 35:1068-1073, 1992

116. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, et al.: Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. *Diabetologia* 38:816-822, 1995
117. Savola K, Sabbah E, Kulmala P, Vahasalo P, Ilonen J, Knip M: Autoantibodies associated with Type I diabetes mellitus persist after diagnosis in children. *Diabetologia* 41:1293-1297, 1998
118. Gorus FK, Goubert P, Semakula C, Vandewalle CL, De Schepper J, Scheen A, Christie MR, Pipeleers DG: IA-2-autoantibodies complement GAD65-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. The Belgian Diabetes Registry. *Diabetologia* 40:95-99, 1997
119. Neufeld M, Maclaren NK, Riley WJ, Lezotte D, McLaughlin JV, Silverstein J, Rosenbloom AL: Islet cell and other organ-specific antibodies in U.S. Caucasians and Blacks with insulin-dependent diabetes mellitus. *Diabetes* 29:589-592, 1980
120. Mustonen A, Knip M, Huttunen NP, Puukka R, Kaar ML, Akerblom HK: Evidence of delayed beta-cell destruction in type 1 (insulin-dependent) diabetic patients with persisting complement-fixing cytoplasmic islet cell antibodies. *Diabetologia* 27:421-426, 1984
121. Urakami T, Miyamoto Y, Matsunaga H, Owada M, Kitagawa T: Serial changes in the prevalence of islet cell antibodies and islet cell antibody titer in children with IDDM of abrupt or slow onset. *Diabetes Care* 18:1095-1099, 1995
122. Gianani R, Eisenbarth GS: Prediction and prevention of type I diabetes. *J Endocrinol Invest* 17:533-543, 1994
123. Spencer KM, Tarn A, Dean BM, Lister J, Bottazzo GF: Fluctuating islet-cell autoimmunity in unaffected relatives of patients with insulin-dependent diabetes. *Lancet* 1:764-766, 1984
124. Lendrum R, Walker G, Cudworth AG, Woodrow JC, Gamble DR: HLA-linked genes and islet-cell antibodies in diabetes mellitus. *Br Med J* 1:1565-1567, 1976
125. Forbes LV, Scott RS, Brown LJ, Darlow BA: Immunogenetic, clinical, and demographic characterization of childhood type I diabetes in New Zealand. *Diabetes Care* 18:1428-1433, 1995
126. Ginsberg-Fellner F, Dobersen MJ, Witt ME, Rayfield EJ, Rubinstein P, Notkins AL: HLA antigens, cytoplasmic islet cell antibodies, and carbohydrate tolerance in families of children with insulin-dependent diabetes mellitus. *Diabetes* 31:292-298, 1982
127. Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipeleers DG, Gorus FK: Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1*0301-DQB1*0302 haplotype at clinical type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years. The Belgian Diabetes Registry. *Diabetologia* 36:1155-1162, 1993
128. Pagano G, Cavallo-Perin P, Cavalot F, Dall'Omo AM, Masciola P, Suriani R, Amoroso A, Curtoni SE, Borelli I, Lenti G: Genetic, immunologic, and environmental heterogeneity of IDDM. Incidence and 12-mo follow-up of an Italian population. *Diabetes* 36:859-863, 1987
129. Gorus FK, Vandewalle CL, Dorchy H, Van Crombrugge P, Schuit FC, Pipeleers DG: Influence of age on the associations among insulin autoantibodies, islet cell antibodies, and HLA DAQ1*0301-DQB1*0302 in siblings of patients with type 1 (insulin-dependent) diabetes mellitus. Belgian Diabetes Registry. *J Clin Endocrinol Metab* 78:1172-1178, 1994
130. Kukko M, Kimpimaki T, Kupila A, Korhonen S, Kulmala P, Savola K, Simell T, Keskinen P, Ilonen J, Simell O, Knip M: Signs of beta-cell autoimmunity and HLA-defined

diabetes susceptibility in the Finnish population: the sib cohort from the Type 1 Diabetes Prediction and Prevention Study. *Diabetologia* 46:65-70, 2003
131. Kulmala P, Savola K, Reijonen H, Veijola R, Vahasalo P, Karjalainen J, Tuomilehto-Wolf E, Ilonen J, Tuomilehto J, Akerblom HK, Knip M: Genetic markers, humoral autoimmunity, and prediction of type 1 diabetes in siblings of affected children. Childhood Diabetes in Finland Study Group. *Diabetes* 49:48-58, 2000
132. Lipton RB, Atchison J, Dorman JS, Duquesnoy RJ, Eckenrode K, Orchard TJ, LaPorte RE, Riley WJ, Kuller LH, Drash AL, et al.: Genetic, immunological, and metabolic determinants of risk for type 1 diabetes mellitus in families. *Diabet Med* 9:224-232, 1992
133. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* 222:1337-1339, 1983
134. Atkinson MA, Maclaren NK, Riley WJ, Winter WE, Fisk DD, Spillar RP: Are insulin autoantibodies markers for insulin-dependent diabetes mellitus? *Diabetes* 35:894-898, 1986
135. Martikainen A, Saukkonen T, Kulmala PK, Reijonen H, Ilonen J, Teramo K, Koskela P, Knip M, Akerblom HK: Disease-associated antibodies in offspring of mothers with IDDM. *Diabetes* 45:1706-1710, 1996
136. Ziegler AG, Hummel M, Schenker M, Bonifacio E: Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 48:460-468, 1999
137. Kimpimaki T, Kulmala P, Savola K, Kupila A, Korhonen S, Simell T, Ilonen J, Simell O, Knip M: Natural history of beta-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. *J Clin Endocrinol Metab* 87:4572-4579, 2002
138. Kulmala P, Rahko J, Savola K, Vahasalo P, Sjoroos M, Reunanen A, Ilonen J, Knip M: Beta-cell autoimmunity, genetic susceptibility, and progression to type 1 diabetes in unaffected schoolchildren. *Diabetes Care* 24:171-173, 2001
139. Strebelow M, Schlosser M, Ziegler B, Rjasanowski I, Ziegler M: Karlsburg Type I diabetes risk study of a general population: frequencies and interactions of the four major Type I diabetes-associated autoantibodies studied in 9419 schoolchildren. *Diabetologia* 42:661-670, 1999
140. Kulmala P, Savola K, Petersen JS, Vahasalo P, Karjalainen J, Lopponen T, Dyrberg T, Akerblom HK, Knip M: Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes. A population-based study. The Childhood Diabetes in Finland Study Group. *J Clin Invest* 101:327-336, 1998
141. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926-933, 1996
142. Karjalainen J, Vahasalo P, Knip M, Tuomilehto-Wolf E, Virtala E, Akerblom HK: Islet cell autoimmunity and progression to insulin-dependent diabetes mellitus in genetically high- and low-risk siblings of diabetic children. The Childhood Diabetes in Finland (DiMe) Study Group. *Eur J Clin Invest* 26:640-649, 1996
143. Krischer JP, Schatz D, Riley WJ, Spillar RP, Silverstein JH, Schwartz S, Malone J, Shah S, Vadheim CM, Rotter JI, et al.: Insulin and islet cell autoantibodies as time-dependent covariates in the development of insulin-dependent diabetes: a prospective study in relatives. *J Clin Endocrinol Metab* 77:743-749, 1993
144. Karjalainen J, Knip M, Mustonen A, Ilonen J, Akerblom HK: Relation between insulin

antibody and complement-fixing islet cell antibody at clinical diagnosis of IDDM. *Diabetes* 35:620-622, 1986
145. Vardi P, Ziegler AG, Mathews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JI, Herskowitz RD, Rabizadeh A, Eisenbarth GS, et al.: Concentration of insulin autoantibodies at onset of type I diabetes. Inverse log-linear correlation with age. *Diabetes Care* 11:736-739, 1988
146. Wilkin T, Hoskins PJ, Armitage M, Rodier M, Casey C, Diaz JL, Pyke DA, Leslie RD: Value of insulin autoantibodies as serum markers for insulin-dependent diabetes mellitus. *Lancet* 1:480-481, 1985
147. Arslanian SA, Becker DJ, Rabin B, Atchison R, Eberhardt M, Cavender D, Dorman J, Drash AL: Correlates of insulin antibodies in newly diagnosed children with insulin-dependent diabetes before insulin therapy. *Diabetes* 34:926-930, 1985
148. Ziegler R, Alper CA, Awdeh ZL, Castano L, Brink SJ, Soeldner JS, Jackson RA, Eisenbarth GS: Specific association of HLA-DR4 with increased prevalence and level of insulin autoantibodies in first-degree relatives of patients with type I diabetes. *Diabetes* 40:709-714, 1991
149. Bonifacio E, Scirpoli M, Kredel K, Fuchtenbusch M, Ziegler AG: Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *J Immunol* 163:525-532, 1999
150. McEvoy RC, Witt ME, Ginsberg-Fellner F, Rubinstein P: Anti-insulin antibodies in children with type I diabetes mellitus. Genetic regulation of production and presence at diagnosis before insulin replacement. *Diabetes* 35:634-641, 1986
151. Bohmer K, Keilacker H, Kuglin B, Hubinger A, Bertrams J, Gries FA, Kolb H: Proinsulin autoantibodies are more closely associated with type 1 (insulin-dependent) diabetes mellitus than insulin autoantibodies. *Diabetologia* 34:830-834, 1991
152. Franke B, Galloway TS, WIlkin TJ: Developments in the prediction of type 1 diabetes mellitus, with special reference to insulin autoantibodies. *Diabetes Metab Res Rev* 21, 2005
153. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 298:167-169, 1982
154. Solimena M, Folli F, Denis-Donini S, Comi GC, Pozza G, De Camilli P, Vicari AM: Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N Engl J Med* 318:1012-1020, 1988
155. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151-156, 1990
156. Petersen JS, Russel S, Marshall MO, Kofod H, Buschard K, Cambon N, Karlsen AE, Boel E, Hagopian WA, Hejnaes KR, et al.: Differential expression of glutamic acid decarboxylase in rat and human islets. *Diabetes* 42:484-495, 1993
157. Seissler J, Amann J, Mauch L, Haubruck H, Wolfahrt S, Bieg S, Richter W, Holl R, Heinze E, Northemann W, et al.: Prevalence of autoantibodies to the 65- and 67-kD isoforms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 92:1394-1399, 1993
158. Vandewalle CL, Falorni A, Svanholm S, Lernmark A, Pipeleers DG, Gorus FK: High diagnostic sensitivity of glutamate decarboxylase autoantibodies in insulin-dependent diabetes mellitus with clinical onset between age 20 and 40 years. The Belgian Diabetes

Registry. *J Clin Endocrinol Metab* 80:846-851, 1995
159. Schmidli RS, DeAizpurua HJ, Harrison LC, Colman PG: Antibodies to glutamic acid decarboxylase in at-risk and clinical insulin-dependent diabetic subjects: relationship to age, sex and islet cell antibody status, and temporal profile. *J Autoimmun* 7:55-66, 1994
160. Gianani R, Pugliese A, Bonner-Weir S, Shiffrin AJ, Soeldner JS, Erlich H, Awdeh Z, Alper CA, Jackson RA, Eisenbarth GS: Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type I diabetes. *Diabetes* 41:347-353, 1992
161. Sabbah E, Kulmala P, Veijola R, Vahasalo P, Karjalainen J, Tuomilehto-Wolf E, Akerblom HK, Knip M: Glutamic acid decarboxylase antibodies in relation to other autoantibodies and genetic risk markers in children with newly diagnosed insulin-dependent diabetes. Childhood Diabetes in Finland Study Group. *J Clin Endocrinol Metab* 81:2455-2459, 1996
162. Christie MR, Daneman D, Champagne P, Delovitch TL: Persistence of serum antibodies to 64,000-Mr islet cell protein after onset of type I diabetes. *Diabetes* 39:653-656, 1990
163. Hermann R, Soltesz G: Prevalence and HLA association of GAD65 antibodies in Hungarian schoolchildren. *Hum Immunol* 64:152-155, 2003
164. Tuomi T, Bjorses P, Falorni A, Partanen J, Perheentupa J, Lernmark A, Miettinen A: Antibodies to glutamic acid decarboxylase and insulin-dependent diabetes in patients with autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 81:1488-1494, 1996
165. Christie MR, Vohra G, Champagne P, Daneman D, Delovitch TL: Distinct antibody specificities to a 64-kD islet cell antigen in type 1 diabetes as revealed by trypsin treatment. *J Exp Med* 172:789-794, 1990
166. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, Goldstein DE, Rae PM: Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* 152:3183-3188, 1994
167. Lan MS, Lu J, Goto Y, Notkins AL: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. *DNA Cell Biol* 13:505-514, 1994
168. Notkins AL, Zhang B, Matsumoto Y, Lan MS: Comparison of IA-2 with IA-2beta and with six other members of the protein tyrosine phosphatase family: recognition of antigenic determinants by IDDM sera. *J Autoimmun* 10:245-250, 1997
169. Lu J, Li Q, Xie H, Chen ZJ, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS: Identification of a second transmembrane protein tyrosine phosphatase, IA-2beta, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. *Proc Natl Acad Sci U S A* 93:2307-2311, 1996
170. Hoppu S, Harkonen T, Ronkainen MS, Akerblom HK, Knip M: IA-2 antibody epitopes and isotypes during the prediabetic process in siblings of children with type 1 diabetes. *J Autoimmun* 23:361-370, 2004
171. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol* 155:5419-5426, 1995
172. Savola K, Bonifacio E, Sabbah E, Kulmala P, Vahasalo P, Karjalainen J, Tuomilehto-Wolf E, Merilainen J, Akerblom HK, Knip M: IA-2 antibodies--a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood Diabetes in Finland

Study Group. *Diabetologia* 41:424-429, 1998
173. Xie H, Zhang B, Matsumoto Y, Li Q, Notkins AL, Lan MS: Autoantibodies to IA-2 and IA-2 beta in insulin-dependent diabetes mellitus recognize conformational epitopes: location of the 37- and 40-kDa fragments determined. *J Immunol* 159:3662-3667, 1997
174. Christie MR, Genovese S, Cassidy D, Bosi E, Brown TJ, Lai M, Bonifacio E, Bottazzo GF: Antibodies to islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. *Diabetes* 43:1254-1259, 1994
175. Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hawkes CJ, Ziegler AG: Perinatal autoimmunity in offspring of diabetic parents. The German Multicenter BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45:967-973, 1996
176. Decochez K, De Leeuw IH, Keymeulen B, Mathieu C, Rottiers R, Weets I, Vandemeulebroucke E, Truyen I, Kaufman L, Schuit FC, Pipeleers DG, Gorus FK: IA-2 autoantibodies predict impending type I diabetes in siblings of patients. *Diabetologia* 45:1658-1666, 2002
177. Genovese S, Bonfanti R, Bazzigaluppi E, Lampasona V, Benazzi E, Bosi E, Chiumello G, Bonifacio E: Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. *Diabetologia* 39:1223-1226, 1996
178. Hawa M, Rowe R, Lan MS, Notkins AL, Pozzilli P, Christie MR, Leslie RD: Value of antibodies to islet protein tyrosine phosphatase-like molecule in predicting type 1 diabetes. *Diabetes* 46:1270-1275, 1997
179. Eisenbarth GS: Type I diabetes mellitus. A chronic autoimmune disease. *N Engl J Med* 314:1360-1368, 1986
180. Itoh N, Hanafusa T, Miyazaki A, Miyagawa J, Yamagata K, Yamamoto K, Waguri M, Imagawa A, Tamura S, Inada M, et al.: Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. *J Clin Invest* 92:2313-2322, 1993
181. Hauben E, Roncarolo MG, Nevo U, Schwartz M: Beneficial autoimmunity in Type 1 diabetes mellitus. *Trends Immunol* 26:248-253, 2005
182. Liblau RS, Singer SM, McDevitt HO: Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 16:34-38, 1995
183. Katz JD, Benoist C, Mathis D: T helper cell subsets in insulin-dependent diabetes. *Science* 268:1185-1188, 1995
184. Andre I, Gonzalez A, Wang B, Katz J, Benoist C, Mathis D: Checkpoints in the progression of autoimmune disease: lessons from diabetes models. *Proc Natl Acad Sci U S A* 93:2260-2263, 1996
185. Mandrup-Poulsen T: Beta cell death and protection. *Ann N Y Acad Sci* 1005:32-42, 2003
186. Cao JY, Wang H: Role of Fas-FasL in insulitis in nonobese diabetic mouse. *Chin Med J (Engl)* 117:615-617, 2004
187. Toyoda H, Formby B: Contribution of T cells to the development of autoimmune diabetes in the NOD mouse model. *Bioessays* 20:750-757, 1998
188. Vreugdenhil GR, Geluk A, Ottenhoff TH, Melchers WJ, Roep BO, Galama JM: Molecular mimicry in diabetes mellitus: the homologous domain in coxsackie B virus protein 2C and islet autoantigen GAD65 is highly conserved in the coxsackie B-like enteroviruses and binds to the diabetes associated HLA-DR3 molecule. *Diabetologia* 41:40-

46, 1998
189. Jaeckel E, Manns M, Von Herrath M: Viruses and diabetes. *Ann N Y Acad Sci* 958:7-25, 2002
190. Gianani R, Sarvetnick N: Viruses, cytokines, antigens, and autoimmunity. *Proc Natl Acad Sci U S A* 93:2257-2259, 1996
191. Kurrer MO, Pakala SV, Hanson HL, Katz JD: Beta cell apoptosis in T cell-mediated autoimmune diabetes. *Proc Natl Acad Sci U S A* 94:213-218, 1997
192. Nerup J, Mandrup-Poulsen T, Helqvist S, Andersen HU, Pociot F, Reimers JI, Cuartero BG, Karlsen AE, Bjerre U, Lorenzen T: On the pathogenesis of IDDM. *Diabetologia* 37 Suppl 2:S82-89, 1994
193. Garza KM, Chan SM, Suri R, Nguyen LT, Odermatt B, Schoenberger SP, Ohashi PS: Role of antigen-presenting cells in mediating tolerance and autoimmunity. *J Exp Med* 191:2021-2027, 2000
194. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M: Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. *Science* 232:1545-1547, 1986
195. Corbett JA, Wang JL, Sweetland MA, Lancaster JR, Jr., McDaniel ML: Interleukin 1 beta induces the formation of nitric oxide by beta-cells purified from rodent islets of Langerhans. Evidence for the beta-cell as a source and site of action of nitric oxide. *J Clin Invest* 90:2384-2391, 1992
196. Kolb H, Kolb-Bachofen V: Type 1 (insulin-dependent) diabetes mellitus and nitric oxide. *Diabetologia* 35:796-797, 1992
197. Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Nieman A, Geller DA, Pipeleers DG, Bendtzen K, Hellerstrom C: Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *J Clin Invest* 93:1968-1974, 1994
198. Foulis AK, McGill M, Farquharson MA: Insulitis in type 1 (insulin-dependent) diabetes mellitus in man--macrophages, lymphocytes, and interferon-gamma containing cells. *J Pathol* 165:97-103, 1991
199. Foulis AK, Farquharson MA, Meager A: Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. *Lancet* 2:1423-1427, 1987
200. Leslie RD, Elliott RB: Early environmental events as a cause of IDDM. Evidence and implications. *Diabetes* 43:843-850, 1994
201. Tattersall RB, Pyke DA: Diabetes in identical twins. *Lancet* 2:1120-1125, 1972
202. Hyoty H, Taylor KW: The role of viruses in human diabetes. *Diabetologia* 45:1353-1361, 2002
203. Yoon JW: A new look at viruses in type 1 diabetes. *Diabetes Metab Rev* 11:83-107, 1995
204. Hyoty H, Leinikki P, Reunanen A, Ilonen J, Surcel HM, Rilva A, Kaar ML, Huupponen T, Hakulinen A, Makela AL, et al.: Mumps infections in the etiology of type 1 (insulin-dependent) diabetes. *Diabetes Res* 9:111-116, 1988
205. Lipman TH, Chang Y, Murphy KM: The epidemiology of type 1 diabetes in children in Philadelphia 1990-1994: evidence of an epidemic. *Diabetes Care* 25:1969-1975, 2002
206. Harris H: A case of diabetes mellitus quickly following mumps. *Boston Med Surg J* 160:465-469, 1899
207. Helmke K, Otten A, Willems WR, Brockhaus R, Mueller-Eckhardt G, Stief T, Bertrams J, Wolf H, Federlin K: Islet cell antibodies and the development of diabetes mellitus in relation to mumps infection and mumps vaccination. *Diabetologia* 29:30-33,

1986
208. Lindberg B, Ahlfors K, Carlsson A, Ericsson UB, Landin-Olsson M, Lernmark A, Ludvigsson J, Sundkvist G, Ivarsson SA: Previous exposure to measles, mumps, and rubella--but not vaccination during adolescence--correlates to the prevalence of pancreatic and thyroid autoantibodies. *Pediatrics* 104:e12, 1999
209. Hyoty H, Hiltunen M, Reunanen A, Leinikki P, Vesikari T, Lounamaa R, Tuomilehto J, Akerblom HK: Decline of mumps antibodies in type 1 (insulin-dependent) diabetic children and a plateau in the rising incidence of type 1 diabetes after introduction of the mumps-measles-rubella vaccine in Finland. Childhood Diabetes in Finland Study Group. *Diabetologia* 36:1303-1308, 1993
210. Menser MA, Forrest JM, Bransby RD: Rubella infection and diabetes mellitus. *Lancet* 1:57-60, 1978
211. McIntosh ED, Menser MA: A fifty-year follow-up of congenital rubella. *Lancet* 340:414-415, 1992
212. Forrest JM, Menser MA, Burgess JA: High frequency of diabetes mellitus in young adults with congenital rubella. *Lancet* 2:332-334, 1971
213. Sibley JT: Concurrent onset of adult onset Still's disease and insulin dependent diabetes mellitus. *Ann Rheum Dis* 49:547-548, 1990
214. Ginsberg-Fellner F, Witt ME, Yagihashi S, Dobersen MJ, Taub F, Fedun B, McEvoy RC, Roman SH, Davies RG, Cooper LZ, et al.: Congenital rubella syndrome as a model for type 1 (insulin-dependent) diabetes mellitus: increased prevalence of islet cell surface antibodies. *Diabetologia* 27 Suppl:87-89, 1984
215. Rubinstein P, Walker ME, Fedun B, Witt ME, Cooper LZ, Ginsberg-Fellner F: The HLA system in congenital rubella patients with and without diabetes. *Diabetes* 31:1088-1091, 1982
216. Rayfield EJ, Kelly KJ, Yoon JW: Rubella virus-induced diabetes in the hamster. *Diabetes* 35:1278-1281, 1986
217. Hviid A, Stellfeld M, Wohlfahrt J, Melbye M: Childhood vaccination and type 1 diabetes. *N Engl J Med* 350:1398-1404, 2004
218. Ward KP, Galloway WH, Auchterlonie IA: Congenital cytomegalovirus infection and diabetes. *Lancet* 1:497, 1979
219. Ivarsson SA, Lindberg B, Nilsson KO, Ahlfors K, Svanberg L: The prevalence of type 1 diabetes mellitus at follow-up of Swedish infants congenitally infected with cytomegalovirus. *Diabet Med* 10:521-523, 1993
220. Hiltunen M, Hyoty H, Karjalainen J, Leinikki P, Knip M, Lounamaa R, Akerblom HK: Serological evaluation of the role of cytomegalovirus in the pathogenesis of IDDM: a prospective study. The Childhood Diabetes in Finland Study Group. *Diabetologia* 38:705-710, 1995
221. Suenaga K, Yoon JW: Association of beta-cell-specific expression of endogenous retrovirus with development of insulitis and diabetes in NOD mouse. *Diabetes* 37:1722-1726, 1988
222. Hao W, Serreze DV, McCulloch DK, Neifing JL, Palmer JP: Insulin (auto)antibodies from human IDDM cross-react with retroviral antigen p73. *J Autoimmun* 6:787-798, 1993
223. Hyoty H, Rasanen L, Hiltunen M, Lehtinen M, Huupponen T, Leinikki P: Decreased antibody reactivity to Epstein-Barr virus capsid antigen in type 1 (insulin-dependent) diabetes mellitus. *Apmis* 99:359-363, 1991
224. Parkkonen P, Hyoty H, Ilonen J, Reijonen H, Yla-Herttuala S, Leinikki P: Antibody

reactivity to an Epstein-Barr virus BERF4-encoded epitope occurring also in Asp-57 region of HLA-DQ8 beta chain. Childhood Diabetes in Finland Study Group. *Clin Exp Immunol* 95:287-293, 1994
225. Hyoty H, Hiltunen M, Knip M, Laakkonen M, Vahasalo P, Karjalainen J, Koskela P, Roivainen M, Leinikki P, Hovi T, et al.: A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes* 44:652-657, 1995
226. Tauriainen S, Salminen K, Hyoty H: Can enteroviruses cause type 1 diabetes? *Ann N Y Acad Sci* 1005:13-22, 2003
227. Gamble DR, Taylor KW: Seasonal incidence of diabetes mellitus. *Br Med J* 3:631-633, 1969
228. Rewers M, LaPorte RE, Walczak M, Dmochowski K, Bogaczynska E: Apparent epidemic of insulin-dependent diabetes mellitus in Midwestern Poland. *Diabetes* 36:106-113, 1987
229. Wagenknecht LE, Roseman JM, Herman WH: Increased incidence of insulin-dependent diabetes mellitus following an epidemic of Coxsackievirus B5. *Am J Epidemiol* 133:1024-1031, 1991
230. Fuchtenbusch M, Irnstetter A, Jager G, Ziegler AG: No evidence for an association of coxsackie virus infections during pregnancy and early childhood with development of islet autoantibodies in offspring of mothers or fathers with type 1 diabetes. *J Autoimmun* 17:333-340, 2001
231. Graves PM, Rotbart HA, Nix WA, Pallansch MA, Erlich HA, Norris JM, Hoffman M, Eisenbarth GS, Rewers M: Prospective study of enteroviral infections and development of beta-cell autoimmunity. Diabetes autoimmunity study in the young (DAISY). *Diabetes Res Clin Pract* 59:51-61, 2003
232. Salminen KK, Vuorinen T, Oikarinen S, Helminen M, Simell S, Knip M, Ilonen J, Simell O, Hyoty H: Isolation of enterovirus strains from children with preclinical Type 1 diabetes. *Diabet Med* 21:156-164, 2004
233. Dahlquist GG, Ivarsson S, Lindberg B, Forsgren M: Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM. A population-based case-control study. *Diabetes* 44:408-413, 1995
234. Hyoty H: Enterovirus infections and type 1 diabetes. *Ann Med* 34:138-147, 2002
235. Hiltunen M, Hyoty H, Knip M, Ilonen J, Reijonen H, Vahasalo P, Roivainen M, Lonnrot M, Leinikki P, Hovi T, Akerblom HK: Islet cell antibody seroconversion in children is temporally associated with enterovirus infections. Childhood Diabetes in Finland (DiMe) Study Group. *J Infect Dis* 175:554-560, 1997
236. Lonnrot M, Korpela K, Knip M, Ilonen J, Simell O, Korhonen S, Savola K, Muona P, Simell T, Koskela P, Hyoty H: Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes* 49:1314-1318, 2000
237. Roivainen M, Rasilainen S, Ylipaasto P, Nissinen R, Ustinov J, Bouwens L, Eizirik DL, Hovi T, Otonkoski T: Mechanisms of coxsackievirus-induced damage to human pancreatic beta-cells. *J Clin Endocrinol Metab* 85:432-440, 2000
238. Roivainen M, Ylipaasto P, Savolainen C, Galama J, Hovi T, Otonkoski T: Functional impairment and killing of human beta cells by enteroviruses: the capacity is shared by a wide range of serotypes, but the extent is a characteristic of individual virus strains. *Diabetologia* 45:693-702, 2002

239. Dahlquist GG, Blom LG, Persson LA, Sandstrom AI, Wall SG: Dietary factors and the risk of developing insulin dependent diabetes in childhood. *Bmj* 300:1302-1306, 1990
240. Elliott RB, Martin JM: Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? *Diabetologia* 26:297-299, 1984
241. Savilahti E, Akerblom HK, Tainio VM, Koskimies S: Children with newly diagnosed insulin dependent diabetes mellitus have increased levels of cow's milk antibodies. *Diabetes Res* 7:137-140, 1988
242. Dahlquist G, Savilahti E, Landin-Olsson M: An increased level of antibodies to beta-lactoglobulin is a risk determinant for early-onset type 1 (insulin-dependent) diabetes mellitus independent of islet cell antibodies and early introduction of cow's milk. *Diabetologia* 35:980-984, 1992
243. Saukkonen T, Savilahti E, Vaarala O, Virtala ET, Tuomilehto J, Akerblom HK: Children with newly diagnosed IDDM have increased levels of antibodies to bovine serum albumin but not to ovalbumin. Childhood Diabetes in Finland Study Group. *Diabetes Care* 17:970-976, 1994
244. Dahlquist G, Blom L, Lonnberg G: The Swedish Childhood Diabetes Study--a multivariate analysis of risk determinants for diabetes in different age groups. *Diabetologia* 34:757-762, 1991
245. Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ: Reduced risk of IDDM among breast-fed children. The Colorado IDDM Registry. *Diabetes* 37:1625-1632, 1988
246. Akerblom HK, Virtanen SM, Ilonen J, Savilahti E, Vaarala O, Reunanen A, Teramo K, Hamalainen AM, Paronen J, Riikjarv MA, Ormisson A, Ludvigsson J, Dosch HM, Hakulinen T, Knip M: Dietary manipulation of beta cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study. *Diabetologia* 48:829-837, 2005
247. Dahl-Jorgensen K, Joner G, Hanssen KF: Relationship between cows' milk consumption and incidence of IDDM in childhood. *Diabetes Care* 14:1081-1083, 1991
248. Virtanen SM, Laara E, Hypponen E, Reijonen H, Rasanen L, Aro A, Knip M, Ilonen J, Akerblom HK: Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood diabetes in Finland study group. *Diabetes* 49:912-917, 2000
249. Ludvigsson J: Cow-milk-free diet during last trimester of pregnancy does not influence diabetes-related autoantibodies in nondiabetic children. *Ann N Y Acad Sci* 1005:275-278, 2003
250. Rakieten N, Rakieten ML, Nadkarni MV: Studies on the diabetogenic action of streptozotocin (NSC-37917). *Cancer Chemother Rep* 29:91-98, 1963
251. Fine DH, Ross R, Rounbehler DP, Silvergleid A, Song L: Formation in vivo of volatile N-nitrosamines in man after ingestion of cooked bacon and spinach. *Nature* 265:753-755, 1977
252. Virtanen SM, Rasanen L, Ylonen K, Aro A, Clayton D, Langholz B, Pitkaniemi J, Savilahti E, Lounamaa R, Tuomilehto J, et al.: Early introduction of dairy products associated with increased risk of IDDM in Finnish children. The Childhood in Diabetes in Finland Study Group. *Diabetes* 42:1786-1790, 1993
253. Scott FW, Daneman D, Martin JM: Evidence for a critical role of diet in the development of insulin-dependent diabetes mellitus. *Diabetes Res* 7:153-157, 1988
254. Nerup J, Mandrup-Poulsen T, Molvig J, Helqvist S, Wogensen L, Egeberg J: Mechanisms of pancreatic beta-cell destruction in type I diabetes. *Diabetes Care* 11 Suppl

1:16-23, 1988
255. Hoorfar J, Buschard K, Dagnaes-Hansen F: Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. *Br J Nutr* 69:597-607, 1993
256. Wilkin TJ: To: Pundziute - Lycka. A et al. (2002). The incidence of type 1 diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. Diabetologia 45: 783-791. *Diabetologia* 45:1734, 2002
257. Wilkin T, Ludvigsson J, Greenbaum C, Palmer J, Becker D, Bruining J: Future intervention trials in type 1 diabetes. *Diabetes Care* 27:996-997, 2004
258. Hypponen E, Virtanen SM, Kenward MG, Knip M, Akerblom HK: Obesity, increased linear growth, and risk of type 1 diabetes in children. *Diabetes Care* 23:1755-1760, 2000
259. Blom L, Nystrom L, Dahlquist G: The Swedish childhood diabetes study. Vaccinations and infections as risk determinants for diabetes in childhood. *Diabetologia* 34:176-181, 1991
260. Luong K, Nguyen LT, Nguyen DN: The role of vitamin D in protecting type 1 diabetes mellitus. *Diabetes Metab Res Rev* 21:338-346, 2005
261. Mathieu C, Gysemans C, Giulietti A, Bouillon R: Vitamin D and diabetes. *Diabetologia* 48:1247-1257, 2005
262. Cosgrove M: Do stressful life events cause type 1 diabetes? *Occup Med (Lond)* 54:250-254, 2004
263. Srikanta S, Ricker AT, McCulloch DK, Soeldner JS, Eisenbarth GS, Palmer JP: Autoimmunity to insulin, beta cell dysfunction, and development of insulin-dependent diabetes mellitus. *Diabetes* 35:139-142, 1986
264. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS: Life-table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with type I diabetes. *Diabetes* 38:1320-1325, 1989
265. Roll U, Christie MR, Standl E, Ziegler AG: Associations of anti-GAD antibodies with islet cell antibodies and insulin autoantibodies in first-degree relatives of type I diabetic patients. *Diabetes* 43:154-160, 1994
266. Borg H, Fernlund P, Sundkvist G: Protein tyrosine phosphatase-like protein IA2-antibodies plus glutamic acid decarboxylase 65 antibodies (GADA) indicates autoimmunity as frequently as islet cell antibodies assay in children with recently diagnosed diabetes mellitus. *Clin Chem* 43:2358-2363, 1997
267. Borg H, Marcus C, Sjoblad S, Fernlund P, Sundkvist G: Islet cell antibody frequency differs from that of glutamic acid decarboxylase antibodies/IA2 antibodies after diagnosis of diabetes. *Acta Paediatr* 89:46-51, 2000
268. Vandewalle CL, Falorni A, Lernmark A, Goubert P, Dorchy H, Coucke W, Semakula C, Van der Auwera B, Kaufman L, Schuit FC, Pipeleers DG, Gorus FK: Associations of GAD65- and IA-2- autoantibodies with genetic risk markers in new-onset IDDM patients and their siblings. The Belgian Diabetes Registry. *Diabetes Care* 20:1547-1552, 1997
269. Yu L, Gianani R, Eisenbarth GS: Quantitation of glutamic acid decarboxylase autoantibody levels in prospectively evaluated relatives of patients with type I diabetes. *Diabetes* 43:1229-1233, 1994
270. Achenbach P, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, Bonifacio E, Ziegler AG: Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes* 53:384-392, 2004
271. Christie MR, Roll U, Payton MA, Hatfield EC, Ziegler AG: Validity of screening for

individuals at risk for type I diabetes by combined analysis of antibodies to recombinant proteins. *Diabetes Care* 20:965-970, 1997
272. Srikanta S, Ganda OP, Gleason RE, Jackson RA, Soeldner JS, Eisenbarth GS: Pre-type I diabetes. Linear loss of beta cell response to intravenous glucose. *Diabetes* 33:717-720, 1984
273. Knip M, Vahasalo P, Karjalainen J, Lounamaa R, Akerblom HK: Natural history of preclinical IDDM in high risk siblings. Childhood Diabetes in Finland Study Group. *Diabetologia* 37:388-393, 1994
274. Thivolet C, Beaufrere B, Geburher L, Chatelain P, Orgiazzi J, Francois R: Autoantibodies and genetic factors associated with the development of type 1 (insulin-dependent) diabetes mellitus in first degree relatives of diabetic patients. *Diabetologia* 34:186-191, 1991
275. Srikanta S, Ganda OP, Rabizadeh A, Soeldner JS, Eisenbarth GS: First-degree relatives of patients with type I diabetes mellitus. Islet-cell antibodies and abnormal insulin secretion. *N Engl J Med* 313:461-464, 1985
276. Vardi P, Crisa L, Jackson RA: Predictive value of intravenous glucose tolerance test insulin secretion less than or greater than the first percentile in islet cell antibody positive relatives of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 34:93-102, 1991
277. Chase HP, Garg SK, Butler-Simon N, Klingensmith G, Norris L, Ruskey CT, O'Brien D: Prediction of the course of pre-type I diabetes. *J Pediatr* 118:838-841, 1991
278. Bingley PJ: Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: the ICARUS data set. Islet Cell Antibody Register Users Study. *Diabetes* 45:1720-1728, 1996
279. Robert JJ, Deschamps I, Chevenne D, Roger M, Mogenet A, Boitard C: Relationship between first-phase insulin secretion and age, HLA, islet cell antibody status, and development of type I diabetes in 220 juvenile first-degree relatives of diabetic patients. *Diabetes Care* 14:718-723, 1991
280. Rowe RE, Leech NJ, Nepom GT, McCulloch DK: High genetic risk for IDDM in the Pacific Northwest. First report from the Washington State Diabetes Prediction Study. *Diabetes* 43:87-94, 1994
281. Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, Leslie RD: Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44:354-362, 2001
282. Gorsuch AN, Spencer KM, Lister J, Wolf E, Bottazzo GF, Cudworth AG: Can future type I diabetes be predicted? A study in families of affected children. *Diabetes* 31:862-866, 1982
283. Cavender DE, Wagener DK, Rabin BS, Becker DJ, Orchard TJ, Eberhardt MS, LaPorte RE, Drash AL, Kuller LH: The Pittsburgh Insulin-Dependent Diabetes Mellitus (IDDM) study. HLA antigens and haplotypes as risk factors for the development of IDDM in IDDM patients and their siblings. *J Chronic Dis* 37:555-568, 1984
284. Lipton RB, Kocova M, LaPorte RE, Dorman JS, Orchard TJ, Riley WJ, Drash AL, Becker DJ, Trucco M: Autoimmunity and genetics contribute to the risk of insulin-dependent diabetes mellitus in families: islet cell antibodies and HLA DQ heterodimers. *Am J Epidemiol* 136:503-512, 1992
285. Pugliese A, Gianani R, Moromisato R, Awdeh ZL, Alper CA, Erlich HA, Jackson RA, Eisenbarth GS: HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM.

Diabetes 44:608-613, 1995
286. Knip M: Prediction and prevention of type 1 diabetes. *Acta Paediatr Suppl* 425:54-62, 1998
287. Bingley PJ, Bonifacio E, Shattock M, Gillmor HA, Sawtell PA, Dunger DB, Scott RD, Bottazzo GF, Gale EA: Can islet cell antibodies predict IDDM in the general population? *Diabetes Care* 16:45-50, 1993
288. Landin-Olsson M, Karlsson A, Dahlquist G, Blom L, Lernmark A, Sundkvist G: Islet cell and other organ-specific autoantibodies in all children developing type 1 (insulin-dependent) diabetes mellitus in Sweden during one year and in matched control children. *Diabetologia* 32:387-395, 1989
289. Boehm BO, Seissler J, Gluck M, Manfras BJ, Thomas H, Schmidt K, Rudert WA, Usadel KH, Trucco M, Scherbaum WA: The level and the persistence of islet cell antibodies in healthy schoolchildren are associated with polymorphic residues of the HLA-DQ beta chain. *Dis Markers* 9:273-280, 1991
290. Seissler J, Hering B, Richter W, Gluck M, Yassin N, Bretzel RG, Boehm BO, Federlin K, Scherbaum WA: Antibodies to the M(r) 64,000 (64K) protein in islet cell antibody positive non-diabetic individuals indicate high risk for impaired beta-cell function. *Diabetologia* 35:550-554, 1992
291. Levy-Marchal C, Tichet J, Fajardy I, Gu XF, Dubois F, Czernichow P: Islet cell antibodies in normal French schoolchildren. *Diabetologia* 35:577-582, 1992
292. Kulmala P, Rahko J, Savola K, Vahasalo P, Veijola R, Sjoroos M, Reunanen A, Ilonen J, Knip M: Stability of autoantibodies and their relation to genetic and metabolic markers of Type I diabetes in initially unaffected schoolchildren. *Diabetologia* 43:457-464, 2000
293. Nejentsev S, Sjoroos M, Soukka T, Knip M, Simell O, Lovgren T, Ilonen J: Population-based genetic screening for the estimation of Type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabet Med* 16:985-992, 1999
294. Schlosser M, Strebelow M, Rjasanowski I, Kerner W, Wassmuth R, Ziegler M: Prevalence of diabetes-associated autoantibodies in schoolchildren: the Karlsburg Type 1 Diabetes Risk Study. *Ann N Y Acad Sci* 1037:114-117, 2004
295. LaGasse JM, Brantley MS, Leech NJ, Rowe RE, Monks S, Palmer JP, Nepom GT, McCulloch DK, Hagopian WA: Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. *Diabetes Care* 25:505-511, 2002
296. Colman PG, McNair PD, Gellert S, Kewming K, Schmidli RS, Steele CE, Harrison LC: Development of autoantibodies to islet antigens during childhood: implications for preclinical type 1 diabetes screening. *Pediatr Diabetes* 3:144-148, 2002
297. Schmidt KD, Valeri C, Leslie RD: Autoantibodies in Type 1 diabetes. *Clin Chim Acta* 354:35-40, 2005
298. Harrison LC: Risk assessment, prediction and prevention of type 1 diabetes. *Pediatr Diabetes* 2:71-82, 2001
299. Knip M: [Can type-1 diabetes in children be prevented?]. *Nord Med* 107:207-210, 1992
300. Pozzilli P: Prevention of insulin-dependent diabetes: where are we now? *Diabetes Metab Rev* 12:127-135, 1996
301. Mandrup-Poulsen T, Reimers JI, Andersen HU, Pociot F, Karlsen AE, Bjerre U, Nerup J: Nicotinamide treatment in the prevention of insulin-dependent diabetes mellitus. *Diabetes*

Metab Rev 9:295-309, 1993
302. Atkinson MA, Leiter EH: The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med* 5:601-604, 1999
303. Hancock WW, Polanski M, Zhang J, Blogg N, Weiner HL: Suppression of insulitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta, and prostaglandin-E. *Am J Pathol* 147:1193-1199, 1995
304. Atkinson MA, Maclaren NK, Luchetta R: Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. *Diabetes* 39:933-937, 1990
305. Fuchtenbusch M, Rabl W, Grassl B, Bachmann W, Standl E, Ziegler AG: Delay of type I diabetes in high risk, first degree relatives by parenteral antigen administration: the Schwabing Insulin Prophylaxis Pilot Trial. *Diabetologia* 41:536-541, 1998
306. Pozzilli P: The DPT-1 trial: a negative result with lessons for future type 1 diabetes prevention. *Diabetes Metab Res Rev* 18:257-259, 2002
307. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E: Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care* 28:1068-1076, 2005
308. Kupila A, Sipila J, Keskinen P, Simell T, Knip M, Pulkki K, Simell O: Intranasally administered insulin intended for prevention of type 1 diabetes--a safety study in healthy adults. *Diabetes Metab Res Rev* 19:415-420, 2003
309. Lacy PE: Pancreatic transplantation as a means of insulin delivery. *Diabetes Care* 5 Suppl 1:93-97, 1982
310. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230-238, 2000
311. Ryan EA, Paty BW, Senior PA, Shapiro AM: Risks and side effects of islet transplantation. *Curr Diab Rep* 4:304-309, 2004
312. Peck AB, Ramiya V: In vitro-generation of surrogate islets from adult stem cells. *Transpl Immunol* 12:259-272, 2004
313. Trucco M: Regeneration of the pancreatic beta cell. *J Clin Invest* 115:5-12, 2005
314. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R: Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 292:1389-1394, 2001
315. Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E, Reunanen A, Virtala E, Kaprio EA, Akerblom HK: Epidemiology of childhood diabetes mellitus in Finland--background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. The Childhood Diabetes in Finland (DiMe) Study Group. *Diabetologia* 35:70-76, 1992
316. WHO Study Group: Diabetes Mellitus. *Technical Report Series* 727:10-12, 1985
317. Ilonen J, Reijonen H, Herva E, Sjoroos M, Iitia A, Lovgren T, Veijola R, Knip M, Akerblom HK: Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. The Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care* 19:795-800, 1996
318. Lernmark A, Molenaar JL, van Beers WA, Yamaguchi Y, Nagataki S, Ludvigsson J, Maclaren NK: The Fourth International Serum Exchange Workshop to standardize cytoplasmic islet cell antibodies. The Immunology and Diabetes Workshops and Participating Laboratories. *Diabetologia* 34:534-535, 1991

319. Greenbaum CJ, Palmer JP, Nagataki S, Yamaguchi Y, Molenaar JL, Van Beers WA, MacLaren NK, Lernmark A: Improved specificity of ICA assays in the Fourth International Immunology of Diabetes Serum Exchange Workshop. *Diabetes* 41:1570-1574, 1992
320. Petersen JS, Hejnaes KR, Moody A, Karlsen AE, Marshall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T: Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes* 43:459-467, 1994
321. Veijola R, Reijonen H, Vahasalo P, Sabbah E, Kulmala P, Ilonen J, Akerblom HK, Knip M: HLA-DQB1-defined genetic susceptibility, beta cell autoimmunity, and metabolic characteristics in familial and nonfamilial insulin-dependent diabetes mellitus. Childhood Diabetes in Finland (DiMe) Study Group. *J Clin Invest* 98:2489-2495, 1996
322. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47:1857-1866, 1998
323. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375-1384, 1965
324. Hjelm M: Enzymatic determination of hexoses in blood and urine. *Scand J Clin Lab Invest Suppl* 18:85-98, 1966
325. Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL: Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. *Pediatr Diabetes* 4:119-125, 2003
326. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419, 1985
327. Tuomilehto-Wolf E, Tuomilehto J, Cepaitis Z, Lounamaa R: New susceptibility haplotype for type 1 diabetes. DIME Study Group. *Lancet* 2:299-302, 1989
328. Sjoroos M, Iitia A, Ilonen J, Reijonen H, Lovgren T: Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *Biotechniques* 18:870-877, 1995
329. Chase HP, Voss MA, Butler-Simon N, Hoops S, O'Brien D, Dobersen MJ: Diagnosis of pre-type I diabetes. *J Pediatr* 111:807-812, 1987
330. Honeyman MC, Harrison LC, Drummond B, Colman PG, Tait BD: Analysis of families at risk for insulin-dependent diabetes mellitus reveals that HLA antigens influence progression to clinical disease. *Mol Med* 1:576-582, 1995
331. Seissler J, Hatziagelaki E, Scherbaum WA: Modern concepts for the prediction of type 1 diabetes. *Exp Clin Endocrinol Diabetes* 109 Suppl 2:S304-316, 2001
332. Eisenbarth GS, Moriyama H, Robles DT, Liu E, Yu L, Babu S, Redondo M, Gottlieb P, Wegmann D, Rewers M: Insulin autoimmunity: prediction/precipitation/prevention type 1A diabetes. *Autoimmun Rev* 1:139-145, 2002
333. Bonifacio E, Hummel M, Walter M, Schmid S, Ziegler AG: IDDM1 and multiple family history of type 1 diabetes combine to identify neonates at high risk for type 1 diabetes. *Diabetes Care* 27:2695-2700, 2004