TYPE I FAMILY HISTORY AND
GAD AUTOANTIBODIES
IN SUBJECTS WITHOUT DIABETES AND
PATIENTS WITH TYPE 2 DIABETES:
PREVALENCE AND ASSOCIATION
WITH INSULIN DEFICIENCY AND
DEVELOPMENT OF DIABETES

Virve Lundgren
Folkhälsan Research Center
and
Institute of Clinical Medicine
Department of Medicine
Faculty of Medicine
University of Helsinki

Academic dissertation

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It is possible to achieve something when you are talented – but usually you need to have passion and devotion that makes you work hard enough to improve your talent and achieve the goal. After this you realize that the goal was only a minor thing and enjoyment of the doing is most important.

To my wonderful family Juha, Marie, Sofie, Anette and Johan
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**ABSTRACT**

**Background and aims.** Evidence is emerging that Type 1 and Type 2 diabetes cluster in families. GAD autoantibodies (GADA) occur more often in families with Type 1 and Type 2 diabetes, are useful to predict diabetes in Type 1 diabetes studies, but their predictive value in adult relatives without diabetes is still unclear. GADA positivity is associated with a more severe insulin deficiency in Type 2 diabetes (LADA), but some patients, especially those with lower GADA levels, are more like Type 2 diabetes patients. The aim of this thesis was to learn whether a Type 1 family history (FH) has any impact on prevalence of GADA, and further, what is the impact of GADA and FH on the development of diabetes, its clinical characteristics, and the development of insulin deficiency.

**Subjects and methods.** 1) Relatives (n=4976) and spouses (n=770) of diabetes patients participating in the Botnia study. Follow-up (median 8.7 years) data were available for 2764 subjects without diabetes. 2) All GADA-positive patients with Type 2 diabetes (n=213) and 648 GADA- negative patients with Type 2 diabetes participating in the Botnia study. 3) Patients with Type 2 diabetes with a family history of both Type 1 and Type 2 diabetes (Type 1 FH, n=196) or Type 2 diabetes only (Type 2 FH, n=139) were recruited through newspaper advertisements (MIX study) and matched for age, sex, body mass index (BMI), and age at diagnosis. All subjects/patients participated in an oral glucose tolerance test with blood samples taken after fasting for measurement of HbA1c, serum lipids, GADA, plasma glucose, serum insulin, and serum C-peptide levels. The homeostasis model assessment, the insulinogenic index, and the disposition index were calculated for estimation of the patients’ insulin secretion and sensitivity. Patients in the MIX study also underwent a glucagon-insulin intolerance test (GITT) for estimation of their insulin secretion and insulin resistance.

**Results.** First-degree Type 1 FH and GADA positivity predicted diabetes in adult relatives, but seroconversion to GADA was the most important risk factor. Three subjects developed Type 1 diabetes, and others had a non-insulin-dependent phenotype 1 year after diagnosis. BMI, age of onset, and insulin secretion and lipids associated with GADA positivity level even before the onset of diabetes and after the diagnosis. LADA patients were leaner, but surprisingly more insulin resistant according to the insulin tolerance test. A Type 1 FH was associated with higher prevalence of GADA and strength of GADA positivity but also...
predisposed to a more severe insulin deficiency with earlier onset not only in patients with LADA, but also in patients with Type 2 diabetes.

**Conclusions.** A family history of Type 1 diabetes was associated with GADA positivity and especially with high GADA positivity. Both GADA and a family history of diabetes affected the risk for developing diabetes. High GADA levels implied a clearly increased risk for diabetes in both the relatives and the control subjects, while low or medium-high levels implied an increased risk only for the relatives of patients with diabetes. The highest risk was for those who converted to GADA positivity during the follow-up period. A combination of GADA and a Type 1 FH was associated in patients with diabetes with the greatest risk for development of insulin deficiency. Most of the patients who developed a marked insulin deficiency had a Type 1 FH, which has clinical implications for screening for insulin deficiency. Further, the heterogeneity of LADA diabetes is in part explained by a Type 1 FH and GADA levels.
ORIGINAL PUBLICATIONS

This thesis is based on the following publications and additional unpublished data.


The articles are cited in the text as their Roman numerals and reprinted here with publishers’ permission.
ABBREVIATIONS

BMI Body mass index
DBP Diastolic blood pressure
FFA Free fatty acids
FH Family history
FH_{T1D} Family history of Type 1 diabetes
FH_{T2D} Family history of Type 2 diabetes
GADA Glutamic acid decarboxylase autoantibodies
GADA^{+/-} GADA positive/negative subject
GADA_{high} GADA subjects within the highest GADA quartile
GADA_{low-med} GADA subjects within the low-medium GADA quartiles
GITT Glucagon-insulin tolerance test
HbA1c Glycosylated hemoglobin A_{1c}
HDL High-density lipoprotein
HLA Human leukocyte antigen
HOMA_{IR} Homeostatic model assessment of basal insulin resistance
IA-2A Autoantibodies to islet antigen 2 (tyrosin phosphatase-like protein
IAA Insulin autoantibodies
ICA Islet cell antibodies
ICAM-1 Intercellular adhesion molecule 1
IDF International Diabetes Federation
IFG Impaired fasting glucose tolerance
IGT Impaired glucose tolerance
LADA Latent autoimmune diabetes in adults
LADA_{high} LADA with GADA levels within the highest quartile
LADA_{low} LADA with GADA levels within the lowest quartile
LADA_{low-med} LADA with GADA levels within the low-medium quartiles
LADA_{med} LADA with GADA levels within the middle quartiles
LDL Low density lipoprotein
MIDD Maternally inherited diabetes and deafness
MIX family Patients with Type 1 and Type 2 family history
MODY Maturity-onset diabetes of the young
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>NGT</td>
<td>Normal glucose tolerance</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>T1D</td>
<td>Type 1 diabetes</td>
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<td>T2D</td>
<td>Type 2 diabetes</td>
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<td>Type 1 FH</td>
<td>Family history of Type 1 diabetes</td>
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<td>Type 2 FH</td>
<td>Family history of Type 2 diabetes</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
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1. INTRODUCTION

Today, about 336 million people have diabetes, and estimates are that by 2030 this number will have risen to 439 million (1). In Finland and Sweden an alarming increase in the incidence of Type 1 and Type 2 diabetes has also already occurred in young adults (2,3). Diabetes is a heterogeneous group of disorders, but with two major phenotypes, Type 1 and Type 2 diabetes, both characterized by β-cell failure. Type 2 diabetes is rapidly increasing, mainly because of overnutrition and lack of exercise, but these and other environmental factors may also explain the increase in Type 1 diabetes. It has been hypothesized, that Type 1 and Type 2 diabetes may represent different stages of the same disease, as many similarities exist in their risk factors, phenotypes, and immune responses.

There exist other types of diabetes, and a particular interest in this thesis is the latent autoimmune diabetes of adults (LADA). LADA patients are often misdiagnosed with Type 2 diabetes, because of the slow development of their insulin deficiency taking years. However, as a marker of autoimmunity they have GAD antibodies (GADA), which usually are detectable in patients with Type 1 diabetes. The phenotypic features of LADA patients are similar to those of both Type 1 and Type 2 diabetes patients. In children considered to be members of risk groups, the presence of multiple autoantibodies predict Type 1 diabetes. In adults they have served in studies as a marker of the strength of the autoimmune process leading to a more severe insulin deficiency, but the predictive value of GADA in adults, in subjects not having diabetes and patients with diabetes, is still unclear. We have hypothesized that GADA is a sign of a subclinical autoimmune process, as Lethagen et al (2002) showed, and that GADA are associated with a decrease in maximal insulin secretory capacity in subjects without diabetes. However, recent studies have indicated that many adult patients with diabetes who develop insulin deficiency but have no common autoantibodies, still have autoreactive T-cells as markers of an activated immune system (4,5).

Obesity, which often occurs along with insulin resistance and Type 2 diabetes, has been observed to be a risk factor also for childhood Type 1 diabetes (6,7). Insulin resistance of various degrees occurs in both types of diabetes and relate also to the age of the subjects and the diabetes duration. Insulin resistance has also been observable even in subjects without
diabetes (8-11) as well as in LADA patients, but the results for the LADA patients have been controversial (8,12-15).

In many studies, Type 1 and Type 2 diabetes seem to cluster in the same families (16-20), but a large UK study on the parents of Type 1 diabetes patients has contradicted this assumption (21). Our previous studies have also indicated that these families contain more LADA patients, and that a Type 1 FH is associated with a more severe defect in insulin secretion (22).

With relatives of patients with Type 1 and Type 2 diabetes and the patients’ healthy spouses without any FH for diabetes, we have conducted a large follow-up study on the prevalence of GADA and its predictive value in diabetes development and explore the possible etiopathological mechanisms behind development of diabetes and insulin deficiency. Further, we have studied the impact of a Type 1 family history on manifestation of GADA and development of insulin deficiency. To further clarify the heterogeneity of LADA, we have studied insulin secretion and insulin resistance and the development of insulin deficiency in a smaller, carefully matched group of patients with Type 2 diabetes, using more exact methods, with the aim of discovering how the differences could be explained by GADA, FH, or both. These studies could also have clinical implications, helping physicians achieve a more precise diagnosis earlier, which would lead to better diabetes management.
2. REVIEW OF THE LITERATURE

2.1 History of diabetes

The first described diabetes cases are believed to be of Type 1 diabetes. Diabetes was for the first time mentioned in an Egyptian manuscript in 1550 BC, described as “too great emptying of the urine”. At about the same time, Indian physicians found the disease and called it “madhumeda” meaning “honey urine”, since the urine attracted ants. The word “diabetes” meaning “to pass through” was established by the Greek physician Aretneus (Appollonius of Memphis) in 230 BC. Indian physicians Sushruta and Charaka were the first to identify Type 1 and Type 2 diabetes as separate conditions in 400-500 AD, Type 1 diabetes being the “diabetes of the youth” and Type 2 diabetes as the disease of “overweight” people. Diabetes “mellitus” (from honey) was first established by the British physician John Rolle in the 1700s to separate it from diabetes insipidus, which was also associated with frequent urination.

At the time of the Roman Empire the disease was rare; the Roman physician Galen commented that “he had seen only two cases during his career”. During the last five decades, diabetes has increased like an epidemic in developed and developing countries, while still remaining rare in less-developed countries. Great differences appear between populations (1); however, these differences are thought to originate from differing lifestyles, a decrease in exercise, and an increase in obesity, but also from the global trend in population aging (23). In 1967, Cerasi et al proposed that in addition to insulin resistance, a failure to secrete insulin also exists in Type 2 diabetes (24). Donath et al observed that hyperglycaemia induces β-cell apoptosis (25).

Both types of diabetes are considered multifactorial diseases with several predisposing genetic and environmental factors. Less prevalent types of diabetes also exist, such as maturity-onset diabetes of the young (MODY) forms of diabetes, gestational diabetes, and maternally inherited diabetes (MIDD). Both Type 1 and Type 2 diabetes are associated with complications affecting various organs due to damage in the small vessels (microangiopathy) and in the larger vessels (macroangiopathy). Complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases affect the quality of life and lead to increased morbidity, causing a burden on the individual as well as on the society as a whole (26-28).
2.2 Type 1 diabetes

Type 1 diabetes (T1D, formerly insulin dependent or juvenile diabetes) is characterized by the autoimmune destruction of β-cells by CD4+ and CD8+ T cells, accompanied by macrophages infiltrating the pancreatic islets and leading to total insulin deficiency (29,30). Patients cannot survive without insulin therapy. Two peaks of onset occur in children, one between the ages of 5 and 9, and the second between the ages of 10 and 14 (31). In a recent study by Thunander et al. in Kroneberg, Sweden, 57% (91 of 161) of all Type 1 patients were diagnosed after age 40; of adults aged 20 to 100 years 83% (91 of 109) were diagnosed after the age of 40 (3). In the same study were two peaks in incidence: diabetes was as high after age 50 to 80 as it was in children aged 5 to 9 years, as reported in several other studies (32,33). No gender difference was observable.

Type 1 diabetes accounts for 5 to 10% of all diabetes cases worldwide (34). In Europeans, the prevalence of Type 1 diabetes in the general population is about 0.4% (3,35), while in siblings of those with Type 1 diabetes, the prevalence is about 6% (35). The incidence of Type 1 diabetes varies geographically, so that a child in Finland is 57 times as likely to acquire the disease as a child in Venezuela or Pakistan (1). In fact, Finland has the highest Type 1 diabetes incidence in the world, together with Sweden; it is twice as high as in Denmark, Norway, Sardinia, Italy and Canada (36-39). A steady increase in incidence of childhood Type 1 diabetes has occurred worldwide, and the age of onset has declined (31,37,40), but Dahlqvist et al claim that this is merely a shift to younger age of onset and reported a decrease in the older 25 to 34 years age group (41).

Risk factors or causative entities for Type 1 diabetes have been studied vigorously, but still remain unresolved. In Finland, incidence has been rising about 4.2% per year (37), indicating that environmental factors influence its pathogenesis. Other indications of environmental influence include the 10-fold difference in occurrence among Caucasians living in different areas of Europe, as well as the tendency for an individual to acquire the incidence of the country of residence rather than of his/her country of origin (42,43). Interestingly, Somali children in Finland show the same prevalence of Type 1 diabetes as Finnish children, despite a different predisposing genetic background (42).
Diverse adhesion molecules participate in various immune responses and therefore have been implicated in the pathogenesis of autoimmune diseases. The intercellular adhesion molecule-1 (ICAM-1), a well-characterized surface glycoprotein, is involved in extravasation of lymphocytes from the circulation into the inflamed pancreas\(^5\). ICAM-1 may act as a receptor for an unknown pathogen that could induce β-cell destruction; ICAM-1 function as a receptor for several rhinoviruses \(^5\).

The Finnish Diabetes Prediction and Prevention study (DIPP) showed that β-cell autoimmunity is induced early in life already before age 3 months \(^4\). Studies of autoantibodies in relatives of childhood Type 1 diabetes patients show an autoantibody response that is multiple and has an occurrence of variable duration before the illness \(^45-49\). The most common autoantibodies include islet cell antibodies (ICA), insulin autoantibodies (IAA), tyrosin phosphatase-like protein autoantibodies (IA-2A) and glutamic acid decarboxylate 65 autoantibodies (GAD65Ab) \(^4\). Number of autoantibodies is related to risk for progression to Type 1 diabetes \(^45,50\). Clinical characteristics as well as combination of autoantibodies correlate with age of diabetes onset \(^32,51-53\). Older-onset patients with Type 1 diabetes more often have GADA, whereas the other types of autoantibodies are more prevalent in younger patients; the combination of autoantibodies seems also to be related to particular HLA risk genotype \(^52,53\). Antibody positivity has therefore served as a criterion for patient recruitment in prevention trials, and also IDDM1 (major susceptibility genes found within the HLA class II region of chromosome 6) genotyping has served to identify newborns at increased risk for developing Type 1 diabetes associated autoimmunity \(^54\).

### 2.3 GAD antibodies

Based on the presence or absence of autoantibodies Type 1 and Type 2 diabetes are now being classified as either autoimmune or not autoimmune diseases \(^57\). There are two types of immune responses: protective or destructive, the latter seen in autoimmune inflammatory diseases \(^57\). The activation of both T and B cells is present in most immune responses, the B-cell activation producing antibodies \(^57\). These have allowed study of disease activity and rate of disease progression, and they help us to classify and predict clinical disease. Still, information is lacking on the relevance of autoantibodies in a healthy adult population.
Autoantibodies against islets (ICA) were first discovered in the sera of patients with Type 1 diabetes at about the same time that the HLA association was first observed (58). First detection of GAD antibodies was by Baekkeskov et al. 1982, who also identified the 64-kDa autoantigen as GAD (59). Later, the association between HLA susceptibility and GADA was detected by the Leslie group (60).

GAD enzymes catalyze the formation of γ-aminobutyric acid (GABA) from glutamate in neurons and islet endocrine cells (61). GABA is located in microvesicles, believed to modulate the release of an islet hormone such as insulin (62). The first sign of β-cell autoimmunity is the appearance of autoantibodies in the blood. GAD antibodies are detectable in about 70 to 90% of Type 1 diabetes patients (3,63-65), occurring in a period of variable duration before the clinical presentation of diabetes (66). This preclinical period can last from a few months to over 10 years. Although autoantibodies to GAD65, IA-2, and insulin are clearly markers for autoimmune diabetes, what is unknown is whether they contribute to the pathogenesis or are simply a response to an existing underlying destructive process. Still, based on extensive studies with animal models, the view is that the cell-mediated immune response is actually responsible for the destruction of β-cells (67,68).

Environmental factors may trigger the initial β-cell damage. The exact triggering events causing a release of GADA into the blood are still unclear; however, damage to β-cells for some reason causes release of GAD into extracellular space and in genetically susceptible individuals, activates the immune system. Many environmental factors have been proposed to cause the false immune response leading to β-cell insult, ones such as viral infections and early access to cow’s milk proteins (69). Hyperglycaemia and fatty acids can promote an inflammatory process and β-cell apoptosis (24,70,71). The latest studies indicate that inflammation occurs in the gut before any appearance of autoantibodies (72) and that in the pathogenesis of Type 1 diabetes, modification of the gut’s immune system is fundamental (73). This inflammation makes the gut more vulnerable to leaks, so that nutrients or other agents such as proteins in food or viruses can induce autoantibody formation, which for some reason lacks suppression by the gut immune system (74). Animal studies have revealed differences in gut bacterial flora in those subjects who develop autoantibodies compared to those who do not (75). Animal studies reveal that autoreactive T-cells, that infiltrate
Langerhans islets and promote destruction of β-cells, are activated by antigen-presenting cells and their pathogenic activity is controlled by genes. (76). Antibodies serve as predictive markers of Type 1 diabetes, but studies of antibody-positive non-diabetic organ donors have revealed that signs of insulitis or a reduced β-cell mass are rare (77-79).

The frequency of GADA in a general population of 14,742 schoolchildren in Germany in the 1990s was 2.1% (IA-2A 1.8%, IAA 2.6% and ICA 0.4%) and for comparison, the multiple autoantibody frequency was 0.7%, (80). In adults, the GADA frequency, less studied, has been about 0.5 to 1.5% in the general population (81-84). In a small study of 114 of adults without diabetes (first-degree relatives of patients with diabetes) the GADA frequency was 9.6% (85). GADA are detectable in 4 to 12% of patients with Type 2 diabetes (12,86-91). The GADA frequency was even higher (32%) in Type 2 diabetes patients with first-degree relatives with diabetes (92). In the German study, in a subgroup of 38 schoolchildren (aged 11.7-15.0 years), autoantibodies occurred in 32% with Type 2 diabetes without insulin requirement over a one-year period, but the GADA frequency lacked details (80).

GADA is the most common autoantibody detected in adult patients with diabetes (87,92). A study of the incidence of diabetes in Kronoberg, Sweden, showed that among 101 antibody-positive adults, GADA were present in 90%, ICA in 71%, and both GADA and ICA in 61% (3). In Type 2 diabetes, GADA seem to enhance the development of insulin dependency (12,87,89,92-96).

GADA are more stable than other autoantibodies detected mostly in children and young adults (97-100) and have the highest diagnostic sensitivity in the age group of 20 to 40 years (98). In children with a single autoantibody, inverse seroconversions were most frequent in the relatives of patients with Type 1 diabetes, and nearly half the children developed no diabetes (101). Moreover, in genetically susceptible children close to half the antibody-positive children (29/63) reverted back to antibody negativity, indicating that autoreactive immune responses can be manipulated and even spontaneously switched off (102). In adults (22/27), 81% of the GADA-positive patients with Type 2 diabetes remained GADA-positive after 12 years (100).
Autoantibodies to other antigens have also been reported in patients with diabetes (carboxypeptidase H, ICA69, GLUT-2, SOX-13, β cell sulfatides, zinc transporter 8), but because they are at a considerably lower frequencies than autoantibodies to ICA/GAD65/IA-2/insulin, they have not proved useful in routine clinical studies. High levels of GAD65-autoantibodies also occur in stiff-man syndrome (103). Autoimmune responses seem, however, to differ in stiff-man syndrome and Type 1 diabetes along Th1 and Th2 pathways (64).

### 2.3.1 Prediction of Type 1 diabetes

As markers of disease activity, with autoantibodies one should be able to predict disease, and this would open up possibilities for secondary prevention (57). To make it more effective, screening should be targeted at risk groups. Moreover, with the higher threshold for autoantibody positivity, the autoantibody assay identifies patients more specifically with the disease, but then it also misses many patients who have low autoantibody signals (57).

Studies on prediabetic subjects have shown variability in rate of progression to clinical diabetes based on the titre and number of antibodies (44). All prospective studies on the relatives of Type 1 diabetes patients have shown that a combination of two or more autoantibodies (IAA, GADA, IA-2A, ZnT8) gives a higher positive predictive value than does any single autoantibody (47,48,104-108).

The predictive values of autoantibodies will likely differ depending on whether assessed in twins, in first-degree relatives, or in the general population (44). A one-time screening for GADA and IA-2A in the general childhood population in Finland would identify 43 to 50% of the individuals who will develop Type 1 diabetes in the next 27 years (35,108). We would need to identify those subjects from the general population who are at increased risk. Many Type 1 diabetes studies have first screened non-diabetic relatives by HLA-risk genotypes (HLA DQ, (109)). In genetically susceptible children recruited from the general population at the age of 2 years, GADA identified 86% of those who persistently tested positive for multiple autoantibodies (110). In the Finnish Type 1 Diabetes Prediction and Prevention study (DIPP) conducted on children having the HLA-DQB1 risk antigens (high risk and
moderate risk) and who were recruited from the general population, 7.1% of the genetically high-risk and 2.3% of the genetically moderate-risk children developed a persistent positivity for multiple autoantibodies by the age of 5 years, and the high-risk children had seven times as high a risk for developing diabetes as did those with a moderate risk (44).

Knowledge of the predictive value of GADA in a healthy adult population is limited, and in two studies, GADA did not predict diabetes (81,111). A study in young (< age 25) relatives of Type 1 diabetes patients suggested that insulin resistance accelerates progression to Type 1 diabetes in ICA-positive relatives whose insulin secretion is markedly reduced, but not in those with well-preserved insulin secretion (112). The large long-term prospective studies that provide us with better knowledge of whether the subjects finally develop diabetes could clarify the role of autoantibodies in development of diabetes or insulin deficiency in adults, help us to understand the aetiopathogenesis of the latent autoimmune diabetes, and reveal whether we can define risk groups in adults with antibodies for the purpose of predicting diabetes or the development of insulin deficiency.

2.4 Type 2 diabetes

Type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM, or adult–onset diabetes) is a metabolic disorder characterized by hyperglycaemia, insulin resistance, and relative insulin deficiency. Type 2 diabetes has increased markedly over the past 50 years in parallel with increased obesity, increase in sedentary lifestyles, and changes in the environment (113). Chronic hyperglycaemia causes micro- and macrovascular complications via several mechanisms and is a great burden to health care systems and also to individuals with diabetes (26-28,114). More than 70% of patients with Type 2 diabetes ultimately die from cardiovascular causes (26). Although it is possible for patients to reduce their risk for diabetes complications by actively participating in their daily health care, their medication must be optimized individually.

Type 2 diabetes has increased markedly in recent years (115) and its prevalence varies greatly between populations as well as among different age groups. In 2010, it was almost 50% in North America in the Pima Indian population, 5 to 10% in Europe and Asia, and only 3.2% in
Africa (1). In Finland, the prevalence is 8.3%, whereas in Germany, Switzerland and Portugal it is already 11 to 12% (1). In Europeans its peak prevalence occurs at 70 to 80 years of age, whereas in high-risk populations such as in the Pima Indians and the Nauruans the peak occurs at 40 to 50 years of age. In the USA, Type 2 diabetes is as common as Type 1 diabetes among children at age 10 to 19 years (116). Some studies there have also shown a male predominance (3,117,118).

Hyperglycaemia develops in association with insulin resistance (decreased insulin sensitivity to glucose), and after years this condition is thought to lead to exhaustion of the insulin secretion ability of the β-cells and finally to insulin deficiency (119). Animal studies have revealed proinflammatory cytokines and chemokines that attract the immune cells to islets, inducing inflammatory processes leading to β-cell dysfunction (24). Well-studied mechanisms underlying β-cell dysfunction also include mitochondrial dysfunction, oxidative stress, ER (endoplasmic reticulum) stress, dysfunctional triglyceride/FFA cycling, and glucolipotoxicity (119). Recently, suggestions have also emerged that some infections are involved in the aetiopathogenesis of Type 2 diabetes (120). Studies have also shown lower first-phase insulin secretion even before diabetes onset (121-123). In postmortem studies on patients with diabetes, the number of β-cells has been decreased by 10 to 30% (124).

Autoantibody screening in clinical studies has proved useful in detecting insulin deficiency in patients initially diagnosed with Type 2 diabetes, since the classification of diabetes is based on clinical symptoms and treatment requirements rather than on knowledge of its actual aetiology.

Insulin resistance occurs commonly in Type 2 diabetes with obesity (119), and inflammation of the adipose tissue is involved in the pathogenesis of insulin resistance (120). HOMA often serves in estimation of insulin resistance, and it correlates also with BMI. In most studies, patients with Type 2 diabetes are also older than patients with Type 1 diabetes, which may also influence their higher incidence of insulin resistance.

Risk factors for developing Type 2 diabetes seem to differ between ethnic groups, but a family history of diabetes has consistently been a risk factor, as well as a low level of physical activity, inducing obesity. Type 2 diabetes incidence, even in individuals in risk groups can be reduced through diet, moderate weight loss, and increased physical activity (125,126). Thus, environmental factors play a key role in the development of the disease, but this does not
diminish the role of genes in diabetes development, because our responses to a changed environment are also genetically modified. Low birth weight leads to increased risk for Type 2 diabetes in adulthood, probably because of intrauterine programming of the thrifty genotypes (127,128). In the modern world, individuals with these genotypes are at increased risk for obesity and also for Type 2 diabetes. These genes are therefore also candidate genes for Type 2 diabetes.

2.5 Latent autoimmune diabetes of adults (LADA)

Patients diagnosed with Type 2 diabetes include a variety of individuals with large differences in their insulin secretory capacity and insulin sensitivity as well as having the clinical phenotype. In 1979, the Irvine group reported that the presence of ICAs in patients with Type 2 diabetes was associated with a higher frequency of insulin-deficiency (129,130). This was later confirmed by other studies (131,132). Tuomi et al (1993) showed that GADA were more frequent in insulin-deficient than in non-insulin-deficient patients with Type 2 diabetes and coined the term LADA to describe slow-onset autoimmune diabetes in adults (133). Other names are: type 1.5 (134), latent Type 1 diabetes (17), and slowly progressive IDDM (125). In the WHO classification of diabetes, LADA is included in the definition of Type 1 autoimmune diabetes (136).

LADA accounts for 5 to 10% of the total diabetes population, the same percentage as with Type 1 diabetes (20,87). In frequency of LADA between different populations, variation is also great.

The definition and even the existence of LADA has been under debate (5,137,138). The definition of LADA includes adult onset of diabetes, positivity for autoantibodies (ICA, GADA, IA-2A) and no insulin treatment within the first 6 months after diagnosis. These criteria are somewhat arbitrary, more useful in studying the disease than in clinical use. Age of onset varies among studies, but an age of onset of over 30 to 35 years has been usual. Patients aged 20 to 35 years more often have other forms of diabetes and especially Type 1 diabetes. Genetic, metabolic and clinical differences, as well as differences in the presentation of antibodies, also emerge between young onset (<35 years) and older-onset (>35 years) patients with Type 1 diabetes. Moreover, using the criteria of >35 years provides more
compatible groups of patients. Recently, an increase has occurred in young-onset Type 2 diabetes, and Reinehr et al observed a group of antibody-positive children classified as having Type 2 diabetes (139).

Selection criteria among studies (for instance, the use of medication when patients enter the study) can have a great influence on the results (140). Requiring that subjects not be on insulin treatment, may mean that one ends by selecting a group of healthier individuals. Interpretation of the length of time before the insulin requirement begins may also be subjective, especially if the insulin treatment starts within the first year after diagnosis. The length of hyperglycaemia is also difficult to assess for patients who have very high glucose values on their first visit, but who underwent no glucose measurements earlier.

Autoantibodies found in LADA are the same found in patients with Type 1 diabetes; IA-2 antibodies are, however, rare in older patients with LADA, but more common in patients with Type 1 diabetes (141). Still, GADA are found also in patients with Type 1 diabetes and are thus not specific for patients with LADA. Because GADA persist longer in the circulation and are more common in older patients than are other antibodies found in patients with Type 1 diabetes, it has become more usual to use GADA in screening for autoimmune diabetes in adults (98,145). Interestingly, ICA staining can be blocked upon the addition of GADA and IA-2A from the sera of Type 1 diabetes, but not from LADA patients (146). This has given rise to a hypothesis that other unidentified autoantibodies occur in LADA patients (147).

The heterogeneity of LADA patients has also raised confusion; several studies have tried to resolve this problem by subgrouping patients with LADA according to their GADA-positivity level. Still, many questions arise involving differing GADA assays in different studies, despite the criterion of a standardization program in an attempt to eliminate the problem. The cut-off limits are often based on children and young adults with Type 1 diabetes and seem not to work as well with adult patients (142,143).

LADA patients have many of the clinical characteristics of Type 1 diabetes and markers of autoimmunity, but are initially non-insulin-requiring, as are patients with Type 2 diabetes (92). Some studies have observed that LADA patients resemble adult patients with Type 1 diabetes more than they do patients with Type 2 diabetes (14). Single autoantibody positivity is more common in LADA than in younger patients with Type 1 diabetes (148). Patients are
often misdiagnosed as having Type 2 diabetes, as they initially retain good metabolic control without insulin for years, distinguishing them from patients with classical Type 1 diabetes. Still, many develop insulin deficiency and may suffer from hyperglycaemia and difficulties with medication for many years before the correct diagnosis is made. LADA patients are younger and leaner than patients with Type 2 diabetes (12,149), but many studies find no difference in age of onset (90,91,150,151). LADA patients also have lower cardiovascular mortality than do patients with Type 2 diabetes (152). Larger studies or studies using special methods for patients with LADA and Type 2 diabetes are presented in Table 1.

Insulin-secretion assessment in patients with LADA has generally been based on fasting C-peptide and insulin measurements. The first studies detecting GADA in patients with Type 2 diabetes had already discovered that LADA patients have lower levels of insulin secretion than do GADA-negative patients with Type 2 diabetes; this was then confirmed by many studies (Table 1). LADA patients have fewer features of the metabolic syndrome than do Type 2 diabetes patients, but more than the recent-onset classical Type 1 diabetes patients, and are similar in this respect to those with Type 1 diabetes with a long disease duration (12,14,140,153). One study found no difference in features of metabolic syndrome between non-insulin requiring patients with LADA and Type 2 diabetes (90).

The extent of insulin resistance in LADA is debatable, because some studies have found LADA patients to be less insulin resistant than are Type 2 diabetes patients (14,140) and some studies have also shown a similar extent of insulin resistance as in patients with Type 2 diabetes (5,8,13,15). These differences may have originated from the study design, the methods used and the overall changes over a period of years in population lifestyles and ageing. Recent data also suggest that lifestyle intervention and medical treatment targeting insulin resistance and CVD risk factors are beneficial in non-obese as well as obese Type 2 diabetes patients (154).

Several LADA studies have attempted to resolve the question of how to predict development of insulin deficiency in patients with LADA. In the UKPDS study, GADA were more sensitive than ICA in predicting insulin requirement (87), and in several studies high GADA levels have been associated with lower fasting C-peptide levels (12,148,155-157), but different cut-off points in different studies makes the comparison of results difficult. In the
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study subjects</th>
<th>Number of subjects (LADA/total T2D)</th>
<th>Analyses of effect of GAD antibody level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turner et al. (1997)</td>
<td>Newly diagnosed T2D patients (UKPDS)</td>
<td>430/1926</td>
<td>Insulin requirement, β-cell function, BMI</td>
</tr>
<tr>
<td>Tuomi et al. (1999)</td>
<td>Finnish T2D patients (Botnia Study)</td>
<td>104/1122</td>
<td>Duration of diabetes, associated autoimmunity, β-cell function, BMI, metabolic syndrome</td>
</tr>
<tr>
<td>Davis et al. (2000)</td>
<td>Diabetes patients aged 11–98 (FDS)</td>
<td>45/1225</td>
<td>Duration of diabetes, insulin requirement, BMI, metabolic syndrome</td>
</tr>
<tr>
<td>Falorni et al. (2000)</td>
<td>T2D patients age &gt; 25 at diagnosis</td>
<td>61/569</td>
<td>Insulin requirement, duration of diabetes, associated autoimmunity</td>
</tr>
<tr>
<td>Gambelunghe et al. (2000)</td>
<td>Italian T2D patients age &gt; 25 diagnosis</td>
<td>67/600</td>
<td>Associated autoimmunity</td>
</tr>
<tr>
<td>Tripathy D et al 2000</td>
<td>LADA, T2D patients</td>
<td>217/744***</td>
<td>Insulin secretion and sensitivity</td>
</tr>
<tr>
<td>Lohmann et al. (2001)</td>
<td>Short-term (&lt; 5 years) DM patients age &gt; 35 at diagnosis</td>
<td>51/312</td>
<td>Associated autoimmunity, β-cell function, BMI, metabolic syndrome, diabetes-associated complications</td>
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<tr>
<td>Kučera et al. (2003)</td>
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<td>58/153*</td>
<td>Associated autoimmunity</td>
</tr>
<tr>
<td>Hamaguchi et al. (2004)</td>
<td>T2D patients aged 31–75</td>
<td>55/835</td>
<td>Age of onset, duration of diabetes, β-cell function, insulin requirement, BMI, associated autoimmunity, HLA genotype</td>
</tr>
<tr>
<td>Li et al. (2004)</td>
<td>LADA patients</td>
<td>145/290*</td>
<td>Age of onset, β-cell function, metabolic syndrome</td>
</tr>
<tr>
<td>Monge et al. (2004)</td>
<td>T2D patients diagnosed over age 50</td>
<td>70/220</td>
<td>Age of onset, duration of diabetes, BMI, β-cell function, insulin requirement</td>
</tr>
<tr>
<td>Authors</td>
<td>Study subjects</td>
<td>Number of subjects (LADA/total T2D)</td>
<td>Analyses of effect of GAD antibody level</td>
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<tr>
<td>Davis et al. (2005)</td>
<td>Newly diagnosed T2D patients age 25–65 (UKPDS)</td>
<td>526/4545</td>
<td>β-cell function</td>
</tr>
<tr>
<td>Yang et al. (2005)</td>
<td>LADA and T2D patients</td>
<td>45/90</td>
<td>β-cell function</td>
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<tr>
<td>Castleden et al. (2006)</td>
<td>T2D patients age 27–84</td>
<td>136/2059</td>
<td>Age of onset, BMI</td>
</tr>
<tr>
<td>Fourlanos et al (2006)</td>
<td>LADA and T2D patients</td>
<td>102/111</td>
<td>Age of onset, BMI, symptoms of hyperglycaemia, personal and FH of autoimmune disease</td>
</tr>
<tr>
<td>Genovese et al. (2006)</td>
<td>Italian T2D patients age &gt; 40 at diagnosis</td>
<td>63/881</td>
<td>BMI, insulin requirement</td>
</tr>
<tr>
<td>Radtke et al (2009)</td>
<td>LADA and T2D patients</td>
<td>106/943</td>
<td>BMI, insulin requirement</td>
</tr>
<tr>
<td>Hawa et al (2009)</td>
<td>LADA and T2D patients</td>
<td>117/1247</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>Lee et al (2011)</td>
<td>LADA and T2D patients age &gt; 25 at diagnosis</td>
<td>87/87*</td>
<td>β-cell function, BMI, weight loss, FH of diabetes</td>
</tr>
</tbody>
</table>

*Alternative study design: known number of LADA patients matched with T2D control group.

**Study of healthy subjects; 23 of 9312 healthy subjects were GADA-positive.

*** A subgroup of patients underwent a intravenous glucose tolerance test (IVGTT; LADA= 9 / T2D n= 20) and euglycaemic clamp (LADA n= 12 / T2D n= 14).

BMI, body mass index; DISS, Diabetes Incidence Study in Sweden; DM, diabetes mellitus; DM2, type 2 diabetes; FDS, Fremantle Diabetes Study; FH, family history; GADA, glutamic acid decarboxylase autoantibody; LADA, latent autoimmune diabetes in adults; UKPDS, UK Prospective Diabetes Study. Modified from van Deutekom et al. (143).

studies mentioned, patients with low GADA levels were more like patients with Type 2 diabetes. Moreover, the early age of onset of diabetes, and low prevalence of markers for the metabolic syndrome including low BMI, hypertension, and dyslipidemia predicted a need for early insulin therapy (156-158), as well as predicted hyperglycaemia, despite good compliance with treatment (Table 1).

LADA patients are at risk for developing other autoimmune conditions (20,149).
2.6 Clinical characteristics of Type 1 and Type 2 diabetes

Patients with Type 1 diabetes have been considered lean, non-insulin-resistant, and insulin-dependent, and patients with Type 2 diabetes to be overweight and insulin-resistant. This characterization has since been updated many times. Here I focus on the main characterizations.

2.6.1 Insulin deficiency

Insulin deficiency means either that insulin secretion is rapidly reduced or has totally ceased within days or weeks (Type 1 diabetes) or has slowly decreased over months or years (LADA) caused by the autoimmune destruction of pancreatic islet β-cells. Clinical Type 1 diabetes occurs when 60 to 80% of the β-cells have been destroyed (29,159,160), but cells secreting glucagon, somatostatin, and pancreatic polypeptide are generally preserved and may be redistributed within the islets.

The mechanisms of β-cell failure in Type 2 diabetes have been clarified recently. Studies of the relatives of patients with diabetes have indicated lower first-phase insulin secretion even before the onset of diabetes. Several factors contribute to β-cell function, including the genetic background and insulin sensitivity of the individual (161). At the prediabetic stage (IGT, IFG), insulin production increases to adapt to the increasing demand of insulin due to increased metabolic stress (overnutrition, obesity). Pancreatic islet pathology is characterized by a decrease in β-cell mass due to apoptosis (162,163) caused by inflammation, which in turn is induced by obesity and insulin resistance (119). Reduced β-cell function together with relative hyperglycaemia, also occurs, due to an increased proportion of α-cells and α-cell dysfunction (164,165).

However, relative insulin deficiency can also occur as a result of many different mutations in different genes participating in insulin secretion or action (MODY). An increasing demand for more insulin, a demand caused by ageing, obesity, or insulin resistance, cannot be compensated any longer, which leads to progressive deterioration of β-cell function over several years (166). This depends on a genetically determined ability of the β-cells to adapt and on the severity of insulin resistance (161). Mechanisms explaining the β-cell failure have been studied extensively, with inflammation as part of these mechanisms, as a causative
agent, or as some type of response (119). In both main diabetes types, immune responses are thus present and play an important role in their pathogenesis.

GADA-positive patients develop insulin deficiency more often than do GADA-negative patients, and several studies have suggested that GADA might serve as a predictive marker for the development of insulin deficiency in patients with Type 2 diabetes {{389 Tuomi,T. 1993; 379 Zimmet,P.Z. 1994; 1207 Abiru,N. 1996; 1446 Turner,R. 1997; 451 Tuomi,Tiinamaija 1999; 449 Carlsson,AsaLinda 2000; 814 Borg,H. 2002; 1605 Lee,S.A. 2011}}. Furthermore, high levels of GADA have been associated with a higher frequency of insulin deficiency (12,91,147,155-157,167). On the other hand, in one US study, 10 to 20% of newly diagnosed CaucasianType 1 diabetes patients and an even higher percentage of African and Hispanic diabetes patients carried no autoantibodies to any of the major autoantigens (168). In a Swedish study of 26 individuals diagnosed with Type 1 or Type 2 diabetes (age-range 26-74 years) who had low fasting C-peptide concentrations (<0.25 nmol/l), 11 (42%) were antibody negative (GADA, ICA, (3)). Auto-reactive T-cells correlated more strongly with impaired β-cell function than with autoantibody positivity (4,146). These findings suggest either that autoantibodies are present in a very low titre or that other still unidentified autoantigens are involved. Alternatively, a fraction of Type 1 diabetes cases may not be autoimmune in nature.

Assessment of insulin secretion from the β-cell is problematic. This may be due to uncertainty as to which defect of insulin secretion is present in the subject still without diabetes: Is it a problem of the capacity of the β-cell mass or a defect in sensitivity of the β-cell to glucose, or in the dynamics of insulin secretion, or a combination of all these factors; we do know that insulin resistance and the presence of chronic hyperglycaemia (glucose toxicity) influences β-cell responses (169).

2.6.2 Insulin resistance

In insulin resistance, the natural hormone insulin becomes less effective at lowering blood sugar, because cells in the body have become insensitive to the effects of insulin, resulting in the blood’s carrying an abnormally large amount of insulin. Glucose uptake in the muscle and fat cells and glycogen synthesis and storage in liver cells are all reduced (169). The liver fails to reduce glucose production and release glucose into the blood (120). These changes can lead
to elevated blood glucose levels in genetically susceptible subjects, when insulin secretion can no longer compensate for insulin resistance. Insulin resistance also causes changes in the fat metabolism by reducing the uptake of circulating lipids and enhancing the hydrolysis of stored triglycerides (170). Insulin resistance is more common in the relatives of patients with Type 2 diabetes than in the controls without a FH of diabetes (40% vs. 20%, (8,132). In Type 1 diabetes and LADA, insulin resistance was earlier a less prevalent feature, but it has become more common also among these patients in Western countries (9,10).

Insulin resistance is thus thought to contribute to the development of Type 2 diabetes. Animal studies have supported the idea of defects in insulin secretion and insulin sensitivity in Type 2 diabetes. Lauro et al demonstrated that a muscle insulin-receptor knockout did not result in a diabetic phenotype, but β-cell upregulation compensated for the insulin resistance (171); however, knockout of the insulin receptor and IRS-1 led to insulin resistance and a β-cell defect (172). High plasma levels of insulin, glucose, and fatty acids are a major component of the metabolic syndrome and of Type 2 diabetes. Insulin resistance seems to be a fundamental disturbance, leading to the metabolic syndrome and further to the endothelial dysfunction involved in the pathogenesis of cardiovascular diseases (169). Progression of insulin resistance either to CVD or Type 2 diabetes can be divided into four stages, in which Type 2 diabetes represents the last stage (169).

Factors such as central obesity, low level of physical activity, genetic diseases, and autoantibodies against insulin receptors can elicit, in some individuals, insulin resistance and contribute to the metabolic syndrome (173). In obese patients with insulin resistance, adipose cells over-secrete a number of adipocytokines such as tumor necrosis factor-α (TNF-α), resistin, plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), and angiotensin, which promote atherosclerosis, vascular inflammation, endothelial dysfunction, and impairment of the action and secretion of insulin (174). As a result, an increase in activation of insulin receptors in the arteries leads to recruitment of vascular smooth muscle cells, inflammatory cells, and the innate immune system, further potentiating atherosclerosis (175). Obesity-induced insulin resistance is the main cause of the metabolic syndrome (71), and sedentary lifestyles, and environmental factors as well as high circulating levels of free fatty acids, have been implicated in its aetiology (169).
The euglycaemic-hyperinsulinemic clamp is thought to be the gold standard in determining insulin resistance (176). In practice, insulin resistance is assessed by homeostatic model assessment (HOMA) or the insulin sensitivity index (ISI), which correlate reasonably well with directly-measured clamp-derived gold-standard values. Results, however, are mixed regarding insulin resistance among patient groups, most likely because these methods are indirect means for measuring insulin resistant. Initially, LADA patients were described to be less insulin resistant than are patients with Type 2 diabetes (12,14,153), but this has since been contradicted, and some of the studies have also used novel methods to assess insulin resistance (8,13,15).

2.7 Family history of diabetes

Clustering has been observable in families showing Type 1 diabetes and Type 2 diabetes (16,18,20,149,177,178), although this has been contradicted in a large UK study on parents of Type 1 diabetic patients (21). Risk for developing Type 1 diabetes in the general population is about 0.4% and in siblings of patients with Type 1 diabetes, 6 to 7% (35). The lifetime cumulative risk for offspring of mothers with Type 1 diabetes is 4.2% (3.0-5.4), and for offspring of fathers 3.8% (2.6-5.0, (179)). In twin studies Type 1 diabetes families with monozycotic twins, diabetes concordance has ranged from 20% to 90% depending on follow-up time (180-184).

In families with Type 2 diabetes, the offspring have a 40% lifetime risk for developing Type 2 diabetes, and when both parents have Type 2 diabetes, the risk is even higher, even as high as 70% (185-187). A high concordance rate, up to 70% to 80% for Type 2 diabetes has emerged in twin studies (188-190). Similarly to the situation in Type 1 diabetes studies, the offspring of mothers are at more risk of developing diabetes than are the offspring of fathers with Type 2 diabetes (187,191-193). The family-history effect was also studied in a large population-based cohort of 10,135 individuals from Wisconsin, in the USA, during 1979-1980 (187). This study found that in younger-onset individuals (taking insulin and with age of onset <30 years, n=1210), the relative risk (RR) for diabetes in a sibling if the mother had diabetes was double the risk for diabetes if the father had the disease, the RR being 2.39 (95% CI, 1.64-3.48) vs. 1.22 (0.72-2.05); if both parents had diabetes, the RR was 5.61 (95% CI, 3.37-9.34).
In the older-onset individuals (age of onset >30 years, n= 1780), the corresponding RR values were 1.69 (95 percent CI, 1.35-2.13) for fathers, 1.72 (95% CI, 1.44-2.06) for mothers, and 2.42 (95% CI, 1.81-3.25) for both parents (187). In Sweden, immigrants from the Middle East show a 2- to 3-fold increased risk for Type 2 diabetes and they tend to have more first-degree relatives with Type 2 diabetes (194).

Li et al compared three groups of Type 2 diabetes patients with Type 2 FH, patients with Type 1 and Type 2 FH and patients without any FH of diabetes. They found that patients with an FH of diabetes were younger at disease onset and had worse β-cell function and lower HDL cholesterol concentrations than did patients without an FH of diabetes. Patients with a Type 2 FH had a higher mean BMI and fasting C-peptide concentration, but a lower frequency of GADA (11% vs. 23%) and \( DQB1 \) risk genotype (37% vs. 54%) than did patients with an FH of both Type 1 and Type 2 diabetes (FHmix, n=240), but some of the patients were related to each other in this study (19,195). This study thus indicated that LADA is more common in FHmix families. Fourlanos et al observed that patients with LADA more often have a Type 1 family FH than do patients with Type 2 diabetes (24% vs. 11%, altogether 35 patients with a Type 1 FH, (149)). Some other studies have indicated that Type 2 families also include more LADA patients (158,178). Middle East immigrant Type 2 diabetes patients in Sweden seem also to have a slightly different form of diabetes with an earlier onset and a lower C-peptide concentration (194). It has also emerged that Type 2 family history is associated with a later onset of Type 1 diabetes and more symptoms of the metabolic syndrome and insulin resistance (196).

A family history of diabetes, together with autoantibodies or risk HLA genotypes or both, has served to identify subjects at risk for Type 1 diabetes. In a nationwide Childhood Diabetes in Finland (DiMe) study of 701 siblings of newly diagnosed patients with Type 1 diabetes, 47 (6.7%) developed the disease during a 15-year follow-up period. In that study, the strongest predictive model for progression to clinical disease included age of the sibling at first sampling, HLA-DR-conferred susceptibility, number of initially detectable autoantibodies, and number of first-degree relatives with Type 1 diabetes (197).

A family history of diabetes is more than just a genetic susceptibility to diabetes, because both main types of diabetes also depend on lifestyle factors, which also can be inherited.
Obesity and eating and exercise habits tend to run in families, and it is often difficult to know which is the most important risk factor. Family history of other autoimmune diseases can be related to immune system vulnerability, which can, as well, expose one to Type 1 diabetes and LADA.

2.8 Genetics of Type 1 and Type 2 diabetes

2.8.1 Genetics of Type 1 diabetes

Identical twins are at much greater risk for developing diabetes within their lifetime than are non-identical siblings although one large study contradicted this. Differences in risk for diabetes have also appeared between ethnic populations living in the same environment (118). Monozygotic and dizygotic twin siblings differ in their expression of islet autoantibodies and in progression to diabetes, dizygotic twins being similar to their non-twin siblings. This observation has led to the suggestion that genetic factors play an important role in determination of islet cell autoimmunity.

The linkage between HLA and Type 1 diabetes was discovered back in the 1970s (198,199). HLA stands for human leukocyte antigen, the major histocompability complex in human beings, involving genes related to immune-system function. The Type 1 Diabetes Genetics Consortium (T1DGC) has assembled data from 17,129 individuals in 3,892 affected sib-pair families, representing the largest family collection of any immune-mediated disease; of the loci that affect the risk of developing Type 1 diabetes they have identified over 50 (200). Foremost among these is the HLA complex, still the most important risk factor in Type 1 diabetes and other immune diseases. The second locus identified was INS, but this and other non-HLA loci have relatively minor risk effects comparable to loci mapped in other common diseases, with risk estimates typically between 1.05 and 2.0 (201). The non-HLA genetic polymorphisms are estimated to explain half, and HLA genes the other half of the genetic susceptibility to Type 1 diabetes (69).

Results of the HLA linkage and association studies support the hypothesis that Type 1 diabetes has an autoimmune component, and HLA genotyping has become an important research tool for identifying subjects at risk for developing Type 1 diabetes. Age-related
differences in risk allele frequencies also exist (202), as well as regional differences. The same risk genotype can also predispose to various autoimmune diseases.

2.8.2 Genetics of Type 2 diabetes

Studies during recent years have revealed dozens of candidate risk genes for Type 2 diabetes, thanks to development of genetic analysis methods and international collaborations (203). What has also become evident is that although genetic factors are important in development of Type 2 diabetes, environmental factors still play a key role. Further sub-grouping of Type 2 diabetes can reveal new information. The transcription factor 7-like 2 (TCF7L2) risk allele has the strongest association with Type 2 diabetes this far (203,204). After 2007, many genome-wide association studies appeared, and many new loci associated with Type 2 diabetes have been identified (205,206). Thereafter, large-scale meta-analyses have revealed over 40 genes associated with diabetes or glycaemic traits (203). Many of the susceptibility loci are associated with insulin secretion; PPARG and FTO have also been associated with insulin resistance (207). Still, such explanations cover a small part of the genetic component of the disease, and most of the variants have very modest effect sizes of about 1.1 to 1.3. Genetic markers have not, this far, proved very useful.

2.8.3 Genetics of LADA

LADA, although an autoimmune disease, differs from Type 1 diabetes, and has a genetic profile that includes genes also found in people who develop Type 2 diabetes. LADA is associated with HLA autoimmunity genes similar to those in people with Type 1 diabetes (12,208,209) as well as with the minor Type 1 diabetes-associated genes INS VNTR and PTPN22 in some, but not in all studies (12,210,211). In some studies, LADA is also linked with the Type 2-associated risk gene TCF7L2 (211-213). Studies have observed differences in Type 2 diabetes risk genotype frequencies among LADA patients based on GADA level, age, and BMI (12,210-214). As LADA shares genetic features with both Type 1 and Type 2 diabetes, this may support the assumption that LADA is an admixture of the two major types of diabetes (211). Studies in patients with LADA have focused on known Type 1 and Type 2 diabetes susceptibility genes.
3. AIMS OF THE STUDIES

The overall objective of these studies was to discover the impact of Type 1 family history and GADA positivity on insulin secretion in subjects before the onset of their diabetes and in patients with diabetes. No genetic data appear in this thesis.

The specific aims were to explore:

1. GADA frequency in relatives without diabetes and in newly diagnosed subjects with diabetes in the prospective Botnia family study, which represents a large cohort with a higher risk for diabetes. A particular focus is the impact of family history of Type 1 diabetes on GADA frequency (Study I).

2. association of GADA with the phenotype, especially β-cell function/insulin secretion in subjects without diabetes and in patients with newly diagnosed diabetes and the effect of family history of Type 1 diabetes on prevalence of GADA, and the clinical characteristics, especially insulin secretion and sensitivity, of patients with diabetes (Studies II and III)

3. association of GADA and family history of Type 1 diabetes with risk for progression to diabetes (Study I)

4. association of family history of Type 1 diabetes and GADA with development of insulin deficiency (Study III)
4. STUDY DESIGN AND SUBJECTS

Study I: GADA positivity predicts non-insulin dependent diabetes in an adult population.

- The prospective study – a follow-up every 3 years of relatives of patients with diabetes, with their spouses without diabetes serving as control subjects.
- Anthropometric and metabolic measurements and collection of family-history data
- Measurement of GADA and IA-2A
- Analyses of family history of diabetes and GADA and whether these are associated with a particular phenotype, with insulin deficiency, or with diabetes

Study II: Latent autoimmune diabetes in adults differs genetically from both Type 2 diabetes and classical Type 1 diabetes diagnosed after the age of 35 years.

- Anthropometric and metabolic measurements and measurements of GADA in LADA patients and patients with Type 2 diabetes
- Comparison of clinical characteristics in a larger group of patients between patients with Type 1 diabetes, Type 2 diabetes, LADA, and the control subjects. The main focus of this thesis is on clinical characteristics of GADA-positive and –negative patients with Type 2 diabetes to discover whether GADA and especially GADA level have any impact on these

Study III: A family history of Type 1 diabetes affects insulin secretion in patients with Type 2 diabetes.

- A cross-sectional study on the effect of a family history of Type 1 diabetes on patients with Type 2 diabetes
- A comparison of clinical characteristics, including measurements of GADA, between patients with Type 2 diabetes with a Type 1 family history or a Type 2 family history
- Analyses of risk factors affecting insulin secretion and the development of insulin deficiency
4.1 Study I

Subjects in this study were relatives of patients with diabetes who participated in the Botnia Study during 1990-2007. Since 1990, families with T2D and T1D have been recruited from western Finland, and since 1994, from all around Finland and Sweden. Subjects without diabetes were invited for a follow-up visit every 3 to 5 years. Spouses without diabetes and without a family history of diabetes served as controls. To GADA-positive subjects unable to travel to the study centers, we mailed a laboratory package for blood samples to be drawn at their nearest health centre. Development of diabetes was verified exclusively by interview for only a few subjects. A total of 4976 relatives without diabetes were included in the study at baseline, and follow-up data were available for 253 (87.5%) of the 289 GADA-positive subjects and 2563 (58.3%) of the 4391 GADA-negative subjects during a median [IQR] follow-up time of 9.3 [5.3] and 8.0 [5.5] years (Study flow chart in Figure 1 in Study I). MODY families were excluded based on the screening for MODY mutations in patients with young-onset Type 2 diabetes.

Family history data were coded from family trees updated on every visit. The subjects participated in a 75-g oral glucose tolerance test (OGTT) after a 12-h overnight fast and their glucose tolerance was assessed according to the WHO 1998 criteria (136). All subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IFG) or impaired glucose tolerance (IGT), were included. GAD- and IA-2 antibodies were measured at baseline for all subjects and GAD antibodies were also measured during the follow-up from GADA-positive subjects, and those with diabetes. Blood samples allowed measurement of plasma glucose (P-glucose), serum insulin (S-insulin), serum total cholesterol (Chol), HDL cholesterol, triglyceride, and serum C-peptide (S-C-peptide) concentrations. Weight, height, waist and hip circumference, fat-free mass (Futrex, Gaithersburg, MD, USA) and blood pressure (mean of two recordings) were also measured. Insulin resistance was estimated by the Homeostasis Model Assessment insulin resistance index (HOMA_{IR}) and β-cell function by the insulinogenic index (see Methods). The disposition index served to adjust insulin secretion for degree of insulin resistance (insulinogenic index/HOMA_{IR}). A structured questionnaire allowed collection of data on other diseases, medication, and lifestyle.

4.2 Study II

The subjects participated in the Botnia family study (See Study I) and The Finnish Diabetic Nephropathy (FinnDiane) study (9). In this study we included all LADA patients from the
Botnia Study (n= 213), as well as all patients with Type 1 diabetes with diabetes onset at age > 35 years (T1D_{old}) from both the Botnia Study and the FinnDiane Study (n=256). For comparison, we included all subjects who had Type 1 diabetes younger than at 20 years (n=158; T1D_{young}) and GADA-negative patients with Type 2 diabetes (n=648) from the Botnia Study.

Subjects not on insulin treatment underwent an OGTT. Patients were either clinically diagnosed with Type 1 diabetes or all Type 2 and LADA patients were classified as having Type 2 diabetes. Additional diagnostic criteria for Type 1 were the following: initiation of permanent insulin treatment within 6 months after diagnosis and fasting serum C-peptide concentration of < 0.2 nmol/l at the time of investigation. LADA was diagnosed as GADA-positive diabetes with age of onset at > 35 years, and with no insulin requirement within the first 6 months of diagnosis. Patients with Type 2 diabetes were GADA negative and received no insulin treatment during the first year. Blood samples were taken for measurements including for P-glucose, S-insulin, serum total cholesterol, HDL cholesterol, triglyceride, and S-C-peptide concentrations, and subjects’ weight, height, waist and hip circumference, and their blood pressure (mean of two recordings) were measured. Insulin resistance was estimated by the HOMA_{IR} and β-cell function by the insulinogenic index.

4.3 Study III

Patients with Type 2 diabetes were recruited through newspaper advertisements and via diabetes nurses around Finland and were interviewed by the study physician (V.L.) during 2002-2008. Inclusion criteria were age at diagnosis > 20 years, Type 2 diabetes managed without insulin treatment during the first year after diagnosis, and a first- to third-degree relative with Type 1 or with Type 2 diabetes. The relatives with Type 1 diabetes were also invited to participate; for those not attending, the diagnosis was confirmed through an interview or medical records (initiation of insulin treatment within 6 months after the diagnosis and a fasting C-peptide concentration of <0.2 nmol/l at the time of investigation). Their median [IQR] age was 36.5 [23] years and age at diagnosis of diabetes 14.5 [14.5] years. In total, 196 unrelated patients with Type 2 diabetes with a Type 1 FH were included; all but six also had relatives with Type 2 diabetes (Mixed families). As a control group, we included 139 unrelated patients with Type 2 diabetes with Type 2 FH and matched them with the patients with a Type 1 FH for age, sex, BMI, and duration of diabetes (Figure 1).
Figure 1. Study chart. Patients with Type 2 diabetes according to family history of Type 1 and Type 2 diabetes, family history, and GADA level, and strength of GADA positivity.

Patients were finally stratified according to GADA level with a cut-off point of >278 IU/ml for the high-GADA group, which was same as in Study I for patients with Type 2 diabetes (Figure 3).

All patients not on multi-dose insulin treatment participated, after an overnight fast, in an OGTT and blood samples were taken for measurement of HbA1c, GADA, plasma glucose and serum insulin, C-peptide and lipids. The HOMA*IR, the insulinogenic index, and the disposition index (DI) were also calculated for estimation of insulin secretion and sensitivity (see Methods). For a better estimation of these, patients also participated in the second visit within 3 months to undergo an intravenous glucagon test, followed by an insulin-tolerance test (Glucagon-Insulin Tolerance Test; GITT, (215)). In GITT, a bolus of glucagon (0.5 mg) was given at time 0 min, and their serum C-peptide levels revealed their insulin secretion. Then they received an iv bolus of short-acting insulin (0.05 U/kg^-1), and their plasma glucose was measured to estimate insulin sensitivity (see also Methods, Figure 3).
5. METHODS

5.1 Metabolic measurements

Oral glucose tolerance test (OGTT, Studies I, II, III)

After a 12-hour overnight fast, each subject ingested 75 g of glucose, with samples taken at -5, 0, 30, 60, and 120 min for the measurement of P-glucose, S-insulin and S-C-peptide. β-cell function was estimated as the ratio of incremental insulin to glucose response during the first 30 min of the OGTT: insulinogenic index = (insulin 30 min – insulin 0 min) / (glucose 30 min- glucose 0 min), Studies I and II (216); as corrected incremental insulin response: CIR = 100*ins30 / gluc30*(gluc30-3.89), Studies II and III (217). The insulinogenic index has been shown to strongly correlate with the first-phase insulin response following IVGTT (r=0.88), (218). Insulin resistance was estimated as the Homeostasis Model Assessment index: HOMAIR =fasting serum insulin * fasting PG / 22.5, Studies I, II, III, (219). The disposition index (DI) was served to adjust insulin secretion for degree of insulin resistance (insulinogenic index/HOMAIR, (220)).

Glucagon-insulin tolerance test (GITT, Study III)

After each subjects’ 12-hr overnight fast, a bolus of glucagon (0.5 mg) was given at 0 minutes intravenously, and P-glucose and S-C-peptide concentrations were measured at -5, 0, 6, and 40 min (Glucagon test). At the time-point of 40 min, each received an intravenous bolus of short-acting insulin (0.05 U/kg⁻¹) and had P-glucose measured at 5, 10, 15, 20, and 30 min (ITT=insulin tolerance test). (Figure 2)

Insulin deficiency was defined as stimulated S-C-peptide <0.7 nmol/l and P-glucose >7.0 mmol/l at 6 min after the glucagon stimulation (95,215,221).

Insulin sensitivity was estimated with the first order-rate constant for glucose disappearance in ITT: $K_{ITT} = \ln2/T_{1/2} \times 100$ from 0 to 30 min P-glucose after the insulin bolus in ITT (215).

Beta-cell function adjusted for insulin sensitivity (DI$_{GITT}$) was calculated during GITT as the product of $K_{ITT}$ and the incremental C-peptide response at 6 min (215).
5.2 Assays

Concentrations of blood glucose were measured by the hexokinase method with a coefficient of variation (CV) of <1% (Boehringer Mannheim, Mannheim, Germany) during the years 1990-1999, and with the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA, USA) during 2000-2007. S-insulin was measured by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) with an interassay CV of 5% (during 1990-1996), double-antibody ELISA (DAKO, Cambridgeshire, UK) during 1996-2003, by a fluoroimmunometric assay (AutoDelfia, Perkin Elmer Finland, Turku, Finland) during 1999-2007. S-C-peptide concentrations were measured by duplicate radioimmunoassay with an interassay CV of 9%, (RIA, Linco Research, Inc, USA (222)), and by a fluoroimmunometric assay during 1999-2007 (AutoDelfia, Perkin Elmer Finland, Turku, Finland,) and the lipids on a Cobra Mira analyzer (Hoffman LaRoche, Basel, Switzerland), and the LDL cholesterol concentration was calculated by the Friedewald formula. HbA1c concentrations were measured by high-pressure liquid chromatography. Reference values for the assay were 5 to 7%.
5.3 Antibody analyses

Extensive studies at international workshops have standardized assays for autoantibodies to GAD65/IA-2A/IAA (223). GADA were measured by a radiobinding assay employing $^{35}$S-labelled recombinant human GAD65 produced from either pEx9 (12) or after 2008 pThGAD65 (143) plasmids by in vitro transcription/translation. IA-2A was measured by a radiobinding assay employing $^{35}$S-labelled IA-2ic produced by in vitro transcription-translation (12).

The GADA result was expressed in relative units; RU= \((\text{sample cpm-mean cpm of 3 negative controls}) / (\text{cpm of a positive internal reference serum-mean cpm of 3 negative controls}) \times 100\), until the year 2000 and as international units/ml (IU/ml) after the introduction of the WHO International Standard. The GADA results expressed as RU or IU/ml showed a linear correlation up to 250 IU/ml. Levels exceeding 5 RU and 32 IU/ml (pEx9)/ 50 IU/ml (pThGAD65) were considered positive for GADA and levels exceeding 2.5 IU/ml positive for IA-2A. The cutoff limit for positivity was > 5 RU for GADA and 2.5 RU for IA-2A, which represent mean + 3 SD of 296 (GADA) or 155 (IA-2A) healthy Finnish control subjects. In 2000-2004, the cut-off limit was >32 IU/ml, which was at the 97.5th percentile in 189 Finnish and Swedish schoolchildren and in 85 Swedish blood donors. After 2004, the cut-off point was > 50 IU/ml, which was at the 98.5th percentile in 398 blood donors in Malmö. In the Combinatorial Autoantibody Workshop of 1998, the sensitivity of the GADA assay was 75% and its specificity, 99%. In the Diabetes Autoantibody Standardization Program Workshops, the GADA assays showed 76 to 88% sensitivity and 91 to 96% specificity (2000 - 2007), and the IA-2A assay 72% sensitivity and 100% specificity (2005).

5.4 Statistical analyses

The data are median [IQR] or mean (SD). Progression-free survival was defined as length of time after diagnosis of diabetes until development of insulin deficiency or until commencement of insulin treatment in the case of patients on a multiple-dose insulin regimen and with a 6-min S-C-peptide of <0.7 nmol/l (Study III). Two-sided P values of <0.05 were
considered statistically significant. Bonferroni-adjusted critical P values were given (Tables 1 and 2, Study III).

Statistical analyses were performed with the Statistical Package for Social Science software: SPSS, version 13.0 (Study I), SPSS version 16.0 (Study II), and SPSS version 17.0 (Study III) (Chicago, IL, USA) by use of the Chi-squared test or Fisher’s exact test to compare group frequencies and a general linear model after logarithmic transformation, and the Mann-Whitney U-test to compare group means. The relative effect of the variables was analysed with logistic regression. Quantitative traits were compared across the risk genotype groups with the Kruskal-Wallis H test. Parameters with skewed distributions were logarithmically transformed (S-Insulin, S-C-peptide, HOMAIR, insulinogenic index, CIR, KITT and DI) for statistical analyses, but untransformed units are presented in tables and text. A linear mixed-effects model served to compare group differences adjusted for age, sex, and BMI, while accounting for the underlying correlation between subjects from the same family (Study I). The cumulative risk for diabetes was analyzed with the Cox proportional hazards model (Study I). Variables significant in univariate analyses were included in the multivariate model, and GADA positivity served as a time-dependent variable, since some subjects became positive during the follow-up period (Study I). Time until development of insulin deficiency was analysed by the Kaplan-Meier method for differing FH and GADA groups, with adjusted hazard ratios (HR) and 95% confidence intervals (CI) computed (Study III).
6. RESULTS

6.1 GADA prevalence and levels in subjects with and without diabetes and the effect on them of FH (Studies I and III)

Among all GADA-positive subjects in the Botnia family study, the patients with Type 1 diabetes had the highest median antibody levels, even after a median duration of diabetes of 17.4 [17.5] years. They were followed by GADA-positive patients with Type 2 diabetes (LADA, median duration 7.5 (11.2) years) and GADA-positive subjects without diabetes (Figure 3).

Figure 3. GADA levels in patients with Type 1 diabetes (on the left), Type 2 diabetes (LADA, middle) and in subjects without diabetes (right side) in the Botnia family study (unpublished data).

The GADA-positive subjects without diabetes were stratified into quartiles, and those who had GADA within the highest quartile (GADA_{high}; >89.25 IU/ml) were compared with those who had lower levels (32.01- 89.25 GADA_{low/med}) or no antibodies (< 32.01, GADA negative, Study I). In the MIX study, patients with Type 2 diabetes were also stratified in quartiles by the same GADA limits as in Study I for GADA-positive patients with Type 2 diabetes; >278
IU/m was a limit for the highest GADA quartile (Studies II and III, Fig 4), and it was also comparable to limits in some earlier studies.

GADA prevalence was 4.7% in subjects without diabetes and in controls at the baseline visit, and 1.1% converted to positive during the follow-up period (Figure 4); 3.9% of the GADA-positive subjects were also IA-2A-positive compared with only one of the GADA-negative subjects without diabetes (p<0.0001). In comparison, of the 393 patients with Type 1 diabetes 47.8% (191), and of the 3231 patients with Type 2 diabetes 8.0% (260) were positive for GADA at a median duration of diabetes of 16.3 (17.5) and of 7.5 (11.2) years. In the Botnia family study, GADA prevalence was much higher in subjects with a first-degree Type 1 FH (9.3%, unpublished information). In the MIX Study, as well patients with diabetes and with a Type 1 FH were significantly more often GADA positive than were patients with only a Type 2 FH (14.3 vs.4.3% p=0.003, Figure 4) at a median duration of diabetes of 7.9 (6.3-7.2) years for both groups.

The Type 1 FH correlated with GADA level, and GADA positivity seemed to cluster in Type 1 families (Figure 5).

*Figure 4. GADA prevalence in subjects without diabetes and patients with Type 2 diabetes according to family history (Studies I and III).*

<table>
<thead>
<tr>
<th>GADA positive %</th>
<th>Controls ND/FHDM</th>
<th>DM-/FHT1D T2D/FHMIX</th>
<th>T2D/FHT1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects without diabetes</td>
<td>0.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Patients with diabetes</td>
<td>0.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Controls, no FH of diabetes; DM-/FHDM, DM-/FHT1D, subjects without diabetes with family history of diabetes or only with a first-degree FH of Type 1 diabetes; T2D/FHT2, T2D/FHT1D, patients with Type 2 diabetes with a family history of only Type 2 or Type 1 (first to third degree).*
GADA\textsubscript{high} subjects without diabetes had significantly more often first-degree relatives with Type 1 diabetes (29.2\% vs. 7.9\%, \(p<0.00001\)), but also GADA-positive relatives without diabetes and GADA-positive relatives with Type 2 diabetes (LADA) than did GADA-negative subjects (Figure 5). Further, in the MIX study, a family history of Type 1 and Type 2 diabetes of patients with Type 2 diabetes correlated with their GADA level (Figure 5); patients with high GADA levels had significantly more often Type 1 FH and significantly less Type 2 FH than did GADA-negative patients. Significantly more patients showed high GADA levels in Type 1 families in the Botnia family study (5.1 vs. 1.0 \%, high GADA > 89 IU/ml, \(p<0.0001\)) and the MIX study (8.7 vs. 1.4\%, high GADA >278 IU7ml, \(p=0.003\), unpublished information) when compared to Type 2 families.

**Figure 5.** Patients with diabetes (A) and subjects without diabetes (B) and the proportion of Type 1 diabetes relatives (blue columns), only GADA-negative relatives with Type 2 diabetes (grey) or GADA- positive relatives with Type 2 diabetes (LADA, light blue) or) according to strength of GADA positivity

6.2 Clinical characteristics according to GADA in subjects without diabetes and at onset of diabetes

Clinically, GADA-positive and -negative subjects without diabetes differed only slightly at baseline. The GADA\textsubscript{high} subjects were younger: (43.9 [17.5] vs. 53.8 [21.3] vs. 48.2 [23.2] years), and had a lower insulin response during the OGTT according to the insulinogenic and disposition indices than did subjects with lower GADA levels or the GADA-negative subjects.
when all were adjusted for age (Figure 6). In analyses including only those subjects who later developed diabetes, GADA-positive subjects had a significantly smaller waist circumference (92.2 vs. 97.6 cm, p = 0.019) and a lower BMI (27.4 vs. 28.9 kg/m², p = 0.059, adjusted for age; data not shown) than did the GADA-negative subjects without diabetes.

Figure 6. Clinical characteristics at baseline according to GADA-positivity level in subjects without diabetes (Study I).

After the diabetes diagnosis, the GADA-positive subjects were still leaner [BMI 27.75 vs. 30.06 kg/m²; p=0.023] than were the GADA-negative subjects. The GADA-positive subjects with diabetes showed significantly less evidence of insulin resistance, as reflected by their a lower fasting insulin concentration and lower HOMAIR index despite similar fasting P-glucose levels (Figure 6, Table 2 in Study I). In both groups, those subjects who did not develop diabetes had equal levels of insulin resistance (Figure 7) and lower levels of insulin resistance than did the GADA-positive or -negative subjects who developed diabetes during follow-up.

The GADA_{high} subjects developed diabetes at a significantly younger age than did GADA_{low-med} or GADA-negative subjects (45.8 [13] vs. 63.1 [13.5] vs. 62 [19.4] years, P < 0.00014). In comparison, subjects with FH of Type 1 or Type 2 developed diabetes at a younger age (59.7 [12.7] versus 71.8 [7.8] years, P < 0.0001) than did subjects without a diabetes FH. Moreover, the subjects with a first-degree Type 1 FH who developed diabetes were also markedly
younger (43.2 [12.7] years) than were the subjects without any FH of diabetes (71.8 [13.2] years, p < 0.005, unpublished information).

**Figure 7.** Fasting insulin and HOMA$_{IR}$ (median, IQR) according to GADA during follow-up in subjects not developing diabetes (left) and in subjects developing diabetes measured at the onset of diabetes (right, Study I).

6.3 Clinical characteristics and insulin secretion of diabetes patients according to FH and GADA

We compared clinical characteristics of patients with Type 2 diabetes according to FH and GADA. In the MIX study, the patients with a Type 1 FH or Type 2 FH were matched for age, age at diagnosis of diabetes, duration of diabetes, and sex distribution. Their medication was similar (no medication 19% vs. 22%, insulin 24.5% vs. 21.6%), except for those with a Type 1 FH who used meal-time insulin more often (10.2% vs. 2.8%; p=0.01). Compared to patients
with a Type 2 FH (Table 1 in Study III), patients with a Type 1 FH had significantly lower insulin secretion, which was evident, based on a lower C-peptide response to glucagon and a lower disposition index during both the OGTT (DI_{OGTT}) and the insulin tolerance test (DI_{GIT}, Table 1 in Study III).

When stratified according to GADA, GADA-positive patients with Type 2 diabetes (LADA) had significantly higher HbA1c and glucose responses during the OGTT as well as lower measures of insulin secretion (S-C-peptide at fasting and at 6 min after the glucagon or the DI_{GIT}) than did GADA-negative patients (Table 2, Study III). When stratified according to GADA level, the GADA_{high} patients had a significantly lower C-peptide response to glucagon than did GADA_{low-med} or GADA-negative patients (p = 0.04, adjusted for sex, age, and duration of diabetes, Figure 8).

**Figure 8. C-peptide response (median, IQR) to glucagon according to A. GADA level (p = 0.04, adjusted for sex, age, and duration of diabetes. B. GADA and FH (GADA+/FH_{TID} vs. others p<0.001, Study III).**

We studied the effect of an FH by stratifying patients according to FH and GADA positivity: The GADA-positive patients with a Type 1 FH had significantly higher HbA1c and glucose responses during the OGTT, as well as lower levels of insulin secretion (S-C-peptide at fasting and at 6 min after glucagon or DI_{GIT}) than did all other patient groups (Figure 8 and Table 2, Study III). Although the patients with GADA+/Type 1 FH were significantly less obese than patients with Type 2 diabetes who had Type 1 FH or Type 2 FH (GADA-/T2D_{T1D}, GADA-/T2D_{T2D}).
T2D\textsubscript{T2D}), they were more insulin resistant (K\textsubscript{ITT} 1.5 [1.4] vs. 1.9 [1.3] and 2.0 [1.3], p=0.018 and p=0.005, adjusted for age, HbA1c, sex, diabetes duration, and BMI).

In Study II with a larger group of patients, the GADA-negative patients with Type 2 diabetes had higher BMI and C-peptide concentrations than did GADA-positive patients (Supplementary Table A1 in Study II). The GADA-positive patients (LADA) had better lipid profiles despite having the same extent of insulin resistance as estimated by HOMA\textsubscript{IR}, and their insulin secretion was lower, according to mean fasting C-peptide concentration, than for GADA-negative patients with Type 2 diabetes (Supplementary Table A1 in Study II). GADA-positive patients were stratified in quartiles; the patients in the lowest quartile were older, and had a longer duration of diabetes, and a slightly lower diastolic blood pressure than did to the patients with Type 2 diabetes (Supplementary Table A2 in Study II). Viewing these from GADA negative to GADA\textsubscript{high}, a significant trend was toward lower insulin secretion based on fasting C-peptide, to a lower BMI, and to higher HDL-cholesterol levels (Figure 9).

**Figure 9.** Fasting C-peptide, BMI, and HDL-cholesterol (median, IQR) in patients with Type 2 diabetes and in GADA-positive patients by GADA quartiles (Study II).
6.4 FH, GADA, and development of diabetes

Altogether 170 subjects (6.2%) in the prospective Botnia study developed diabetes. Surprisingly, only 3 male subjects, aged 31 to 44, were diagnosed with Type 1 diabetes, all the other 167 patients were diagnosed with Type 2 diabetes, and were not treated with insulin during the first year. Two of the subjects had been highly GADA positive 4.0 and 5.2 years earlier, and the third subject was ICA- and IA-2A-positive at diagnosis. Moreover, of the 11 IA-2A-positive subjects, 5 (46%) developed diabetes.

Although subjects with an FH of diabetes developed diabetes more often (6.9%, vs. 4.0%; p < 0.028) than did subjects without any FH of diabetes, no significant difference appeared in diabetes development between subjects with a Type 1 FH (7.9%, first to third degree) and Type 2 FH (6.2%, first to third degree), which was contrary to our hypothesis. Still, incidence of diabetes was higher in those with first degree Type 1 FH (15 of 167, 9.0%), first-degree dg Type 1 and Type 2 FH (12 of 109, 11.0%) and first-degree Type 2 FH (139 of 1993, 7.0%), but because most subjects with a Type 1 FH also had a Type 2 FH, we could not analyze the pure effect of a Type 1 FH. The proportion of subjects not developing diabetes during the follow-up period according to FH of diabetes and GADA was analyzed by Cox survival analyses, and it appears in Figure 10.

Figure 10. Development of diabetes according to a family history of diabetes (A) and GADA (B).

Control, no FH of diabetes; Type 2 FH, FH of Type 2 diabetes; Type 1 FH, a first-degree FH of Type 1 diabetes (unpublished data, Study I).
The GADA-positive subjects developed diabetes significantly more often than the GADA-negative subjects (14.2% vs. 5.3%, p < 0.00001). Moreover, higher GADA levels were associated with higher risk (Figure 3 in Study I). Among subjects with an FH of diabetes, diabetes incidence was doubled between subjects with no GADA and GADA\textsubscript{low-med}, and further doubled between GADA\textsubscript{low-med} and GADA\textsubscript{high} (5.6 vs. 13.0 vs. 23.3%, p<0.0001). In subjects without any FH of diabetes, GADA level also seemed to affect diabetes incidence in the GADA\textsubscript{high} subjects (25%; p=0.035) compared with GADA-negative subjects, but not in the GADA\textsubscript{low-med} subjects (GADA\textsubscript{low-med} vs. GADA-negative, 2.9 vs. 3.9%).

Thus, both GADA level and family history of diabetes influenced risk for diabetes, and high GADA levels were also associated with a Type 1 FH. High GADA levels implied a clearly increased risk for diabetes in both the relatives and the control subjects, while low or medium-high levels implied an increased risk only in relatives of patients with diabetes.

Table 3. Cox time-dependent regression analysis of factors affecting the development of diabetes during a median follow-up of 8.1 [5.5] years.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95.0% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GADA conversion</td>
<td>6.52</td>
<td>2.80-15.17</td>
<td>0.00001</td>
</tr>
<tr>
<td>GADA\textsubscript{high}</td>
<td>4.88</td>
<td>2.80-8.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of Type 1 diabetes</td>
<td>2.24</td>
<td>1.23-4.09</td>
<td>0.009</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>2.42</td>
<td>1.87-3.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.48</td>
<td>1.09-2.04</td>
<td>0.013</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.13</td>
<td>1.09-1.17</td>
<td>0.0002</td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.01-1.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of Type 2 diabetes</td>
<td>1.64</td>
<td>0.99-2.75</td>
<td>0.057</td>
</tr>
<tr>
<td>GADA\textsubscript{low/med}</td>
<td>1.23</td>
<td>0.72-2.12</td>
<td>0.450</td>
</tr>
</tbody>
</table>

GADA conversion, GADA-negative subjects converting to GADA positivity; GADA\textsubscript{high}, subjects within high GADA levels; GADA\textsubscript{low-med}, subjects within three lower quartiles; Type 1 or Type 2 family history, subjects with a first-degree Type 1 FH or Type 2 FH, fasting plasma glucose; BMI, body mass index (Study I).

Having shown that age, sex, BMI, GADA, and an FH for Type 1 or Type 2 diabetes affected risk for diabetes, we tested the relative effects of those variables on risk for future diabetes using Cox time-dependent regression analyses; we included GADA as a time-dependent variable. The traditional risk factors (age, sex, BMI, and fasting glucose at baseline) were
independent determinants of risk, and a first degree FH of Type 1 diabetes conferred a 2.2-fold risk. High GADA levels implied a 4.9-fold risk, but unexpectedly, the highest (6.5-fold) risk was associated with a seroconversion to GADA positivity during follow-up (Table 3).

6.5 FH and GADA in prediction of insulin deficiency

Insulin deficiency was defined as serum C-peptide level of < 0.7 nmol/l after glucagon-stimulation. Most of the patients considered insulin-deficient had a Type 1 FH (30 of 36; 83%). While 38% of the GADA-positive and 7% of GADA-negative patients were insulin deficient, those who had both GADA and a Type 1 FH were associated with the highest prevalence of insulin deficiency (GADA+/Type 1 FH 46.4% vs. GADA-/Type 1 FH 9.5% and GADA-/Type 2 FH 4.5% ; p<0.00001). Notably, none of the six patients with GADA+/Type 2 FH had a low C-peptide response. We made a logistic regression analysis of the risk for insulin deficiency, including the HbA1c and the duration of diabetes as covariates, together with either an FH of diabetes, GADA positivity, a risk HLA genotype or a combination of GADA positivity and Type 1 FH.

A Type 1 FH implied a 3.7-fold risk (95% CI 1.4-10.3, p=0.01), HLA risk genotypes a 3.6-fold risk (1.5-8.3, p=0.003), GADA positivity a 5.5-fold risk (2.0-15.1, p=0.001), and a combination of GADA positivity and a Type 1 FH a 17-fold risk (4.7-58.2, p=1.3*10^{-5}). When all variables were entered into the same model, a combination of GADA and a Type 1 FH implied a 12.3-fold risk (95% CI 3.2-44.1, p= 2.1*10^{-4}) for insulin deficiency, whereas HLA risk genotypes did not reach significance. Moreover, high serum HDL and low triglyceride values correlated with development of insulin deficiency. In another logistic regression analysis with the same covariates as in the first analysis, but taking into account GADA level, a Type 1 FH implied a 2.9-fold risk, high GADA levels a 6.2-fold risk, HbA1c a 2.2-fold risk, and a combination of GADA and a Type 1 implied a 9.4-fold risk for insulin deficiency.

Development of insulin deficiency in relation to duration of diabetes we analyzed using Kaplan-Meier survival analysis, stratified for FH or GADA positivity (Figure 2 in Study III). In 9 years, 20% of the patients with a Type 1 FH developed insulin deficiency, compared to only 2% of the Type 2 FH patients. When analyzing only the GADA effect, 20% of the GADA-positive patients developed insulin deficiency in 5 years, whereas 20% of the GADA-
negative patients developed insulin deficiency in 20 years. We also looked at development of insulin deficiency according to GADA/FH using Kaplan Meier analyses. Patients with GADA+/Type 1 FH and GADA-/Type 1 FH developed insulin deficiency earlier and more often than did patients with GADA-/Type 2 FH. For 20% of the patients to develop insulin deficiency, it took 4 years for patients with GADA+/Type 1 FH compared to the 10 years for patients with GADA-/Type 1 FH (Figure 11). In 5 years, 10% of the GADA-negative patients with a Type 1 FH, but none of the GADA-negative patients with a Type 2 FH developed insulin deficiency.

**Figure 11.** Kaplan-Meier survival analyses of development of insulin deficiency following development of diabetes according to FH and GADA (Study III).

We could not accomplish Kaplan-Meier analyses for different GADA-level groups, because of the small groups. Insulin deficiency was most prevalent in patients with high GADA levels (42%); those patients with high GADA levels who were not insulin-deficient at the time of investigation were approximately 10 years younger than were other non-insulin-deficient patients (unpublished data).
We have shown in a large cohort already having a risk for the development of diabetes that Type 1 FH and GADA positivity both raise their diabetes incidence. We have also shown that level of GADA positivity has an effect here and also affects LADA patients’ clinical characteristics. Finally, we showed, by more exact methods, that Type 1 FH and GADA are independent risk factors in patients with Type 2 diabetes for development of a more severe insulin deficiency. These studies help us to understand the heterogeneity of LADA and also of Type 2 diabetes, which is important in searching for the aetiopathogenesis of these diseases.

Since autoimmunity is common in twins in Type 1 diabetes families, and in siblings of Type 1 diabetic patients (181), the assumption is that genetic factors contribute to development of autoimmunity. We have therefore assumed that a Type 1 FH would also contribute to development of diabetes in MIX families and to development of insulin deficiency in patients with Type 2 diabetes.

Relatives without diabetes in the Botnia family study represent a large cohort at higher risk for diabetes. Their diabetes incidence was, however, lower than were the frequencies recently published for the Finnish population (6.2% vs. 8.7%, (1)). The Botnia study began over 20 years ago, and some of those individuals were followed only in the 1990s. Most of the individuals lived in the western part of Finland, where the Botnia study originated more than 20 years ago. A long follow-up time was one of the strengths of Study I, but it did cause challenges as well, due to changes in laboratory methods and in GADA limits. In the prospective Study I was therefore great variation in follow-up time, as the patients were recruited over a long period of time. Another strength of our study was its use of OGTT for glycaemic-state assessment, which is a more reliable method than are the fasting values used in most large studies.

Here we showed that risk for diabetes was significantly higher in adult high-GADA-positive subjects; the same was true also for control subjects (patients without a family history of diabetes), but not for subjects with lower GADA levels. The latter needs confirmation in a larger study, as we had only 38 GADA-positive control subjects, including 4 with high GADA levels. Thus, diabetes incidence was associated with GADA positivity level at least in those with an FH of diabetes. Adult patients with GADA developed diabetes significantly
more often, as observed earlier (149), but our results suggest that a first-degree Type 1 FH has the same effect. The highest incidence of diabetes was in subjects seroconverting to GADA positivity during follow-up, also suggesting that β-cell autoimmunity can be induced at any age. It may also be, that those who were high-GADA-positive or who seroconverted to positivity, had real ongoing autoimmune process, and that there were more false positive in those with lower GADA levels. These findings bear some similarity to data on those with Type 1 diabetes, for whom the number of Type 1 relatives has predicted diabetes (197).

**GADA prevalence**

GADA prevalence (4.7%) in non-diabetic subjects with and without an FH of diabetes was much higher in the general population (0.5-1.5%, (81,111)), but is comparable to findings for at-risk individuals (224). High GADA frequency in the control subjects may be related to the overall high prevalence of Type 1 diabetes in Finland and may be associated with genetic factors such as the HLA risk genotypes more frequent in Scandinavia, but also with environmental factors. The influence of environmental factors is supported by immigrants’ tendency to adopt the incidence of the disease of the destination country as shown by Somali children in Finland, despite their different risk genotypes (42).

Type 1 FH was associated with a high prevalence of GADA positivity in patients with Type 2 diabetes, as Li et al has shown (19). Prevalence of GADA positivity in patients with a Type 1 and also with a Type 2 FH was slightly lower (14.3% vs. 18.0% and 4.3% vs. 8.0%, respectively) than in Li et al (19). This could be due to their study design, since some of their patients were related, while in the MIX study all patients were unrelated. Still, the prevalence of GADA in patients with a Type 1 FH was higher than in most studies on patients with Type 2 diabetes, although the overall GADA prevalence (10%) in our MIX study was similar to other studies’ prevalence (12, 86-91).

**Insulin resistance**

Subjects without diabetes became more insulin resistant according to HOMA$_{IR}$ during the follow-up period, but the GADA-positive subjects were still less insulin resistant at the onset of their diabetes than were the GADA-negative subjects. Surprisingly, the GADA-positive patients with a Type 1 FH were more insulin resistant than other patients, according to insulin
tolerance testing, even when sensitivity was adjusted for level of hyperglycaemia, BMI, and age.

In earlier studies, LADA patients have been less insulin resistant (14,139) than were patients with Type 2 diabetes or at least had an equivalent insulin resistance level (5,8,13,15). Tripathy et al (2002) measured insulin sensitivity directly with a euglycaemic hyperinsulinemic clamp, while Carlsson et al used the glucose-arginine test; neither observed any difference between LADA and Type 2 diabetes (13). The other studies have used the HOMA model based on fasting insulin and glucose. HOMA will, however, give a false finding of insulin sensitivity if glucose concentration increases while insulin concentration decreases, as in insulin deficiency. The ITT is likely to be more useful for measuring insulin resistance in patients with diabetes than is HOMAIR, which is mostly used with normoglycaemic subjects. LADA patients usually have fewer features of the metabolic syndrome, and this has also been considered a sign of higher insulin sensitivity. Furthermore, here we observed an increase in the BMI of patients with LADA compared to BMI in patients in our Finnish study more than 10 years earlier (28.5 vs. 26.0 kg/m², (12)), which may reflect a change in lifestyle and can affect the insulin sensitivity. Still, insulin resistance may contribute to development of insulin deficiency in patients with LADA.

**Insulin secretion in GADA-positive subjects without diabetes**

The GADA-positive subjects differed from GADA-negative subjects only in their lower measures of insulin secretion before diabetes onset. Subjects with high GADA levels had a blunted insulin response during the OGTT, and after their diagnosis they were also less insulin resistant than were our GADA-negative subjects. These findings indirectly support the hypothesis that GADA may be associated with a defect in insulin secretion even before diabetes onset, as has been suggested by Lethagen et al (2002). The defect is presumably mild and seen only when the β-cells are maximally stressed. The follow-up data came from the visit at the onset of diabetes, which means that diabetes was probably discovered earlier than usual, because of the regular follow-up.

**Effect of GADA level and FH on clinical characteristics in patients with diabetes**

GADA levels seemed to have some effect on insulin secretion; a significant trend was toward lower insulin secretion observed from the lowest GADA quartile to the highest, as suggested
by others (225-227). Li X et al (2004) tried to find the optimal cut-off point in attempting to discriminate those LADA patients who were at greater risk for insulin deficiency, but actually the different GADA level groups did not much differ in this respect (156).

We could not determine the role of FH of diabetes in Study II. However, since HLA risk genotypes correlated with GADA level in Studies II and III, one could assume that this would also be the case with a Type 1 FH, which correlated with GADA level in Study III, but without enough patients for anymore subgroups. Our studies thus suggest that a Type 1 FH is also associated with GADA level, as was true Studies I and III, in subjects without diabetes, and in patients with Type 2 diabetes. However, in Study III, GADA-negative patients with insulin deficiency also seemed to be clinically more like patients with Type 1 diabetes (unpublished information), but this issue requires study in a larger group of patients.

In Study II, clinical characteristics of patients in the lower quartiles differed only slightly from the clinical characteristics of GADA-negative patients with Type 2 diabetes. A Type 2 FH has been shown to lead to increased features of metabolic syndrome and age of onset in patients with Type 1 diabetes (196,228). The same phenomenon was evident also in Study III, where patients in lower quartiles more often had only a Type 2 FH (10%, 27% vs 44%, Figure 5, unpublished) and patients with lower GADA levels more often had features of metabolic syndrome than did patients with higher GADA levels.

We attempted to study the pure effect of Type 1 FH in the MIX study by stratifying patients according to GADA and FH. Lower levels of insulin secretion were apparent in patients with a Type 1 FH and GADA positivity, but since most of the patients with high GADA levels also had a Type 1 FH, we discerned no effect of FH from GADA. The GADA-positive patients with Type 1 FH more often had a first-degree Type 1 FH than did the GADA-negative patients with a Type 1 FH.

**Development of insulin deficiency**

GADA-positive patients with Type 1 FH differed not only from the GADA-negative patients but also from other GADA-positive patients with Type 2 FH by being more insulin-deficient and having more severe hyperglycaemia despite being leaner. In fact, the GADA-positive patients with a Type 1 FH had the highest risk for developing insulin deficiency. Even though the proportion of insulin-deficient patients was especially great among the high-GADA group, many patients with a Type 1 FH developed insulin deficiency irrespective of their GADA. In
In this respect, Type 1 FH seemed to predispose to a more severe state of insulin deficiency. It is also interesting that we found no insulin-deficient patients in GADA-positive/Type 2 FH group, but it included only six patients.

In a study by Li H et al, Type 2 diabetes patients with a Type 1 FH had lower fasting C-peptide concentrations and more often used insulin treatment than did patients with a Type 2 FH, even when GADA-positive patients were excluded from analyses (22). The GADA-positive patients and especially those with high GADA levels more often developed insulin-deficiency than did GADA-negative patients with Type 2 diabetes (13,87,89,95,96,156). The glucagon test was their choice in a prospective study by Niskanen et al; however, they studied with a glucagon test only those patients already using insulin therapy or with signs of lower insulin secretion in the OGTT, and made no study of any FH of diabetes. Tuomi et al (1993) also measured a stimulated C-peptide response in outpatient clinic patients, but had only eight patients with Type 1 FH. It is also difficult to compare our findings with those of other studies with insulin treatment as a surrogate marker of insulin deficiency, studies which made no attempt to measure insulin secretion. Measuring fasting C-peptide or insulin levels seems insufficiently sensitive as a method, although it is adequate to detect the most severe cases of insulin deficiency.

In addition to the matched study design, another strength of Study III (MIX study) was that it used several methods to assess β-cell function and insulin sensitivity. One weakness of the MIX study was the small number of patients when stratified into subgroups, even though we had a large number of subjects overall. The MIX study faced some difficulties recruiting patients with a Type 2 FH because of its matching criteria. Patients with a Type 1 FH tended to show a younger age of onset and a lower BMI, but by matching the patients we could diminish the statistical bias resulting from these factors. This setting may also explain differences from other studies. In the MIX study, median age differed between groups when patients were stratified according to GADA quartiles. We also could have had more insulin-deficient patients in the high-GADA group, as those who did not develop insulin deficiency were 10 years younger than non-developers in other groups. More GADA-positive patients were in Study II, but insulin secretion and sensitivity assessments were based only on fasting C-peptide and –insulin values.
Both a Type 1 FH and GADA were associated with development of diabetes and insulin deficiency. However, a Type 1 FH was independently associated with insulin deficiency, and we observed that almost half the insulin-deficient patients had neither GADA nor HLA risk genotypes, although most had a Type 1 FH. Presumably genetic factors outside the loci studied contribute to this association, perhaps by autoimmune processes not associated with GADA, such as islet T-cell reactivity, which leads to an increase in insulin deficiency among Type 2 diabetes patients negative for GADA and the HLA risk genotypes (4). The suggestion that GADA are present at a very low titre is a less likely explanation, since insulin deficiency seemed to be associated especially with high GADA levels, which are likely to persist many years. It would also be important to study more intensely the effect of degree of family history.

Finally, our study suggests that patients with Type 2 diabetes and with a Type 1 FH inherit a vulnerability to immune-system failure predisposing them to GADA positivity and also earlier and more severe insulin deficiency, as seen in patients with LADA. Subjects with higher GADA levels are clinically more like patients with Type 1 diabetes, and those with lower levels are like patients with Type 2 diabetes. If we combine with this the knowledge that there exist differences in autoantibody presentation and HLA risk genotypes according to age of onset, the aetiopathogenesis of LADA seems to differ from younger-onset as well as from older-onset Type 1 diabetes.

The clinical picture in adult patients with LADA is milder than in classical Type 1 diabetes, depending in part on FH of diabetes or on GADA level. Still, patients with LADA were at least as insulin resistant as were patients with Type 2 diabetes irrespective of GADA level. Whether insulin resistance is involved in the aetiopathogenesis of LADA more than in classical Type 1 diabetes is an interesting question needing more study. However, insulin deficiency was only partially explained by GADA and the most common Type 1 HLA risk genotypes; there may exist unknown Type 1 FH-related inherited factors predisposing patients with Type 1 FH to a more severe insulin deficiency. Furthermore, the fact that most patients who developed marked insulin deficiency had a Type 1 FH has clinical implications for screening for insulin deficiency. Thus, heterogeneity of LADA is in part explained by a Type 1 FH and GADA level.
8. SUMMARY OF RESULTS AND CONCLUSIONS

Results of Studies I-III can be summarized as follows:

1. GADA prevalence was 4.7% in subjects without diabetes at the baseline visit. Of all the subjects, 1.1% converted to positive during the follow-up period.

2. GADA positivity clustered in families with Type 1 and Type 2 diabetes (MIX) in relatives, and in patients with a family history of Type 1 diabetes. High GADA levels were especially evident in patients with a Type 1 FH.

3. GADA positivity and especially high GADA positivity elevated the risk for diabetes, and a first-degree Type 1 FH was associated with development of diabetes. Those converting to GADA positivity during the follow-up period had the highest risk for diabetes. Subjects with high GADA levels and with a first-degree Type 1 FH developed diabetes at a significantly younger age.

4. Clinical characteristics of GADA-positive subjects differed from those of GADA-negative subjects without diabetes only by their lower levels of insulin secretion before diabetes onset. At follow-up, the GADA-positive subjects were leaner, and the subjects with high GADA levels had lower insulin responses and, according to their HOMA$_{IR}$, less evidence of insulin resistance at diabetes onset.

5. In patients with diabetes, GADA positivity and a Type 1 FH were associated, according to the glucagon test, with lower levels of insulin secretion. According to the insulin tolerance test, GADA-positive patients with a Type 1 FH were less insulin sensitive than were other patients, even when sensitivity was adjusted for the level of hyperglycaemia, BMI, and age.

6. High GADA positivity and a Type 1 FH were independently associated with development of insulin deficiency defined by the glucagon test, and the highest risk was for GADA-positive patients with Type 1 FH. Of the patients who developed
insulin deficiency, 83% had a Type 1 FH, but only among of these patients did 37% test as GADA positive.

GADA positivity was associated in the relatives of patients with diabetes with development of diabetes and in LADA patients with insulin deficiency. GADA positivity predicted diabetes in adult relatives independently of FH, but a seroconversion to GADA positivity was the most important risk factor. Still, a Type 1 FH not only led to increased the rate and strength of GADA positivity but also predisposed to a more severe and earlier insulin deficiency in patients with Type 2 diabetes. There also seemed to be unknown Type 1 diabetes-related, possibly inherited factors predisposing to insulin deficiency. Most of the patients who developed a marked insulin deficiency had a Type 1 FH, a fact with clinical implications for screening for insulin deficiency. Furthermore, the heterogeneity of LADA diabetes is in part explained by a Type 1 FH and the GADA level.

Lapsilla riskiä sairastua tyypin 1 diabetekseen voidaan ennustaa diabetes sukutaustan ja HLA- ja autovasta-ainemääristyksen avulla. Tyyppi I diabeteksessa esiintyy vasta-aineita merkkinä haiman insuliinia tuottavien solujen tuhosta. GAD (Glutamic acid decarboxylase) -vasta-aineita esiintyy yleisesti nuorilla tyypin I diabeetikoilla muiden vasta-aineiden ohella, mutta niiden merkityksestä terveillä ja tyypin II diabeetikoilla on vähemmän tiedoa. Aikuisilla tyyppin I diabeetikoilla sen sijaan esiintyy harvemmin mitä kuin GAD–vasta-aineita. GAD vasta-aineita löydetään ajoittain myös Tyyppin II diabeetikoilla ja niiden tiedetään ennustavan insuliinipuutteen kehittymistä, mutta insuliinipuute selvästi hitaammin kuin klassisessa tyyppin I diabeteksessa ja sen vuoksi heidät aluksi helposti luokitellaan tyyppin II diabeetikoiksi. Kansainvälisessä diabetesluokituksessa tämä diabetestyyppi onkin erotettu omaksi tyyppin I diabeteksen alaryhmäksi nimeltä LADA (latent autoimmune diabetes of adults). Tyyppin I ja tyyppin II diabetes esiintyvät usein samoissa perheissä ja on havaittu, että näissä perheissä on enemmän myös LADA diabetesta.

Tässä väitöskirjassa tutkin tyyppin I sukutaustan vaikutusta GAD-vasta-aineiden esiintymiseen tyyppin II diabeetikoilla ja heidän sukulaissiilaan, sekä sukutaustan ja vasta-aineiden merkitystä sairastumisriskiin huomioiden myös muut tunnetut riskitekijät. Lisäksi tutkin tyyppin II diabeetikoilla, miten tyyppin I sukutausta ja GAD-vasta-aineet vaikuttavat insuliinipuutteen kehittymiseen ja muihin kliiniisiin muuttuihin eli diabeteksen taudinkuvaan. GAD-vasta-
aineiden esiintymistä tutkin 4976 henkilöllä, joilla oli diabetes-sukutausta ja joista 2764:ää oli seurattu keskimäärin 8.7 vuotta diabeteksen kehittymisen suhteen Botnia-tutkimuksessa. LADA diabeteksen monimuotoisuuden selventämiseksi vertasin Botnia-tutkimuksen 213 GAD-positiivisen ja 648 GAD-negatiivisen tyypin II diabeetikon kliinisää muuttujia. Tyypin I sukutaustan vaikutuksen selvittämiseksi tutkin yhteensä 335 tyypin I ja tyypin II sukutaustaista tyypin II diabeetikkoa (MIX-tutkimus) tarkemmin insuliinierityksen ja insuliniresistenssin suhteen.

Tutkimuksissa todettiin, että GAD vasta-aineita esiintyi tyypin I sukutaustaisilla henkilöillä ja diabeetikoilla merkittävästi useammin ja korkeampia pitoisuuksia verrattuna niihin, joilla oli tyypin II sukutausta. GAD-positiivisilla henkilöillä ja erityisesti niiillä, joilla oli korkeat vastaaine-pitoisuudet, oli viitteitä heikommasta insuliinierityksestä jo ennen diabetekseen sairastumista. GAD-vasta-aineet ja myös tyypin I sukutausta, etenkin I:asteen sukutausta, lisäivät sairastumisriskiä ja suurin riski oli niiillä, joille vasta-aineita kehitettiin seurannan aikana.

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REFERENCES


(2) Lammi N, Blomstedt PA, Moltchanova E, Eriksson JG, Tuomilehto J, Karvonen M. Marked temporal increase in the incidence of type 1 and type 2 diabetes among young adults in Finland. Diabetologia 2008 May;51(5):897-899.


(19) Li HBM, Isomaa B, Taskinen MRPHD, Groop LPHD, Tuomi TPHD. Consequences of a Family History of Type 1 and Type 2 Diabetes on the Phenotype of Patients With Type 2 Diabetes.[Article]. Diabetes Care 2000 May;23(5):589-594.


(22) Li H, Isomaa B, Taskinen MR, Groop L, Tuomi T. Consequences of a family history of type 1 and type 2 diabetes on the phenotype of patients with type 2 diabetes.[see comment]. Diabetes Care 2000 May;23(5):589-594.


(28) Fu AZ, Qiu Y, Radican L, Wells BJ. Health care and productivity costs associated with diabetic patients with macrovascular comorbid conditions. Diabetes Care 2009 Dec;32(12):2187-2192.


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(90) Radtke MA, Midthjell K, Nilsen TI, Grill V. Heterogeneity of patients with latent autoimmune diabetes in adults: linkage to autoimmunity is apparent only in those with perceived need for insulin treatment: results from the Nord-Trondelag Health (HUNT) study. Diabetes Care 2009 Feb;32(2):245-250.


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74


(202) 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 1993 Nov;36(11):1155-1162.


