

# Clinical and Genetic Characteristics of Type 2 Diabetes With and Without GAD Antibodies

Tiinamaija Tuomi, Åsa Linda Carlsson, Haiyan Li, Bo Isomaa, Aaro Miettinen, Anita Nilsson, Michael Nissén, Björn-Olof Ehrnström, Björn Forsén, Börje Snickars, Kaj Lahti, Carol Forsblom, Carola Saloranta, Marja-Riitta Taskinen, and Leif C. Groop

The aim of the study was 1) to establish the prevalence of GAD antibodies (GADab) in a population-based study of type 2 diabetes in western Finland, 2) to genetically and phenotypically characterize this subgroup, and 3) to provide a definition for latent autoimmune diabetes in adults (LADA). The prevalence of GADab was 9.3% among 1,122 type 2 diabetic patients, 3.6% among 558 impaired glucose tolerance (IGT) subjects, and 4.4% among 383 nondiabetic control subjects. Islet antigen 2 antibodies (IA2ab) or islet cell antibodies were detected in only 0.5% of the GADab<sup>-</sup> patients. The GADab<sup>+</sup> patients had lower fasting C-peptide concentrations (median [interquartile range]: 0.46 [0.45] vs. 0.62 [0.44] nmol/l,  $P = 0.0002$ ) and lower insulin response to oral glucose compared with GADab<sup>-</sup> patients. With respect to features of the metabolic syndrome, the GADab<sup>+</sup> patients had lower systolic (140 [29.1] vs. 148 [26.0] mmHg,  $P = 0.009$ ) and diastolic (79.2 [17.6] vs. 81.0 [13.1] mmHg,  $P = 0.030$ ) blood pressure values, as well as lower triglyceride concentrations (1.40 [1.18] vs. 1.75 [1.25] mmol/l,  $P = 0.003$ ). GADab<sup>+</sup> men had a lower waist-to-hip ratio compared with GADab<sup>-</sup> patients. Compared with GADab<sup>-</sup> patients and control subjects, the GADab<sup>+</sup> patients had an increased frequency HLA-DQB1\*0201/0302 (13 vs. 4%;  $P = 0.002$ ) and other genotypes containing the \*0302 allele (22 vs. 12%;  $P = 0.010$ ). However, the frequency of these high-risk genotypes was significantly lower in GADab<sup>+</sup> type 2 patients than in type 1 diabetes of young or adult onset (0201/0302 or 0302/X: 36 vs. 66 vs. 64%,  $P < 0.001$ ). The GADab<sup>+</sup> type 2 group did not differ from control subjects with respect to genotypes containing the protective DQB1-alleles \*0602 or \*0603, nor with respect to the type 1 high-risk genotype in the IDDM1 (Hph1 +/+). We conclude that GADab<sup>+</sup> patients differ from both GADab<sup>-</sup>

type 2 diabetic patients and type 1 diabetic patients with respect to  $\beta$ -cell function, features of the metabolic syndrome, and type 1 diabetes susceptibility genes. Further, we propose that LADA be defined as GADab positivity (>5 relative units) in patients older than 35 years at onset of type 2 diabetes. *Diabetes* 48:150–157, 1999

**A** subgroup of patients diagnosed with type 2 diabetes has circulating antibodies to islet cell cytoplasmic antigens (ICA) (1–4) and more frequently to GAD (GADab) (5–7). Several studies have shown that positivity for ICA (3,4,8) or GADab (5–7, 9,10) correlates with insulin deficiency or relative insulin requirement in Caucasians, and also in Asians (11,12). Fifty percent of newly diagnosed GADab<sup>+</sup> patients developed relative insulin deficiency, defined as glucagon-stimulated C-peptide concentration <0.7 nmol/l, after 10 years compared with 3% of GADab<sup>-</sup> patients (10). In consequence, 52% of GADab<sup>+</sup> patients required insulin therapy after 6 years in the U.K. Prospective Diabetes Study (UKPDS) (7). Autoantibody positivity together with the subsequent development of insulin deficiency lead to introduction of the eponym latent autoimmune diabetes in adults (LADA) for this subgroup (5), which now has been included in the proposal for the new World Health Organization (WHO) criteria for diabetes as its own subgroup (13).

However, except for the UKPDS (7), the studies on LADA have been small and, at least partly, restricted to hospital outpatient clinics, which may have led to selection of more serious cases. The data on features of the metabolic syndrome in LADA are limited to one small study (14), although the patients with LADA may be indistinguishable from type 2 diabetes at onset of disease. The key question is whether LADA represents a late manifestation of type 1 diabetes or whether it can be considered a unique disease entity. One way to approach the problem is—in addition to comparing the phenotype between patients with LADA, type 1 diabetes, and type 2 diabetes—study whether LADA patients share a genetic background with type 1 diabetic patients.

Of the familial risk for type 1 diabetes, 42% has been ascribed to the IDDM1-locus in the HLA Class II region on chromosome 6p21, and 10% has been ascribed to the IDDM2-locus in the promotor region of the insulin gene on chromosome 11p15.5 (15). In addition, several other gene loci have been suggested to contribute to the genetic susceptibility to type 1 diabetes (16–23). Whether the type 1 diabetes susceptibility genes play a role in the etiology of polygenic type

From the Wallenberg Laboratory (T.T., Å.L.C., H.L., A.N., L.C.G.), Department of Endocrinology, Lund University, Lund, Sweden; Jakobstad Hospital (B.I.), Jakobstad; Helsinki University Central Hospital and Haartman Institute (A.M.), University of Helsinki; the Department of Internal Medicine (C.F., C.S., M.-R.T.), Helsinki University Central Hospital, Helsinki; Primary Health Care Centers (B.-O.E., B.F., B.S., K.L.) in Korsholm, Närpes, Malax-Korsnäs, and Vaasa; and the Vaasa Central Hospital (M.N.), Vaasa, Finland.

Address correspondence and reprint requests to Dr. T. Tuomi, Wallenberg Laboratory, Department of Endocrinology, Lund University, S-20502 Malmö, Sweden. E-mail: tiinamaija.tuomi@endo.mas.lu.se.

Received for publication 14 October 1997 and accepted in revised form 28 September 1998.

CV, coefficient of variation; FBG, fasting blood glucose; FS, fasting serum; IA2, islet antigen 2; ICA, islet cell antibody; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; LADA, latent autoimmune diabetes in adults; PCA, parietal cell antibody; PCR, polymerase chain reaction; RU, relative unit; TGA, thyroglobulin antibody; TMSA, anti-thyroid microsomal antibody; UKPDS, U.K. Prospective Diabetes Study; WHO, World Health Organization.

2 diabetes is unclear. An increased frequency of HLA-DR4 has been reported in type 2 diabetes (24–26), but the increase seems to be restricted to patients with relative insulin deficiency (24).

The aim of this study was 1) to establish the prevalence of LADA in a population-based study on type 2 diabetes in western Finland, 2) to phenotypically and genetically characterize this subgroup, and 3) to provide a definition for LADA based on the unique characteristics of the patients identified in this study.

## RESEARCH DESIGN AND METHODS

**Subjects.** The subjects participated in the Botnia study in Western Finland, into which patients with type 2 diabetes and their family members have been recruited since 1990 (27). Nondiabetic spouses without family history of diabetes served as control subjects. Glucose tolerance was assessed according to the revised WHO and American Diabetes Association criteria (28). Thus, subjects with fasting blood glucose (FBG)  $\geq 6.1$  mmol/l or 2-h blood glucose  $\geq 10$  mmol/l during an oral glucose tolerance test (OGTT) were diagnosed with diabetes, and subjects with FBG  $< 5.5$  mmol/l and 2-h blood glucose  $< 6.7$  mmol/l as normal glucose tolerance, and subjects with intermediate blood glucose levels as impaired glucose tolerance (IGT). After exclusion of families with maturity-onset diabetes of the young (29), GADabs were analyzed for 2,063 subjects, including 383 control subjects, 558 IGT subjects, and 1,122 patients diagnosed with type 2 diabetes.

The clinical characteristics were compared with those in 194 patients (108 men, 86 women) with classic type 1 diabetes, including 82 patients from the Botnia Study (the age at onset of diabetes was  $\leq 20$  years in 50 and  $> 20$  years in 32 patients) and 112 patients diagnosed before the age of 35 years who attended the Jakostad Hospital outpatient clinic located in the Botnia region (30). Their mean age was 36.5 [20.8] years; age at onset of diabetes was 15 [14.8] years; duration was 21.0 [17.5] years; HbA<sub>1c</sub> was 8.4 [1.9]%; fasting serum (FS) C-peptide was 0.01 [0.1] nmol/l; and BMI was 24.3 [3.5] kg/m<sup>2</sup>.

Genetic analyses were performed for all unrelated GADab<sup>+</sup> patients diagnosed with type 2 diabetes for whom DNA was available ( $n = 95$ ). In case of more than one GADab<sup>+</sup> patient in the same family (eight families), the patient who was first recruited in the study was included. Genotype frequencies were compared with those in 172 GADab<sup>-</sup> control subjects (vide supra; 76 M/95 F; age [mean  $\pm$  SD] 57.1  $\pm$  12.9 years) and in 191 (88 M/103 F) unrelated GADab<sup>-</sup> type 2 diabetic patients with an age at onset of diabetes  $> 40$  years (61  $\pm$  10 years), who were randomly chosen to be representative of the whole Botnia study population (age 67.5  $\pm$  9.9 years; duration of diabetes 6.5  $\pm$  6.4 years, BMI 29.3  $\pm$  4.5 kg/m<sup>2</sup>, HbA<sub>1c</sub> 7.21  $\pm$  1.5%, 19% treated with insulin).

**Antibody tests.** GADab and islet antigen 2 antibodies (IA2ab) were measured by a radioimmunoprecipitation method employing <sup>35</sup>S-labeled recombinant human GAD65 produced by in vitro transcription/translation (31,32). The *Escherichia coli* clones with complementary DNA for full-length human GAD65 or intracellular domain of IA2 were gifts from Drs. Allan E. Karlens and Catherine E. Grubin, University of Washington (Seattle, WA) and Dr. Michael Christie, Kings College (London, U.K.), respectively. The results are expressed as rela-

tive units (RU): RU = (sample cpm – mean cpm of 3 negative controls)/(cpm of a positive internal reference serum – mean cpm of 3 negative controls)  $\cdot$  100. The cutoff limit for positivity was 5 RU for GADab and 2.5 RU for IA2ab, which represent mean + 3 SD of 296 (GADab) or 155 (IA2ab) healthy Finnish control subjects. At the Combined Autoantibody Workshop (33), the specificity and sensitivity of the GADab assay were 99 and 75%, respectively.

ICA (34) and parietal cell antibodies (PCAs) (8) were determined by an indirect immunofluorescence technique. Anti-thyroid microsomal antibodies (TMSAs) and thyroglobulin antibodies (TGAs) were measured using Serodia-ATM or -ATG particle agglutination kits, respectively (Fujirebio, Tokyo, Japan) (8). The cutoff value for ICA was 5 Juvenile Diabetes Foundation units. The screening titers for PCA, TMSA, and TGA were 1:10, 1:400, and 1:100, respectively. ICA, PCA, TMSA, and TGA tests were performed for the first 517 type 2 diabetes patients recruited in the study and for all GADab<sup>+</sup> patients for whom serum was available (Table 1). Of the control subjects, 1/122 (0.8%) was positive for ICA (0/36 M, 1/86 W), and 11/115 (9.6%) were positive for PCA (2/29 M, 9/86 W), 12/105 (11.4%) for TMSA (2/27 M, 10/78 W), and 4/105 (3.8%) for TGA (0/27 M, 4/78 W).

**Metabolic measurements.** An OGTT was performed for all subjects  $\geq 15$  years old who had FBG  $< 10$  mmol/l and were not treated with insulin. After 12 h of overnight fasting, the subjects ingested 75 g of glucose in a volume of 300 ml. Samples for measurements of blood glucose and serum insulin were drawn at –10, 0, 30, 60, and 120 min. Blood glucose was measured with a hexokinase method with a coefficient of variation (CV) of  $< 1\%$  (Boehringer Mannheim, Mannheim, Germany). Serum insulin concentrations were measured by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) with an interassay CV of 5%.

FS C-peptide concentrations were measured in duplicate by a radioimmunoassay with an interassay CV of 9% (35). HbA<sub>1c</sub> concentrations were measured by high-pressure liquid chromatography. The reference values for the assay were 5–7%. Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured on a Cobas Mira analyser (Hoffman LaRoche, Basel).

**HLA-DQB1-genotypes.** The second exon of the DQB1 gene was amplified by polymerase chain reaction (PCR) followed by dot-blotting onto positively charged nylon membranes (HybondTM-N+; Amersham, U.K.) and hybridization with sequence-specific oligonucleotide probes at 54°C in 50 mmol/l Tris-Base, 2 mmol/l EDTA, 0.1% SDS, 3 mol/l tetramethylammonium chloride, 5 $\times$  Denhardt's, and 0.1 mg/ml herring sperm DNA. The sequence-specific oligonucleotide probes originating from the 11th International Histocompatibility Workshop (36) were labeled with digoxigenin (DIG Oligonucleotide 3'-End Labelling Kit; Boehringer Mannheim), and the bound probes were detected with alkaline phosphatase conjugated antidigoxigenin antibody and chemiluminescent substrate CSPD (DIG Luminescent Detection Kit; Boehringer Mannheim). Three DQB1 probes were used to distinguish the DQB1 alleles \*0201, \*0302, and either \*0602 or \*0603 [\*0602(3)]. Genotypes are presented as 0201/0302, 0201/X, 0302/X, 0201/0602(3), 0302/0602(3), 0602(3)/X, or X/X, where X could mean either a homozygous allele or any allele other than 0201, 0302, or 0602(3).

**Hph1-polymorphism in the insulin gene.** We used a restriction fragment length polymorphism method involving digestion of the PCR-amplified DNA (37) with Hph1 according to manufacturer's instructions (Amersham, Buckinghamshire, U.K.) to produce fragments of 229 bp + 125 bp (–/– genotype), or 190 bp + 125 bp + 39 bp (+/+ genotype).

TABLE 1  
Frequency of ICA, IA2ab, PCA, TMSA, and TGA in relation to GADab levels in patients diagnosed with type 2 diabetes

	Negative GADab ( $\leq 5$ RU)	Low GADab ( $> 5$ –38 RU)	High GADab ( $> 38$ RU)
<i>n</i> (M/W)	517 (221/296)	64 (31/39)	29 (14/15)
IA2ab	0.5	10.1*	33*
ICA	0.6	1.4	43†
PCA			
Men	9	16	29‡
Women	19	15	20
TMSA			
Men	7.2	3.5	14§
Women	10	16	40
TGA			
Men	3.2	3.7	0
Women	6.7	8.8	6.7

Data are % unless otherwise indicated. \* $P < 0.00001$  (all three groups); † $P < 0.00001$  (high GADab vs. other groups); ‡ $P = 0.056$  (high GADab vs. other groups); § $P = 0.002$  (high GADab vs. other groups).

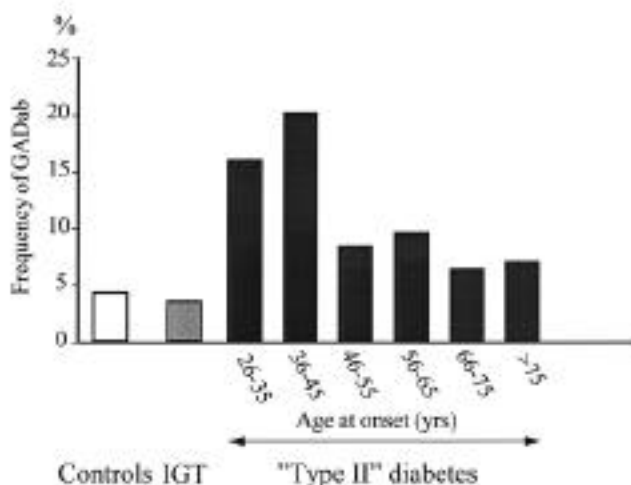
**Statistical analysis.** The statistical significance of the differences between group frequencies was tested by the  $\chi^2$  test (with Yates correction when appropriate) or Fisher's exact test. The differences in continuous variables between groups were tested by the Mann-Whitney test, the Kruskal-Wallis test for group means, or GLM analysis of variance using either the BMDP or SOLO statistical software (Biomedical Data Processing, Los Angeles, CA). Logarithmic transformation of data was used for the covariate analysis. Incremental glucose and insulin areas under the curve during the OGTT were calculated by the trapezoidal rule. For the statistical analysis, the insulin and C-peptide concentrations were adjusted for age, BMI, and duration of diabetes. The blood pressure and lipid data were adjusted for age and BMI. Data were analyzed separately for men and women, but the sex-specific data are given only when differences between the sexes were seen. Data are given as means  $\pm$  SD or medians [75–25% interquartile ranges]. The *P* values are given uncorrected in the text and both uncorrected and corrected for the number of alleles/genotypes analyzed in the tables.

## RESULTS

**Prevalence of autoantibodies.** GADabs were found in 104 of 1,122 patients diagnosed with type 2 diabetes (9.3%), 20 of 558 subjects with IGT (3.6%), and 17 of 383 control subjects (4.4%) ( $P < 0.0001$ ). The frequency of GADab was significantly higher in patients who had been  $\leq 45$  years old at onset of diabetes than in patients  $>45$  years old (19.3 vs. 8.2%,  $P = 0.0001$ ; Fig. 1). After an age of 45 years at onset, the frequency of GADab was rather stable and uninfluenced by the age at onset. The frequency and levels of GADab were similar between men and women in both diabetic and nondiabetic subjects. The frequency or level of GADab was not associated with the duration of diabetes.

The 104 GADab<sup>+</sup> type 2 diabetic patients came from 96 families. Thus, eight families included two GADab<sup>+</sup> patients who were first-degree relatives. Seven GADab<sup>+</sup> patients had a first-degree relative with type 1 diabetes, and three had a second-degree relative with type 1 diabetes.

IA2abs and ICA were detected in 17 and 16%, respectively, of the GADab<sup>+</sup> patients, whereas they were rare (0.4 and 0.5%) in type 2 diabetic patients without GADab. Table 1 shows the antibody frequencies according to the level of



**FIG. 1.** The prevalence of GADab in control subjects, subjects with IGT, and type 2 diabetic patients divided into subgroups according to age at onset.  $P < 0.0001$  for the difference between type 2 diabetic patients and control or IGT subjects.  $P < 0.0001$  for the difference between diabetic patients  $\leq 45$  years old compared with those  $>45$  years old at onset of diabetes.

GADab. The GADab<sup>+</sup> patients were divided into tertiles of GADab positivity, and the patients in the highest tertile ( $>38$  RU) were compared with those belonging to the two lower tertiles ( $>5$ – $38$  RU) or without GADab ( $< 5$  RU). Both IA2ab and ICA were more common in patients with high GADab levels compared with the group with intermediate GADab levels (IA2ab: 33 vs. 10%,  $P = 0.009$ ; ICA 43 vs. 1.4%,  $P < 0.00001$ ). Of the other organ-specific autoantibodies, PCAs were increased in frequency in men with high GADab levels and TMSAs were increased in women with high GADab levels, whereas the overall frequencies of these antibodies were not associated with GADab positivity. The frequency of thyroid disease was not associated with GADab positivity (3 vs. 9 vs. 8%,  $P = 0.59$ ).

**Clinical comparison of GADab<sup>+</sup> and GADab<sup>-</sup> individuals IGT and control subjects.** Table 2 shows the clinical characteristics for the groups according to glucose tolerance. No difference was seen between either GADab<sup>+</sup> and GADab<sup>-</sup> control subjects or GADab<sup>+</sup> and GADab<sup>-</sup> IGT subjects with respect to age, BMI, FBG, incremental insulin area, or lipid concentrations.

**Patients diagnosed with type 2 diabetes.** The GADab<sup>+</sup> patients were between 28 and 83 years at diagnosis of diabetes, with a median age at onset slightly lower than that for the GADab<sup>-</sup> patients (58.5 [19.0] vs. 63.0 [15.0] years;  $P = 0.002$ ) (Table 2). In the GADab<sup>+</sup> group, 4.8% were diagnosed before the age of 35 years and 13.5% before the age of 40 years, compared with 2.6 and 5.4% in the GADab<sup>-</sup> group. The fasting glucose and insulin levels did not significantly differ between the antibody-positive and antibody-negative groups, but the GADab<sup>+</sup> patients had lower FS C-peptide concentrations (0.46 [0.45] vs. 0.62 [0.44] nmol/l,  $P = 0.0002$ ) (Table 2). During the OGTT, no difference was seen in the glucose response, but the insulin response was lower in the GADab<sup>+</sup> compared with the GADab<sup>-</sup> patients ( $P = 0.039$ ). In accordance with the lower C-peptide and insulin concentrations, the GADab<sup>+</sup> patients were assigned insulin therapy more often (30 vs. 12%,  $P < 0.0001$ ) and earlier (median duration of diabetes: 5.0 [10.3] vs. 11.0 [8.8] years,  $P = 0.009$ ) than were the GADab<sup>-</sup> patients.

The GADab<sup>+</sup> patients had lower systolic (140 [29.1] vs. 148 [26.0] mmHg,  $P = 0.009$ ) and diastolic (79.2 [17.6] vs. 81.0 [13.1] mmHg,  $P = 0.009$ ) blood pressure values, as well as lower triglyceride concentrations (1.40 [1.18] vs. 1.75 [1.25] mmol/l,  $P = 0.003$ ) (Table 2). The HDL cholesterol concentrations were higher in GADab<sup>+</sup> than in GADab<sup>-</sup> patients ( $P = 0.019$ ), but the difference was not statistically significant when corrected for BMI (1.26 [0.36] vs. 1.19 [0.38] mmol/l,  $P = 0.055$ ). The total cholesterol concentrations were similar between the two groups. Also, GADab<sup>+</sup> men had a lower waist-to-hip ratio than did GADab<sup>-</sup> men (Table 2).

**Association between GADab levels and clinical characteristics.** There was an inverse correlation between the FS C-peptide concentration and the GADab level ( $R = -0.25$ ,  $P = 0.029$ ). As shown in Fig. 2, the GADab<sup>-</sup> patients differed only slightly from the low GADab<sup>+</sup> group with respect to C-peptide concentration (median 0.62 vs. 0.55 nmol/l,  $P = 0.045$ ), whereas the high GADab<sup>+</sup> patients had significantly lower FS C-peptide levels (0.27 nmol/l,  $P = 0.0001$ ). Only the high GADab<sup>+</sup> group differed from the type 2 diabetic patients with respect to BMI (Fig. 2) and insulin response during the OGTT (data not shown).

TABLE 2  
Clinical characteristics of the subjects

	Control subjects		IGT subjects		Type 2 diabetic subjects		
	GADab <sup>-</sup>	GADab <sup>+</sup>	GADab <sup>-</sup>	GADab <sup>+</sup>	GADab <sup>-</sup>	<i>P</i>	GADab <sup>+</sup>
<i>n</i> (M/W)	366 (168/198)	17 (7/10)	538 (231/307)	20 (5/15)	1,017 (446/571)	—	104 (49/55)
Age at onset of diabetes (years)	NA	NA	NA	NA	63.0 (15.0)	0.002	58.5 (19.0)
Age (years)	50.4 (20.5)	49.7 (11.7)	59.7 (23.3)	63.1 (30.3)	69.9 (13.8)	—	67.6 (17.2)
Duration (years)	NA	NA	NA	NA	5.7 (9.5)	0.029	6.8 (10.6)
FBG (mmol/l)	4.9 (0.7)	4.8 (0.7)	5.3 (0.7)	5.2 (0.6)	7.7 (3.6)	—	8.1 (4.7)
HbA <sub>1c</sub> (%)	5.4 (0.6)	5.5 (0.6)	5.5 (0.7)	5.7 (0.7)	7.1 (2.4)	—	7.8 (3.0)
FS C-peptide (nmol/l)	0.34 (0.25)	0.22 (0.15)	—	—	0.62 (0.44)	0.0002*†‡	0.46 (0.45)
Insulin area (mU/l)	3,997 (2,824)	2,953 (3,513)	5,762 (5,399)	4,282 (4,360)	3,793 (4,468)	0.039*†‡§	2,512 (3,795)
BMI (kg/m <sup>2</sup> )	25.1 (4.5)	26.1 (5.3)	26.8 (5.0)	27.0 (10.2)	27.6 (5.6)	0.007†	26.0 (6.1)
Systolic blood pressure (mmHg)	126.7 (21)	126.0 (14)	139.3 (27)	136.0 (37)	148.0 (26)	0.009*	140.0 (29)
Diastolic blood pressure (mmHg)	78.0 (14)	75.0 (11)	81.0 (13)	80.0 (16)	81.0 (13.1)	0.030*	79.2 (17.6)
Waist-to-hip ratio							
Men	0.93 (0.07)	0.93 (0.03)	0.96 (0.08)	0.97 (0.06)	0.98 (0.08)	0.012*	0.96 (0.10)
Women	0.82 (0.08)	0.81 (0.13)	0.84 (0.08)	0.85 (0.06)	0.87 (0.08)	—	0.87 (0.08)
HDL cholesterol							
Men	1.28 (0.33)	1.26 (0.28)	1.17 (0.36)	1.20 (0.15)	1.07 (0.31)	0.093	1.23 (0.45)
Women	1.56 (0.53)	1.38 (0.41)	1.38 (0.43)	1.47 (0.70)	1.23 (0.45)	0.073	1.30 (0.59)
Triglycerides							
Men	1.19 (0.84)	1.29 (2.85)	1.49 (1.11)	1.83 (1.81)	1.73 (1.22)	0.024*	1.21 (1.27)
Women	0.98 (0.64)	1.14 (0.76)	1.40 (0.8)	1.5 (1.7)	1.76 (1.27)	0.040*	1.51 (1.14)
Cholesterol							
Men	5.79 (1.4)	5.10 (3.65)	5.66 (1.33)	5.70 (2.26)	5.70 (1.56)	—	5.27 (1.26)
Women	5.56 (1.75)	5.24 (1.36)	6.0 (1.5)	6.8 (1.2)	6.14 (1.62)	—	6.13 (1.47)

Data are medians (interquartile ranges). For insulin area,  $n = 544$  for type 2 diabetes, and  $n = 54$  for LADA. Data are corrected for \*BMI, †duration, ‡age, and §FBG.

A different picture was seen with respect to the features of the metabolic syndrome. Both the patients with high and low GADab<sup>+</sup> levels differed from the GADab<sup>-</sup> patients with respect to lower blood pressure (systolic 141.3 [28.3] vs. 139.2 [30.5] vs. 148.0 [25.8] mmHg,  $P = 0.009$ ; diastolic 78.8 [14.6] vs. 79.3 [17.2] vs. 81.0 [13.1] mmHg,  $P = 0.030$ ), serum lipid concentrations (Fig. 2), and waist-to-hip ratio (men: 0.93 [0.14] vs. 0.97 [0.08] vs. 0.98 [0.08],  $P = 0.044$ ).

**Clinical comparison between GADab<sup>+</sup> type 2 diabetes and type 1 diabetes.** Although the type 2 diabetic patients with the highest GADab levels had lower FS C-peptide concentrations than did patients with low GADab levels or no GADab, their FS C-peptide concentration was significantly higher than in type 1 diabetic patients ( $P < 0.0001$ ; Fig. 2). The type 1 diabetic patients and the high GADab<sup>+</sup> group had a comparable mean BMI (24.3 [3.5] vs. 23.5 [4.0] kg/m<sup>2</sup>), which was significantly lower than that in the patients with low GADab<sup>+</sup> or no GADab (27.0 [5.7] and 27.6 [5.6] kg/m<sup>2</sup>, respectively). Irrespective of the strength of GADab positivity, the GADab<sup>+</sup> patients had lower blood pressure and a better lipid profile than did the GADab<sup>-</sup> patients (see above). However, compared with type 1 diabetic patients, the GADab<sup>+</sup> type 2 patients had higher serum triglyceride and total cholesterol concentrations as well as lower serum HDL cholesterol concentrations ( $P < 0.001$ ).

**Assigned treatment at diagnosis of diabetes.** At diagnosis of diabetes, 10 (10%) GADab<sup>+</sup> and 9 (1%) GADab<sup>-</sup> patients were treated with insulin ( $P < 0.0001$ ). Of them, 9/10 GADab<sup>+</sup> and 4/9 GADab<sup>-</sup> patients continued insulin treatment thereafter. Compared with all other GADab<sup>+</sup> patients ( $n = 95$ ),

the GADab<sup>+</sup> patients with onset of permanent insulin treatment within the 1st year ( $n = 9$ ) more often had IA2ab (5/9 [55%] vs. 13/93 [14%],  $P = 0.002$ ), PCA (4/7 [57%] vs. 14/93 [15%],  $P = 0.01$ ), and TMSA (4/7 [57%] vs. 11/89 [12%],  $P = 0.01$ ), whereas the frequencies of ICA (2/7 [29%] vs. 12/92 [13%]) and TGA (1/7 [14%] vs. 5/89 [6%]) did not significantly differ. Also, they had a lower age at onset ( $43.1 \pm 12$  vs.  $58.1 \pm 12$ ,  $P = 0.004$ ), lower fasting C-peptide ( $0.08 \pm 0.11$  vs.  $0.53 \pm 0.39$ ,  $P = 0.0014$ ), higher serum HDL cholesterol ( $1.84 \pm 0.26$  vs.  $1.26 \pm 0.36$ ,  $P = 0.005$ ), and lower serum triglyceride concentrations ( $0.82 \pm 0.11$  vs.  $1.67 \pm 0.86$ ,  $P = 0.009$ ). However, the frequency of type 1 diabetes susceptibility genotypes, either DQB1 or insulin gene, did not differ from the rest of the GADab<sup>+</sup> patients. Thus, the GADab<sup>+</sup> patients who had been assigned permanent insulin treatment at onset of diabetes clinically resembled type 1 diabetes more than the other GADab<sup>+</sup> patients did.

**Genetic comparison between GADab<sup>+</sup> and GADab<sup>-</sup> individuals**

**IDDM1: HLA-DQB1 genotypes.** The proportion of subjects with the type 1 susceptibility allele \*0302 was twice as high in GADab<sup>+</sup> type 2 diabetic patients (41%) as in both GADab<sup>-</sup> patients (21%) and control subjects (24%) ( $P = 0.007$ ). As shown in Table 3, both 0201/0302 and 0302/X genotypes were increased in frequency in GADab<sup>+</sup> patients compared with GADab<sup>-</sup> patients and control subjects (0201/0302: 13 vs. 4 vs. 4%,  $P = 0.002$ ; 0302/X: 22 vs. 12 vs. 12%,  $P = 0.010$ ). No difference was seen in the frequency of genotypes comprising the \*0602(3) allele considered protective from type 1 diabetes (Table 3).

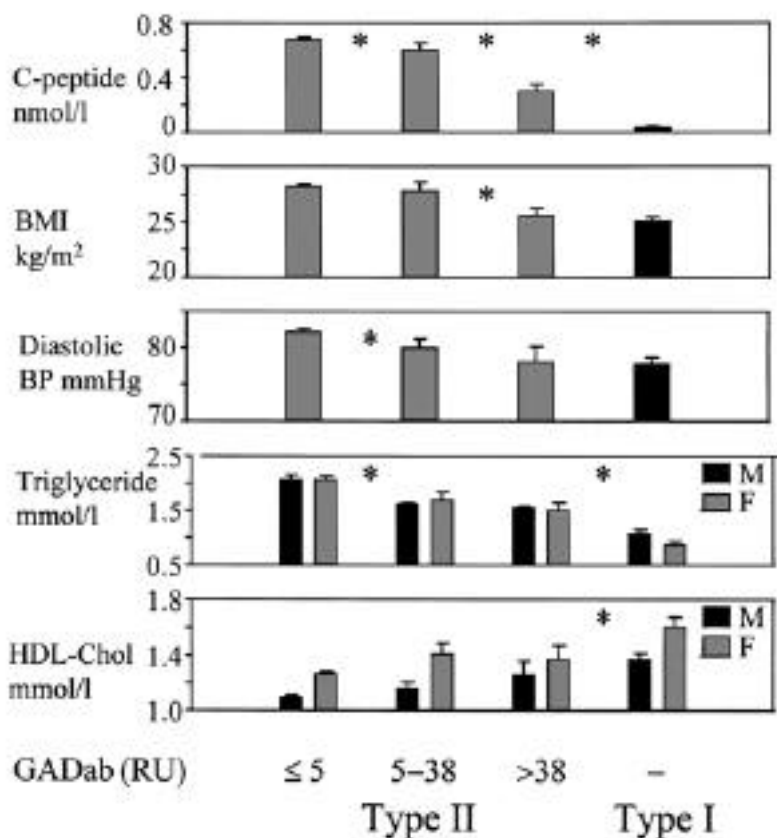


FIG. 2. Comparison of patients with type 2 diabetes ( $n = 1,121$ ) who have no GADab ( $\leq 5$  RU;  $n = 1,017$ ), low GADab levels ( $>5$ – $38$  RU;  $n = 70$ ), or high GADab levels ( $>38$  RU;  $n = 34$ ), and patients with type 1 diabetes ( $n = 194$ ). Data are shown as means  $\pm$  SE; M, males; F, females. Significant differences between groups ( $P < 0.05$ ) are indicated with an asterisk. FS C-peptide:  $P = 0.045$  for GADab<sup>-</sup> vs. low GADab<sup>+</sup>;  $P = 0.0001$  for low GADab<sup>+</sup> vs. high GADab<sup>+</sup> and for high GADab<sup>+</sup> vs. type 1 diabetes. BMI:  $P = 0.030$  for low GADab<sup>+</sup> vs. high GADab<sup>+</sup>. Diastolic blood pressure:  $P = 0.030$  for GADab<sup>-</sup> vs. low and high GADab<sup>+</sup>. Fasting serum triglyceride concentration:  $P = 0.0086$  for GADab<sup>-</sup> vs. low and high GADab<sup>+</sup>;  $P < 0.0001$  for high GADab<sup>+</sup> vs. type 1 diabetes. FS HDL cholesterol:  $P = 0.08$  for GADab<sup>-</sup> vs. low and high GADab<sup>+</sup> (females);  $P < 0.0001$  for high GADab<sup>+</sup> vs. type 1 diabetes (females).

The frequency of genotypes comprising the \*0302 allele was significantly lower in GADab<sup>+</sup> type 2 diabetes than in type 1 diabetes (41 vs. 71%,  $P < 0.0001$ ). This was mainly due to a lower frequency of the 0201/0302 genotype in the GADab<sup>+</sup> type 2 patients (13%) compared with type 1 diabetic patients, either young-onset (34%) or adult-onset (41%) (Table 3). The difference in the frequency of 0302/X between GADab<sup>+</sup> type 2 (22%) and young-onset (30%) or adult-onset (25%) type 1 diabetes was not statistically significant.

Genotypes comprising the protective allele 0602(3) were rare in type 1 diabetic patients, whereas GADab<sup>+</sup> type 2 patients did not differ from control subjects (Table 3). Thus, the proportion of subjects with 0602(3) was significantly lower in type 1 diabetes (17% in Botnia) than in GADab<sup>+</sup> type 2 diabetes (36%,  $P = 0.009$ ). No significant difference was observed between the young- and adult-onset type 1 diabetic patients (18 vs. 13%).

In both type 1 diabetes and GADab<sup>+</sup> type 2 diabetes, the frequency of genotypes comprising the 0201 allele was increased only in combination with the high-risk allele 0302.

**Association between HLA DQB1 alleles and GADab levels.** Figure 2 shows the frequency of type 1 high-risk genotypes 0201/0302 and 0302/X stratified according to tertiles of GADab levels. Among type 2 diabetic patients, the frequency of the high-risk genotypes increased along with higher GADab levels: 15% in GADab<sup>-</sup> patients versus 29% in patients with low GADab levels versus 47% in patients with high GADab levels ( $P = 0.0011$ ; df 2).

**IDDM2: The frequency of restriction fragment length polymorphism genotypes of the Hph1-polymorphism in the insulin gene.** The GADab<sup>+</sup> type 2 diabetic patients did

not differ from the GADab<sup>-</sup> patients or control subjects with respect to the frequency of the +/+ genotype associated with type 1 diabetes (47 vs. 52 vs. 55%). The results were similar when subjects with the DQB1 high-risk genotypes 0201/0302 and 0302/X were excluded (data not shown). As expected, the frequency of the +/+ genotype was high in the type 1 diabetic patients with young (82%) or adult (69%) onset in the Botnia Study ( $P = 0.0002$  vs. all other groups).

## DISCUSSION

The WHO group on the classification and diagnosis of diabetes acknowledged LADA as a separate entity by dividing type 1 diabetes into an autoimmune and idiopathic form. The former was further subdivided into a rapidly (classic type 1 diabetes) and slowly (LADA) progressive form. Unfortunately, there is no consensus regarding diagnostic criteria of LADA. This study was undertaken 1) to establish the prevalence of GADab<sup>+</sup> patients among Finnish type 2 diabetic patients, 2) to clinically and genetically characterize this subgroup, and 3) to use this information to provide a definition for LADA. The subjects participated in the Botnia Study, which is a population-based type 2 diabetes family collection study in western Finland (27). Several subjects were diagnosed with diabetes during the OGTT at the examination. Thus, the present study covers most diabetic patients in the area. According to the revised WHO and American Diabetes Association criteria, an FBG of 6.1 mmol/l was used to define diabetes (28). Five percent of all patients with diabetes (53/1,122) and 4% of the GADab<sup>+</sup> patients (4/104) would have been classified as nondiabetic (50 as IGT and 3 as normal glucose tolerant) using a FBG level of 6.7 mmol/l as diagnostic

TABLE 3  
HLA DQB1 genotype frequencies according to diabetic subgroups in the Botnia study

DQB1 genotype	Control	Type 2 diabetes				Type 1 diabetes
		GADab <sup>-</sup>	$P_1$	GADab <sup>+</sup>	$P_2^*$	
0201/0302	7 (4)	7 (4)	0.002 (0.0012)	12 (13)	0.0004 (0.002)	30 (37)
0201/0602(3)	23 (13)	22 (12)	—	10 (11)	—	1 (1)
0201/X	26 (15)	37 (19)	—	13 (14)	—	11 (13)
0302/0602(3)	13 (8)	11 (6)	—	6 (6)	—	5 (6)
0302/X	21 (12)	22 (12)	0.010 (0.060)	21 (22)	—	23 (28)
0602(3)/X	51 (30)	51 (27)	—	20 (21)	0.036 (0.216)	7 (9)
X/X	31 (18)	41 (22)	—	13 (14)	—	5 (6)
Total <i>n</i>	172	191		95		82

Data are *n* (%).  $P_1$  GADab<sup>+</sup> type 2 diabetic vs. GADab<sup>-</sup> type 2 diabetic and control subjects;  $P_2$  GADab<sup>+</sup> type 2 diabetic vs. type 1 diabetic subjects. *P* values in parentheses are corrected for multiple comparisons.

for diabetes. However, using the WHO 1986 criteria did not change either the prevalence of GADab positivity or the clinical characteristics of GADab<sup>+</sup> patients (data not shown).

Overall, 9.4% of the patients diagnosed with type 2 diabetes were positive for GADab. The prevalence of GADab was higher among patients with age at onset of diabetes between 28 and 45 years (19%), whereas it remained rather stable after 45 years (mean 8.2%, range 6.5–9.7%). These figures agree with the data from the UKPDS, where 34% of patients diagnosed before 35 years, 14% of those between 35–44 years, and 7–9% thereafter were GADab<sup>+</sup> (7). The other pancreatic autoantibodies, ICA and IA2ab, were less frequent, and their presence was associated with GADab positivity and GADab levels. That they were detected in only 0.5% of the GADab<sup>-</sup> patients, a figure comparable to the 2% ICA positivity in GADab<sup>-</sup> patients in the UKPDS, suggests that screening for them in patients with type 2 diabetes would have a marginal effect, at most, on increasing the sensitivity to diagnose LADA.

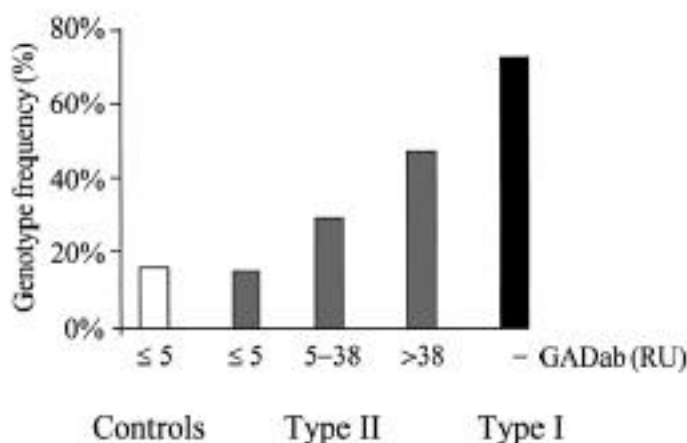


FIG. 3. The proportion of subjects with HLA-DQB1\*0201/0302 or 0302/X. The type 2 diabetic patients are divided into three groups according to their GADab level.

As expected, the GADab<sup>+</sup> patients were more insulin deficient than were the GADab<sup>-</sup> patients, as evidenced by lower fasting C-peptide concentrations and lower insulin concentrations during the OGTT. The lowest values were seen in the patients belonging to the highest tertile of GADab, whereas the patients with lower GADab levels had only marginally lower fasting C-peptide concentrations compared with the GADab<sup>-</sup> patients. Similar to our previous results in Finnish patients (5), but differing from the UKPDS (7), the mean BMI and the age at onset of diabetes of the GADab<sup>+</sup> patients differed only slightly from those in GADab<sup>-</sup> diabetes. Again, the differences were mainly due to the high GADab<sup>+</sup> group. However, it should be kept in mind that even the high GADab<sup>+</sup> group had significantly higher C-peptide levels than did patients diagnosed with type 1 diabetes. Of note, the “low” GADab positivity was hardly due to a technical problem since 29% of GADab<sup>+</sup> type 1 diabetic patients participating in the Combined Autoantibody Workshop (33) had GADab levels comparable to those referred to as “low” in this study.

The metabolic differences between GADab<sup>+</sup> patients, GADab<sup>-</sup> type 2 diabetic patients, and type 1 diabetic patients were reinforced by the finding that the GADab<sup>+</sup> patients also differed genetically from the two classic diabetes types. In the GADab<sup>+</sup> type 2 patients, the frequencies of high-risk HLA-DQB1 genotypes, 0201/0302, and other genotypes with 0302, were significantly lower than in patients with type 1 diabetes but higher than in GADab<sup>-</sup> patients with type 2 diabetes. The frequency of the high-risk genotypes (0201/0302 and 0302/X) in the type 1 diabetic patients from the Botnia study (65%) were similar to those reported from the Finnish DiMe study (68%) (38). Another feature distinguishing both type 2 groups from type 1 diabetes was normal, i.e., not decreased, frequency of the DQB1\*0602(3) allele, which confers protection against type 1 diabetes. Moreover, the GADab<sup>+</sup> type 2 patients did not show the expected increased frequency of IDDM2 (insulin gene Hph1 polymorphism) susceptibility genotype typical for type 1 diabetes. Homozygosity for the short variable number of tandem repeat alleles in the promoter region of the insulin gene—in linkage disequilibrium with the +/+ Hph1-genotype—is associated with an increased risk for IDDM in Caucasoid populations (37,39). In contrast

to the frequency of 71% in both children and adults with type 1 diabetes in the Botnia study, which compares with 76% reported in the DiMe study (40,41), only 53% of GADab<sup>+</sup> type 2 patients had the +/+ Hph1-genotype.

Is the different genetic background of GADab<sup>+</sup> type 2 diabetes simply an age-related phenomenon? This could explain the diversity of type 1 diabetes in the 30% of patients whose disease is diagnosed after the age of 35 years. Among young-onset type 1 diabetes, the prevalence of 0201/0302 and DR3/DR4 seems to be age-related (42,43). Also, compared with younger patients, those older than 20 years at onset of type 1 diabetes have been reported to be less often heterozygous for DQB1\*0201/0302 (20%) (42) and DR3/DR4 (12.5–24%) (44,45). However, in our study, no difference in genotype frequencies was observed between the young-onset and adult-onset type 1 diabetic patients. Of note, the frequency of DQB1\*0201/0302 was lower in GADab<sup>+</sup> type 2 patients (12%) compared with both young-onset (34%) and adult-onset (41%) type 1 patients in the Botnia study, although it did not differ from the frequency of DR3/DR4 in adult-onset patients in another Finnish study (12.5%) (45). Also, the proportion of GADab<sup>+</sup> type 2 patients having genotypes comprising DQB1\*0302 or 0602(3) was clearly different from type 1 diabetes, whereas no difference was observed in the genotype frequencies between the two type 1 diabetic groups. This confirms previous data that age at onset of diabetes does not affect the frequency of either the protective DR2 and DQB1\*0602(3) or the susceptibility-increasing DR4 and DQB1\*0302 (44,45). However, in contrast to the studies mentioned above, a recent small study from Germany reported that only 17% of 24 adults over 40 years old at onset of type 1 diabetes had DQB1\*0302, whereas 21% had DQB1\*0602 (46). It is unclear whether these frequencies could be affected by the low antibody positivity rate in the study patients. All in all, it seems that the prevalence of the DQB1\*0201/0302 (DR3/DR4) genotype may reflect the age at onset of (autoimmune) diabetes, whereas the 0302 genotype appears to be associated with type 1 diabetes and only marginally affected by the age at onset. Of note, there are no age-related differences in the genotype frequencies in the normal population. Whether these age-dependent differences reflect a longer or repeated exposure to environmental influences remains to be shown. The ultimate answer to this question would be to identify and compare LADA patients with a sufficient number of adult-onset patients with rapidly progressing autoimmune diabetes requiring insulin treatment from the beginning. Unfortunately, these patients seem to be rare, even in Scandinavian countries with a high incidence of type 1 diabetes.

The third aim of the study was to provide a definition for LADA. We have shown that with respect to IDDM1 and IDDM2 type 1 diabetes susceptibility loci, GADab<sup>+</sup> patients differed genetically from both type 1 and type 2 diabetes. Further, GADab levels above 5 RU distinguish a subgroup of type 2 diabetic patients characterized by fewer features of the metabolic syndrome. Within this group of GADab<sup>+</sup> patients, those with high GADab levels (>38 RU or the highest tertile) are also characterized by a significant impairment in their  $\beta$ -cell function. GADab<sup>+</sup> type 2 diabetic patients differed from patients with classic type 1 diabetes by having higher C-peptide concentrations and more features of the metabolic syndrome. Only 3% of type 2 diabetic patients were diagnosed before the age of 35 years, whereas 70% of type 1 dia-

betic patients are diagnosed before that age. Given that only 5% of all LADA patients were diagnosed before the age of 35 years and the median age at diagnosis was 58.5 years, it may be reasonable to define LADA as GADab positivity (>5 RU) in patients with an age at onset of diabetes of >35 years and who do not initially (at least 6 months) require insulin.

#### ACKNOWLEDGMENTS

The study was financially supported by the Sigrid Juselius Foundation, the Pahlsson Foundation, the Medical Faculty of the Lund University, the Malmö University Hospital, the Swedish National Board of Health and Welfare, The Swedish Society of Medicine, the Crafoord Foundation, the Novo Nordisk Foundation, the Swedish Medical Research Council, the Swedish Medical Doctors Association, the European Community Grant BMH4-CT95-0662, and the Finnish Medical Society (B.I.).

Britt Bruveris-Svenburg is acknowledged for technical assistance, and the Botnia Research Group is acknowledged for recruiting and clinically studying the subjects.

#### REFERENCES

- Irvine WJ, Gray RS, McCallum CJ, Duncan LJP: Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetics treated with oral hypoglycemic agents. *Lancet* i:1025–1027, 1977
- Groop L, Bottazzo GF, Doniach D: Islet cell antibodies identify latent type 1 diabetes in patients aged 35–75 years at diagnosis. *Diabetes* 35:237–241, 1986
- Landin-Olsson M, Nilsson KO, Lernmark Å, Sundkvist G: Islet cell antibodies and fasting C-peptide predict insulin requirement at diagnosis of diabetes mellitus. *Diabetologia* 33:561–568, 1990
- Gottsäter A, Landin-Olsson M, Fernlund P, Lernmark Å, Sundkvist G: Beta-cell function in relation to islet cell antibodies during the first 3 yr after clinical diagnosis of diabetes in type II diabetic patients. *Diabetes Care* 16:902–910, 1993
- Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes* 42:359–362, 1993
- Hagopian WA, Karlsen AE, Gottsäter A, Landin-Olsson M, Grubin CE, Sundkvist G, Petersen JS, Boel E, Dyrberg T, Lernmark Å: Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD65) shows that 64K autoantibody positivity at onset predicts diabetes type. *J Clin Invest* 91:368–374, 1993
- Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R, for UK Prospective Diabetes Study (UKPDS) Group: UKPDS 25: autoantibodies to islet cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288–1293, 1997
- Groop L, Miettinen A, Groop P, Meri S, Koskimies S, Bottazzo G: Organ-specific autoimmunity and HLA-DR antigens as markers for beta-cell destruction in patients with type II diabetes. *Diabetes* 37:99–103, 1988
- Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ, Knowles W, Cohen M, Lang A: Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabet Med* 11:299–303, 1994
- Niskanen LK, Tuomi T, Karjalainen J, Groop LC, Uusitupa MI: GAD antibodies in NIDDM: ten-year follow-up from the diagnosis. *Diabetes Care* 18:1557–1565, 1995
- Abiru N, Takino H, Yano M, Kawasaki E, Yamasaki H, Yamaguchi Y, Akazawa S, Nagataki S: Clinical evaluation of non-insulin-dependent diabetes mellitus patients with autoantibodies to glutamic acid decarboxylase. *J Autoimmun* 9:683–688, 1996
- Fukui M, Nakano K, Shigeta H, Yoshimori K, Fujii M, Kitagawa Y, Mori H, Kajiyama S, Nakamura N, Abe N, Obayashi H, Fukui I, Ohta K, Ohta M, Kondo M: Antibodies to glutamic acid decarboxylase in Japanese diabetic patients with secondary failure of oral hypoglycaemic therapy. *Diabet Med* 14:148–152, 1997
- Alberti KGMM, Zimmet PZ, Consultation W: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
- Gottsäter A, Afmed M, Lilja B, Fernlund P, Sundkvist G: Islet cell antibodies at diagnosis, but not leanness, relate to a better cardiovascular risk factor profile 5 years after diagnosis of NIDDM. *Diabetes Care* 19:60–63, 1996

15. Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BR, Farrall M, Barnett AH, Bain SC, Todd JA: A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371:130-136, 1994
16. Field LL, Tobias R, Magnus T: A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. *Nat Genet* 8:189-194, 1994
17. Zamani M, Pociot F, Raeymaekers P, Nerup J, Cassiman JJ: Linkage of type 1 diabetes to 15q26 (IDDM3) in the Danish population. *Hum Genet* 98:491-496, 1996
18. Hashimoto L, Habita C, Beressi J, Delepine M, Besse C, Cambon-Thomsen A, Deschamps I, Rotter JI, Djoulah S, James MR, Froguel P, Weissenbach J, Lathrop GM, Julier C: Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. *Nature* 371:161-164, 1994
19. Davies JL, Cucca F, Goy JV, Atta ZAA, Merriman ME, Wilson A, Barnett AH, Bain SC, Todd JA: Saturation multipoint linkage mapping of chromosome 6q in type 1 diabetes. *Hum Mol Gen* 5:1071-1074, 1996
20. Copeman JB, Cucca F, Hearne CM, Cornall RJ, Reed PW, Rønningen KS, Undlien DE, Nisticò L, Buzzetti R, Tosi R, Pociot F, Nerup J, Cornélis F, Barnett AH, Bain SC, Todd JA: Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31-q33. *Nat Genet* 9:80-85, 1995
21. Owerbach D, Gabbay KH: The HOXD8 locus (2q31) is linked to type 1 diabetes: interaction with chromosome 6 and 11 disease susceptibility genes. *Diabetes* 44:132-136, 1995
22. Luo DF, Maclaren NK, Huang HS, Muir A, She JX: Intrafamilial and case-control association analysis of D2S152 in insulin-dependent diabetes. *Autoimmunity* 21:143-147, 1995
23. Nisticò L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Martínez Larrad MT, Serrano Rios M, Chow CC, Cockram CS, Jacobs K, Mijovic C, Boin SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Belgian Diabetes Registry, Tosi R, Pozzilli P, Todd JA: The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum Mol Gen* 5:1075-1080, 1996
24. Groop L, Groop PH, Koskimies S: Relationship between B-cell function and HLA antigens in patients with type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29:757-760, 1986
25. Rich SS, French LR, Sprafka JM, Clements JP, Goetz FC: HLA-associated susceptibility to type 2 (non-insulin-dependent) diabetes mellitus: the Wadena City Health Study. *Diabetologia* 36:234-238, 1993
26. Tuomilehto Wolf E, Tuomilehto J, Hitman GA, Nissinan A, Stengard J, Pekkanen J, Kivinen P, Kaarsalo E, Karvonen MJ: Genetic susceptibility to non-insulin dependent diabetes mellitus and glucose intolerance are located in HLA region. *BMJ* 307:155-159, 1993
27. Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M, Ehrnstrom BO, Forsen B, Isomaa B, Snickars B, Taskinen MR: Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. *Diabetes* 45:1585-1593, 1996
28. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997
29. Lehto M, Tuomi T, Mahtani MM, Widen E, Forsblom C, Sarelin L, Gullström M, Isomaa B, Lehtovirta M, Hyrkkö A, Kanninen T, Orho M, Manley S, Turner RC, Brettn T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen M, Groop L: Characterization of the MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582-591, 1997
30. Isomaa B, Henricsson M, Lehto M, Forsblom C, Karanko S, Sarelin L, Häggblom M, Groop L: Chronic diabetic complications in patients with MODY3 diabetes. *Diabetologia* 41:467-473, 1998
31. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian W, Li L, Karlens AE, Boel E, Michelsen B, Lernmark Å: A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia* 37:344-350, 1994
32. Falorni A, Grubin CE, Takei I, Shimada A, Kasuga A, Maruyama T, Ozawa Y, Kasatani T, Saruta T, Li L, Lernmark Å: Radioimmunoassay detects the frequent occurrence of autoantibodies to the Mr 65,000 isoform of glutamic acid decarboxylase in Japanese insulin-dependent diabetes. *Autoimmunity* 19:113-125, 1994
33. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS, and participating laboratories: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetologia* 11:541-548, 1975
34. Miettinen A, Holthöfer H, Kontiainen S, Miettinen M, Andersson LC: Antibodies against gastrointestinal carcinoid tumors in IDDM. *Diabetes* 38:667-669, 1989
35. Heding LG: Radioimmunological determination of human C-peptide in serum. *Diabetologia* 11:541-548, 1975
36. Kimura A, Sasazuki T: Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In *HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol. 1. Tsuji K, Aizawa M, Sasazuki T, Eds. Oxford, U.K., Oxford University Press, 1993, p. 391-419
37. Bennett ST, Lucassen AM, Gough SC, Powell EE, Undlien DE, Pritchard LE, Merriman ME, Kawaguchi Y, Dronsfield MJ, Pociot F, Nerup J, Bouzekri N, Cambon-Thomsen A, Rønningen KS, Barnett AH, Bain SC, Todd JA: Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet* 9:284-292, 1995
38. Ilonen J, Reijonen H, Herva E, Sjöröos M, Iitiä A, Lövgren T, Veijola R, Knip M, Åkerblom HK: Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population: the Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care* 19:795-800, 1996
39. Bell GI, Horita S, Karam JH: A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes* 33:176-183, 1984
40. Metcalfe KA, Hitman GA, Fennessy MJ, McCarthy MI, Tuomilehto J, Tuomilehto Wolf E: In Finland insulin gene region encoded susceptibility to IDDM exerts maximum effect when there is low HLA-DR associated risk: DiMe (Childhood Diabetes in Finland) Study Group. *Diabetologia* 38:1223-1229, 1995
41. Halminen M, Veijola R, Reijonen H, Ilonen J, Åkerblom HK, Knip M: Effect of polymorphism in the insulin gene region on IDDM susceptibility and insulin secretion: the Childhood Diabetes in Finland (DiMe) Study Group. *Eur J Clin Invest* 26:847-852, 1996
42. Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipeleers DG, Gorus FK: Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1\*0301-DQB1\*0302 haplotype at clinical type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years: the Belgian Diabetes Registry. *Diabetologia* 36:1155-1162, 1993
43. Mustonen A, Ilonen J, Tiilikainen A, Kataja M, Åkerblom HK: An analysis of epidemiological data in HLA-typed diabetic children. *Diabetologia* 28:397-400, 1985
44. Svejgaard A, Jakobsen BK, Platz P, Ryder LP, Nerup J, Christy M, Borch-Johnsen K, Parving H, Deckert T, Mølsted-Pedersen L, Kühl C, Buschard K, Green A: HLA associations in insulin-dependent diabetes: search for heterogeneity in different groups of patients from a homogeneous population. *Tissue Antigens* 28:237-244, 1986
45. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M: A comparison of childhood and adult type 1 diabetes mellitus. *N Engl J Med* 320:881-886, 1989
46. Lohmann T, Seissler J, Verloren H, Schröder A, Rötger J, Dähn K, Morgenthaler N, Scherbaum WA: Distinct genetic and immunological features in patients with onset of IDDM before and after age 40. *Diabetes Care* 20:524-529, 1997

Author Queries (please see Q in margin and underlined text)

Q1: ÅsaLinda correct with no space?

Q2: In Fig. 2, please designate what asterisks refer to and add in legend in appropriate places.

Q3: Please spell out VNTR

Please give the journal volume number for Ref. 1.

Please provide all authors' names for the following references:

6,7,9,11,12,15,18,19,20,23,26,29,31,32,36,43,and 45.

Do you have any updated information for ref. 30?

Please add all authors to ref. 37.

Please add all authors' names to ref. 45.