GAD Antibody Positivity Predicts Type 2 Diabetes in an Adult Population

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OBJECTIVE—To evaluate the significance of GAD antibodies (GADAs) and family history for type 1 diabetes (FH_{T1}) or type 2 diabetes (FH_{T2}) in nondiabetic subjects.

RESEARCH DESIGN AND METHODS—GADAs were analyzed in 4,976 nondiabetic relatives of type 2 diabetic patients or control subjects from Finland. Altogether, 289 (5.9%) were $GADA^+$—a total of 253 $GADA^+$ and 2,511 $GADA^-$ subjects participated in repeated oral glucose tolerance tests during a median time of 8.1 years. The risk of progression to diabetes was assessed using Cox regression analysis.

RESULTS—Subjects within the highest quartile of $GADA^+$ ($GADA^+_{high}$) had more often first-degree FH_{T1} (29.2 vs. 7.9%, $P < 0.00001$) and $GADA^+$ type 2 diabetic (21.3 vs. 13.7%, $P = 0.002$) or nondiabetic (26.4 vs. 13.3%, $P = 0.010$) relatives than $GADA^-$ subjects. During the follow-up, the $GADA^+$ subjects developed diabetes significantly more often than the $GADA^-$ subjects (36/253 [14.2%] vs. 134/2,511 [5.3%], $P < 0.00001$). $GADA^+_{high}$ conferred a 4.9-fold increased risk of diabetes (95% CI 2.8–8.5) compared with $GADA^-$—seroconversion to positive during the follow-up was associated with 6.5-fold (2.8–15.2) and first-degree FH_{T1} with 2.2-fold (1.2–4.1) risk of diabetes. Only three subjects developed type 1 diabetes, and others had a non–insulin-dependent phenotype 1 year after diagnosis. $GADA^+$ and $GADA^-$ subjects did not clinically differ at baseline, but they were leaner and less insulin resistant after the diagnosis of diabetes.

CONCLUSIONS—GADA positivity clusters in families with type 1 diabetes or latent autoimmune diabetes in adults. GADA positivity predicts diabetes independently of family history of diabetes, and this risk was further increased with high GADA concentrations. ***Diabetes*** **59:416–422, 2010**

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Latent autoimmune diabetes in adults (LADA) was introduced nearly 2 decades ago to separate a GAD antibody (GADA)-positive subgroup of adult patients initially diagnosed with type 2 diabetes (1,2). Using this definition with the add-on criteria of no exogenous insulin during the first 6–12 months, the prevalence of LADA among unselected "type 2 diabetic patients" is ~25% in subjects younger than 35 years and between 4 and 13% in subjects older than 35 years at diagnosis in populations of European origin (3–9). In follow-up studies, a progressive defect in insulin secretion was observed in ~50–60% of LADA patients within 6–10 years (3,10), which led to the inclusion of these patients as a slowly progressing form of type 1 diabetes in the last World Health Organization (WHO) classification of diabetes (11). However, both the existence of LADA as a distinct subgroup of diabetes and the criteria that should be used to diagnose it have been challenged (e.g., (12,13). The LADA group is heterogeneous, and most studies have been cross-sectional, whereas prospective studies including patients at or before diagnosis and population-based studies are few (3,4,14–16). Genetic background, especially for type 1 diabetes, may be a confounding factor, and we have shown that LADA was more frequent in families with both type 1 and type 2 diabetes than in families with type 2 diabetes only (17). Moreover, some data support that type 1 and type 2 diabetes cluster in same families (17–20), although this has been contradicted in a large U.K. study on parents of type 1 diabetic patients (21).

In children, progression to diabetes has been associated with high antibody levels and early development of multiple autoantibodies, whereas subjects with a later appearance of antibodies had a slower progression (22–25). We have previously hypothesized that GADAs would be a marker of a subclinical autoimmune process and showed that GADA positivity was associated with a decrease in maximal insulin secretory capacity in nondiabetic subjects (26). If that is the case, GADAs should also be a predictor of future diabetes in adults. This was not supported by two studies on the general population (16,27), but a Swedish study reported a sixfold increased risk for diabetes in $GADA^+$ subjects (15).

In a prospective follow-up study of a large cohort of relatives of type 2 diabetic patients and population control subjects from Finland, we have now evaluated the predictive value of GADAs and family history for type 1 or type 2 diabetes in conjunction with the traditional risk factors for diabetes.

RESEARCH DESIGN AND METHODS

The Botnia Study is a study recruiting type 2 diabetic patients and their family members from Western Finland since 1990, as well as families with type 2

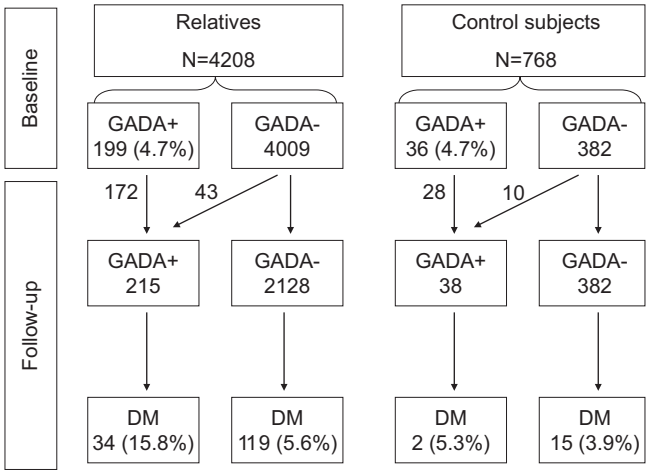

FIG. 1. Flowchart showing the number of relatives and control subjects at baseline and during the follow-up according to GADA positivity and progression to diabetes (DM).

diabetes from all over Finland and type 1 diabetic patients from Western Finland since 1994 (28,29). The study was subsequently extended to other parts of Finland and southern Sweden. The nondiabetic subjects were invited for follow-up examinations approximately every 3 years (29). GADA data were available for 4,976 nondiabetic subjects over 20 years of age at the baseline examination: 4,208 relatives diabetic patients including 92 patients with only family history for type 1 diabetes (FH_{T1}) and 768 control subjects without family history of diabetes (spouses of the diabetic patients). Altogether 289 nondiabetic subjects were GADA positive ($GADA^+$). Follow-up data were available for 253 (87.5%) of the 289 $GADA^+$ and 2,511 (53.6%) of the 4,687 $GADA^-$ subjects during a median (interquartile range [IQR]) follow-up time of 9.3 (5.3) and 8.0 (5.5) years, respectively (Fig. 1). IA2 antibody (IA2ab) measurements were available for 249 of the 253 $GADA^+$ subjects and for 2,049 of the 2,511 $GADA^-$ subjects who participated in the follow-up.

The GADA concentrations of the nondiabetic subjects were compared with those of patients previously diagnosed with type 1 [193 males/200 females, median age 38.3 (15.9) years, age at diagnosis 19.0 (19.0) years] or type 2 diabetes [1,496 males, 636 females, age 63.4 (17.7) years, age at diagnosis 55.0 (18.0) years]. A total of 191/393 (47.8%) of the type 1 diabetic patients and 260/3,231 (8.0%) of the type 2 diabetic patients were positive for GADA at a median duration of diabetes of 16.3 (17.5) and 7.5 (11.2) years, respectively.

Information on family history of type 1 or type 2 diabetes in first- to third-degree relatives was obtained from the pedigrees, which were drawn according to information received from both the subjects and their relatives through questionnaires or clinical investigation. Frequency of GADA positivity in the relatives was also analyzed.

Anthropometric and metabolic measurements. As explained in detail elsewhere (28), we measured the subjects' weight, height, waist and hip circumference, fat-free mass (Futrex, Gaithersburg, MD), and blood pressure (mean of two recordings). BMI was calculated as weight (kg) divided by height (kg/m^2). The subjects participated in a 75-g oral glucose tolerance test (OGTT) after a 12-h overnight fast. Glucose tolerance was classified according to the WHO criteria (11). The diagnosis of type 1 or type 2 diabetes had been made on clinical grounds by the patients' own physician. In addition, as a criterion for type 2 diabetes, we used treatment with diet or oral antidiabetic agents for at least 6 months after the diagnosis and, for type 1 diabetes, treatment with insulin from diagnosis and a serum C-peptide concentration <0.2 nmol/l at the time of baseline investigation. Blood samples were drawn at fasting for the measurement of, e.g., serum total cholesterol, HDL cholesterol, triglyceride, and C-peptide concentrations, and at −10, 0, 30, 60, and 120 min for the measurement of plasma glucose and serum insulin. Insulin resistance was estimated as the homeostasis model assessment index ($HOMA_{IR}$ = fasting serum insulin × fasting plasma glucose/22.5) and β-cell function as the ratio of incremental insulin to glucose responses during the first 30 min of the OGTT (also called the insulinogenic index). The disposition index was used to adjust insulin secretion for the degree of insulin resistance (insulinogenic index/$HOMA_{IR}$).

A structured questionnaire was used to collect data on other diseases, medication, and lifestyle.

Assays. GADAs and IA2abs were determined by a radio-binding assay using ^{35}S-labeled recombinant human GAD65 or IA-2ic produced by coupled in vitro transcription-translation as described earlier (4). The result was expressed as relative units until the year 2000 (for GADAs) and as international units/ml (IU/ml) after the introduction of the WHO International Standard. The GADA results expressed as relative units or IU/ml had a linear correlation up to a concentration of 250 IU/ml. Levels exceeding 5 relative units or 32 IU/ml were considered positive for GADAs and levels exceeding 5 IU/ml were considered positive for IA2abs. In the Combinatorial Autoantibody Workshop 1998, the sensitivity and specificity of the GADA assay were 75 and 99%, respectively. In the Diabetes Autoantibody Standardization Program Workshops, the GADA assay showed a sensitivity of 76–88% and a specificity of 91–96% (years 2000, 2002, 2003, and 2005), and the IA2ab assay showed a sensitivity of 72% and a specificity of 100% (year 2005).

We measured the concentration of plasma glucose with a glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA). Serum insulin was first measured by radioimmunoassay (RIA, Linco; Pharmacia, Uppsala, Sweden) then by an enzyme immunoassay (EIA; DAKO, Cambridgeshire, U.K.) and finally with fluoroimmunometric assay (FIA, AutoDelfia; Perkin Elmer Finland, Turku, Finland). For statistical analysis, the insulin concentrations obtained using the other two assays were transformed to cohere with the insulin concentrations obtained using the enzyme immunoassay. The correlation coefficient between RIA and EIA as well as FIA and EIA was 0.98 ($P < 0.0001$). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured on a Cobas Mira analyzer (Hoffman LaRoche, Basel, Switzerland), and LDL cholesterol concentration was calculated using the Friedewald formula.

Statistical analysis. All statistical analyses were performed using SPSS statistical software version 13 (SPSS, Chicago, IL). Data are expressed as frequencies, mean ± SD, or median (IQR) in the case of non-normally distributed values. The Mann-Whitney test was used to compare group means and the χ^2 test (Pearson) was used to compare group frequencies. The insulin data were logarithmically transformed and a linear mixed-effects model was used to compare group differences adjusted for age, sex, and BMI while accounting for the underlying correlation between subjects from the same family. Cumulative risk for diabetes was analyzed with the Cox proportional hazards model. Variables that were found to be significant in univariate analyses were included in the multivariate model, and GADA positivity was used as a time-dependent variable, since some subjects became positive during the follow-up. Two-sided P values <0.05 were considered statistically significant.

RESULTS

Altogether, 236 (4.7%) nondiabetic subjects were $GADA^+$ at the baseline visit and 53 converted to positive during the follow-up (Fig. 1). A total of 3.9% (11/281) of the $GADA^+$ subjects were also $IA2ab^+$ compared with only one of the $GADA^-$ subjects ($P < 0.0001$). The $GADA^+$ and $GADA^-$ groups had similar age [50.6 (22.0) versus 48.2 (23.3) years] and sex distribution, with women predominating (55 vs. 54%). The majority of the subjects in both groups had normal glucose tolerance (69.6 vs. 71.9%), and about one-third had impaired glucose tolerance or impaired fasting glucose (30.4 vs. 28.1%). A total of 15.7 and 15.4% of the $GADA^+$ and $GADA^-$ subjects, respectively, were control subjects without family history of diabetes.

The GADA concentrations of the nondiabetic subjects were compared with those of diabetic patients. Among all $GADA^+$ subjects in the Botnia Study, the type 1 diabetic patients had the highest median antibody concentrations [239 (1,865) IU/ml], even after a median duration of diabetes of 17.4 (17.5) years followed by $GADA^+$ type 2 diabetic patients [i.e., LADA, 74 (365) IU/ml; median duration 7.5 (11.2) years] and $GADA^+$ nondiabetic subjects [46 (48) IU/ml] ($P < 0.00001$, 2 df). However, the GADA concentrations of these three groups overlapped, and 13.9% of the $GADA^+$ nondiabetic subjects had concentrations exceeding the median of the type 1 diabetic subjects. In further analyses, the $GADA^+$ nondiabetic subjects were stratified into quartiles, and individuals having GADA within the highest quartile ($GADA^+_{high}$; >89 IU/ml) were compared with individuals having lower concentrations ($GADA^+_{low/med}$) or no antibodies ($GADA^-$).

GADA positivity and type 1 diabetes clustered in fami-

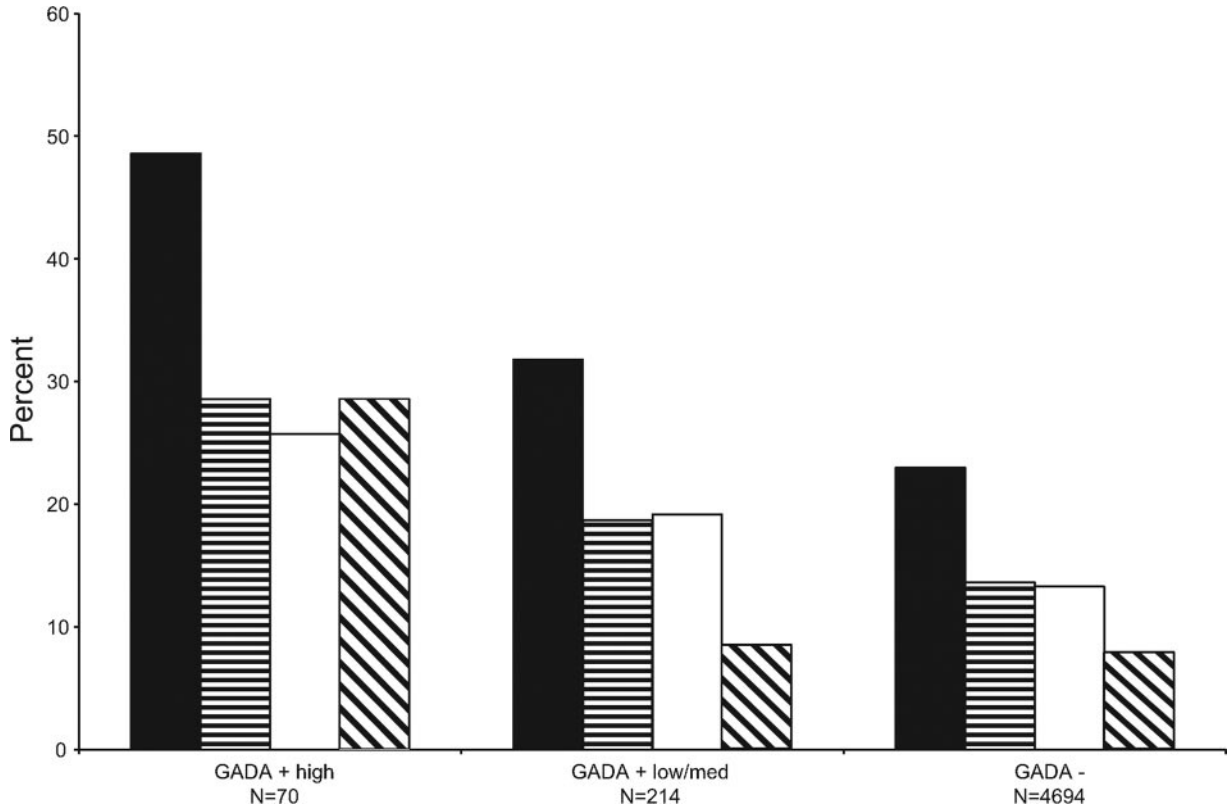

FIG. 2. The proportion of nondiabetic subjects having any GADA$^+$ relatives (■), GADA$^+$ nondiabetic relatives (▤), GADA$^+$ type 2 diabetic relatives (LADA, □), or type 1 diabetic relatives (▧) according to strength of GADA positivity. $P < 0.0001$, GADA^{+high} versus GADA$^-$ regarding subjects having any GADA$^+$ relatives or type 1 diabetic relatives; $P = 0.002$, GADA^{+high} versus GADA$^-$ regarding subjects having GADA$^+$ type 2 diabetic relatives; $P = 0.01$, GADA^{+high} versus GADA$^-$ regarding subjects having nondiabetic GADA$^+$ relatives.

lies (Fig. 2). Compared with the GADA$^-$ subjects, particularly the GADA$^+_{high}$ subjects had first-degree relatives with type 1 diabetes significantly more often (29.2 vs. 7.9%, $P < 0.00001$) as well as GADA$^+$ relatives (50 vs. 23%, $P < 0.00001$): both nondiabetic GADA$^+$ relatives (26.4 vs. 13.3%, $P = 0.010$) and GADA$^+$ relatives diagnosed with type 2 diabetes (LADA; 21.3 vs. 13.7%, $P = 0.002$) (Fig. 2). The subjects with low or medium GADA concentrations did not differ from the GADA$^-$ subjects with respect to family history for type 1 diabetes, but the GADA$^+_{low/med}$ group had GADA$^+$ relatives more often (33 vs. 23%, $P = 0.002$).

Overall, the clinical characteristics of the GADA$^+$ and GADA$^-$ subjects did not differ much at baseline, but the GADA$^-$ subjects were a bit younger (Table 1). However, GADA$^+_{high}$ subjects were younger and had a blunted insulin response during OGTT (i.e., lower insulinogenic and disposition indexes) than subjects with lower GADA concentrations or no GADAs (Table 1).

Development of diabetes. The GADA$^+$ subjects developed diabetes significantly more often than the GADA$^-$ subjects (36/253 [14.2%] vs. 134/2,511 [5.3%], $P < 0.00001$), and higher GADA concentrations were associated with a higher risk (Fig. 3). Surprisingly, type 1 diabetes was diagnosed in only three male subjects aged 31–44 years. Two had been highly GADA$^+$ 4.0 and 5.2 years earlier and the third was ICA$^+$ and IA2ab$^+$ at diagnosis. Altogether, 5 of the 11 (45.5%) IA2ab$^+$ subjects developed diabetes. Except for these three type 1 diabetic patients, all the other 167 patients were diagnosed with type 2 diabetes, and they were not treated with insulin during the first year.

At the baseline visit, there was no difference between the GADA$^+$ and GADA$^-$ subjects who were later to develop diabetes (data not shown), except that the GADA$^+$ pre-diabetic subjects had a reduced waist circumference (92.2 vs. 97.6 cm, $P = 0.019$) and lower BMI (27.4 vs. 28.9 kg/m^2, $P = 0.059$). We have previously shown that both fasting plasma glucose and BMI were strong predictors of diabetes (29), and this applied also to the GADA$^+$ group (data not shown).

As shown in Table 2, after the diagnosis of diabetes, GADA$^+$ patients were leaner than GADA$^-$ patients [BMI 27.75 ± 2.7 vs. 30.06 ± 5.3 kg/m^2; $P = 0.023$], but the groups had had similar weight gain. GADA$^+$ patients also had less evidence of insulin resistance, as reflected by a lower fasting insulin concentration [7.24 (7.9) vs. 13.13 (11.4) IU/ml, $P = 0.008$] and lower $HOMA_{IR}$ index [2.09 (2.6) vs. 4.04 (3.9), $P = 0.008$], despite similar fasting plasma glucose (Table 2). GADA$^+_{high}$ subjects developed diabetes at a significantly younger age than GADA$^+_{low/med}$ or GADA$^-$ subjects [45.8 (13) vs. 63.1 (13.5) vs. 62 (19.4) years, $P = 0.00014$]. At diagnosis, the GADA$^-$ diabetic patients were comparable to other type 2 diabetic patients included in the Botnia study, except for higher C-peptide and lower fasting plasma glucose concentrations.

Subjects with family history for type 1 or type 2 diabetes developed diabetes more often (143/2062, 6.9%, vs. 17/420, 4.0%; $P = 0.028$) and at a younger age [59.7 (12.7) versus 71.8 (7.8) years, $P = 0.0001$] than subjects without any family history. There was no significant difference between individuals with FH_{T1} (7.9%) and FH_{T2} (6.2%). However, the majority of subjects with FH_{T1} also had type 2 diabetic relatives, so we could not analyze the effect of pure type 1 diabetes family history. At diagnosis of diabe-

TABLE 1
Clinical characteristics of the nondiabetic subjects at baseline according to the strength of GADA positivity

	GADA$^-$	*P*	GADA$^+_{low/med}$	*P*	GADA$^+_{high}$	*P* (high vs. negative)
n (male/female)	4,687 (2,107/2,580)		216 (101/115)		73 (33/40)	
NGT/IGT (%)	72/28		79/30		67/33	
Age at baseline (years)	48.2 (23.2)	0.036	53.8 (21.4)	0.014	43.9 (17.5)	
FPG (mmol/l)	5.5 ± 0.6		5.4 ± 0.7		5.4 ± 0.7	
Plasma glucose 30 min (mU/l)	8.5 ± 1.6		8.6 ± 1.7		8.6 ± 1.8	
Plasma glucose 120 min (mU/l)	6.3 ± 1.5	0.002	6.1 ± 1.6	0.04	6.4 ± 1.8	
Fasting serum insulin (mU/l)	4.7 (4)		4.7 (3.5)		4.7 (4.5)	
Serum insulin 30 min (mU/l)	35.9 (37.8)		41.7 (35.5)	0.053	31.9 (31.9)	0.04
Serum insulin 120 min (mU/l)	26.7 (33.2)	0.029	26.0 (31.0)		23.7 (16.7)	
Fasting serum C-peptide (nmol/l)	0.5 ± 0.3		0.5 ± 0.3		0.5 ± 0.3)	
Insulinogenic index	12.4 (14.6)		13.5 (16)	0.032	11 (7.3)	0.013
HOMA	1.1 (1.0)		1.1 (0.9)		1.1 (1.2)	
Disposition index	10.2 (12.7)		10.3 (13.3)	0.042	7.8 (11.1)	0.019
BMI (kg/m^2)	26.1 ± 4.1		26.3 ± 3.8		26.3 ± 4.3	
Systolic blood pressure (mmHg)	130.2 ± 18.6		134.5 ± 20.2		127.4 ± 19.8	
Diastolic blood pressure (mmHg)	79 ± 10.4		80.4 ± 10.7		78.6 ± 10.4	
A1C (%)	5.3 ± 0.5		5.4 ± 0.5		5.3 ± 0.7	
LDL cholesterol (mmol/l)	3.6 ± 1.0		3.6 ± 1.1		3.4 ± 1.0	
HDL cholesterol (mmol/l)	1.4 ± 0.4		1.4 ± 0.3		1.4 ± 0.4	
Triglycerides (mmol/l)	1.4 ± 0.9		1.3 ± 0.7		1.2 ± 0.6	

Data are means ± SD or median (IQR). GADA$^-$, no GADAs; GADA$^+_{low/med}$, GADAs within the three lower quartiles of positivity; GADA$^+_{high}$, GADAs within the highest quartile. In the statistical analyses, a linear mixed-effects model was used to compare group differences adjusted for age, sex, and BMI while accounting for the underlying correlation between subjects from the same family when appropriate.

tes, patients with first-degree FH$_{T1}$ were markedly younger [43.2 (12.7) years] than individuals without any family history for diabetes [71.8 (13.2) years] ($P < 0.005$, FH$^-$ versus all other groups).

Thus, both GADA concentration and family history for diabetes affected the risk of diabetes. However, high GADAs was also associated with FH$_{T1}$. Among control subjects without family history for diabetes, low or medium levels of GADA had no effect on diabetes incidence (GADA$^+_{low/med}$ vs. GADA$^-$, 2.9 vs. 3.9%), but one of four control subjects with high GADAs developed diabetes (25%; $P = 0.035$ vs. GADA$^-$). However, among subjects with family history for diabetes, the incidence of diabetes was doubled between subjects with no GADA and GADA$^+_{low/med}$, and it further doubled between GADA$^+_{low/med}$ and GADA$^+_{high}$ (5.6 vs. 13.0 vs. 23.3%, $P < 0.0001$). Contrary to our hypothesis, in this respect, there was no difference between individuals with family history for type 1 diabetes and individuals with family history for type 2 diabetes. Thus, high GADA concentrations implied a clearly increased risk of diabetes in both relatives and control subjects, while low or medium-high levels implied an increased risk only in relatives of diabetic patients.

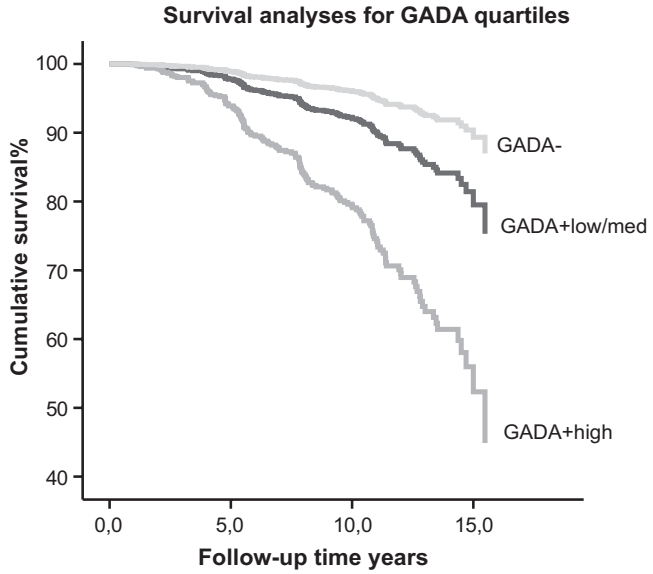

FIG. 3. Development of diabetes according to strength of GADA positivity: GADAs in the highest quartile (GADA^{+high}) or three lower quartiles of positivity (GADA$^{+low/med}$) or no GADA (GADA$^-$). The data are adjusted for age, sex, and BMI. The *y*-axis shows the cumulative proportion of subjects without diabetes; the *x*-axis shows the follow-up time in years ($P < 0.00001$, Cox proportional hazards model).

Having shown that age, sex, BMI, GADAs, and family history of type 1 or type 2 diabetes affected the risk of diabetes, we tested the relative effects of those variables on the risk of future diabetes using Cox time-dependent regression analyses and included GADAs as a time-dependent variable. The traditional risk factors age, sex, BMI, and fasting glucose at baseline were independent determinants of risk (hazard ratio 1.03–2.42), and first-degree family history for type 1 diabetes conferred a 2.2-fold risk (95% CI 1.23–4.09, $P = 0.009$). GADA$^+_{high}$ implied a 4.9-fold risk (2.80–8.51, $P < 0.0001$), but unexpectedly the highest risk (6.5-fold, 2.8–15.17, $P < 0.00001$) was associated with seroconversion to GADA$^+$ during the follow-up. Figure 2 shows the proportion of subjects surviving without diabetes during the follow-up according to GADA positivity, including age, sex, and BMI in the model.

DISCUSSION

In this large population-based family study from Finland, we have shown that in addition to the traditional risk factors for type 2 diabetes, GADA positivity significantly increased the risk of diabetes. The incidence of diabetes

TABLE 2
Clinical characteristics of the GADA$^-$ and GADA$^+$ subjects at follow-up according to progression to diabetes (DM$^+$)

	GADA$^-$			GADA$^+$			
	DM$^-$	*P*	DM$^+$	DM$^-$	*P*	DM$^+$	*P**
n	2,377		134	216		36	
Age (years)	54.6 (20.6)	<0.0001	61.9 (19.4)	56.9 (20.9)		60.1 (19)	
Follow-up time (years)†	8.0 (5.6)		7.7 (5.4)	9.6 (5.4)	0.001	6.3 (4.9)	0.039
A1C (%)	5.5 ± 0.5	<0.0001	6.3 ± 0.6	5.7 ± 0.4	<0.0001	6.7 ± 1.1	0.004
FPG (mmol/l)	5.3 ± 0.6	<0.0001	6.9 ± 1.0	5.3 ± 0.6	<0.0001	6.9 ± 0.9	
Plasma glucose 30 min (mmol/l)	8.5 ± 1.8	<0.0001	11.5 ± 2.2	8.4 ± 1.7	<0.0001	11.5 ± 1.4	
Plasma glucose 120 min (mmol/l)	5.9 ± 1.7	<0.0001	11.3 ± 2.9	5.8 ± 1.7	<0.0001	10.5 ± 2.8	
Fasting insulin (mU/l)	7.3 (6.5)	<0.0001	13.1 (11.4)	7.0 (7.1)		7.2 (7.9)	0.008
Serum insulin 30 min (mU/l)	53.5 (43.5)	0.0003	50.5 (46.6)	53.4 (38.0)	0.006	36.8 (37.1)	
Serum insulin 120 min (mU/l)	30.9 (37.2)	<0.0001	74.6 (91.9)	29.3 (33.8)	0.009	61.2 (74.7)	
Fasting serum C-peptide (nmol/l)	0.5 ± 0.3	<0.0001	0.9 ± 0.5	0.5 ± 0.3	0.073	0.7 ± 0.4	
Insulinogenic index	14.6 (16.2)	<0.0001	8.1 (10.9)	15.6 (13.2)	<0.0001	6.4 (7.3)	
HOMA	1.7 (1.6)	<0.0001	4.0 (3.9)	1.7 (1.7)	0.036	2.1 (2.6)	0.005
Disposition index	8.8 (11.2)	<0.0001	2.3 (1.9)	8.8 (10.2)	<0.0001	2.9 (2.5)	
Waist (cm)	91.8 ± 12.5	<0.0001	102.9 ± 12.6	91.1 ± 11.5	0.026	98.0 ± 9.6	
BMI (kg/m)	26.7 ± 4.2	<0.0001	30.1 ± 5.3	26.6 ± 4.3		27.8 ± 2.7	0.023
Fat %	28.3 ± 7.3	<0.0001	30.2 ± 6.4	28.8 ± 9.3		30.0 ± 6.2	
Systolic blood pressure (mmHg)	133.5 ± 19.6	<0.0001	145.3 ± 22	135.0 ± 19.2	0.055	146.6 ± 27.4	
Diastolic blood pressure (mmHg)	81.4 ± 9.9	<0.0001	85.7 ± 10.2	82.0 ± 9.3		83.6 ± 9.7	
HDL cholesterol (mmol/l)	1.3 ± 0.4		1.1 ± 0.3	1.4 ± 0.4		1.3 ± 0.4	
Triglycerides (mmol/l)	1.4 ± 0.8		1.8 ± 1.0	1.4 ± 0.8		1.8 ± 0.9	
LDL cholesterol (mmol/l)	3.4 ± 1.0		3.5 ± 1.1	3.2 ± 0.8		3.4 ± 1.0	

Data are means ± SD or median (IQR). In the statistical analyses, a linear mixed-effects model was used to compare group differences adjusted for age, sex, and BMI while accounting for the underlying correlation between subjects from the same family. BMI was adjusted for age and sex. OGTT data were available for 60% of the GADA$^+$ subjects and 87% of the GADA$^-$ subjects who developed diabetes. *Difference between GADA$^+$ DM$^+$ and GADA$^-$ DM$^+$. †Time until diagnosis of diabetes or until last visit.

was highest in individuals with GADAs in the highest quartile of positivity (23.3%; HR 4.9, 95% CI 2.80–8.51), intermediate in individuals having GADAs within the three lower quartiles (13.0%), and lowest in GADA$^-$ subjects (5.6%). The younger age of the GADA$^+_{high}$ group and the shorter follow-up of the GADA$^+$ subjects reflects their increased rate of progression to diabetes, since the follow-up was terminated at diagnosis of diabetes. Although the increased risk was clearly associated with strength of GADA reactivity, we could not distinguish a GADA cutoff, under which diabetes would be less likely, and we observed no bimodality in the GADA distribution, as has been suggested (9). Also, seroconversion from GADA$^-$ to GADA$^+$ during the follow-up conferred an increased risk of diabetes, but IA2abs were too rare in this population to have a high impact. GADA concentrations have been associated with lower C-peptide concentration in cross-sectional studies of LADA (4,30,31) as well as development of complete β-cell failure in adults with newly diagnosed diabetes of any type (32), although data from the U.K. Prospective Diabetes Study did not support an association between GADA level and need of insulin (33). Phenotypically, only three of our patients who developed diabetes had type 1 diabetes, whereas all other GADA$^+$ patients had type 2 diabetes. We could not see a decreased insulin response to glucose, but the GADA$^+$ diabetic patients were less insulin resistant than the GADA$^-$ patients, which indirectly supports the hypothesis that GADAs might be associated with a defect in insulin secretion. We have previously shown in nondiabetic subjects with thyroiditis that GADA$^+$ subjects had a decreased maximal insulin secretory capacity, as estimated with an intravenous glucose-arginine test compared with GADA$^-$ subjects (26). Apparently, the insulin secretory defect associated with GADAs is mild and can only be seen with a test that stresses the β-cells maximally.

GADA positivity and type 1 diabetes clustered in families. In concert with the high prevalence of type 1 diabetes in Finland (34), FH_{T1} was found in ~8% of GADA$^-$ and GADA$^+_{low/med}$ subjects, whereas almost one-third (29%) of the GADA$^+_{high}$ group had type 1 diabetic relatives. Moreover, 50% in the GADA$^+_{high}$ and 30% of the GADA$^+_{low/med}$ groups had GADA$^+$ relatives. It would be important to study how much the known type 1 diabetes susceptibility genes, such as HLA and PTPN22, explain of this clustering. With this background, the high frequency of GADAs (4.7%) among the nondiabetic relatives and even control subjects without any family history for diabetes was not that surprising. In a 6-year follow-up study of this population, family history for type 2 diabetes together with BMI >30 kg/m^2 and fasting plasma glucose >5.5 mmol/l conferred a 3.7-fold risk of diabetes (29). We now hypothesized that FH_{T1} would increase the risk of type 2 diabetes (or LADA) through an effect on insulin secretory capacity. We were reassured to find that in conjunction with the other risk factors, FH_{T1} doubled the risk of diabetes (HR 2.2, CI 1.23–4.01). However, even when FH_{T1} was in the model, high GADAs implied an even stronger risk (HR 4.9, CI 2.8–8.5). Further, there seemed to be a difference in incidence rates between the population control subjects without any family history for diabetes, in whom high GADAs only affected diabetes risk, and subjects with family history for type 1 or type 2 diabetes, whose diabetes risk was doubled also with low or medium-high GADAs. Mild autoimmunity might not be sufficient to cause diabetes in the absence of other factors decreasing insulin secretion or increasing insulin resistance. One such factor could be having inherited the risk allele of the gene with

strongest association with type 2 diabetes, TCF7L2, which has been shown to decrease insulin secretion and which was as common in LADA as in type 2 diabetes (35,36). Although the low number of $GADA^+$ population control subjects precludes any firm conclusions on the difference in risk, it could explain the difference between our study and the two previous studies looking at the predictive value of GADAs for diabetes in the general population, where no increased risk was found during a comparable 8-year follow-up (16,27). Another difference between the studies was the number of $GADA^+$ subjects, which was only 18 in the Italian Cremona Health Study (16) and 23 in the Swedish Västerbotten County Health Project. However, in another part of the Västerbotten Study, 7 of 25 (28%) initially $GADA^+$ subjects were reported to have developed diabetes after a mean time of 9.2 ± 2.9 years compared with 86 of 2,209 (3.9%) of $GADA^-$ subjects ($P < 0.0001$). Only one of the seven was diagnosed with type 1 diabetes (15).

In conclusion, GADA positivity clustered in families with type 1 diabetes or LADA. GADA positivity predicted diabetes independently of family history of diabetes, and this risk increased with high GADA concentrations.

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