

GADA positivity predicts non-insulin dependent diabetes in an adult population

Running title: GADA positivity predicts diabetes in adults

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Submitted 18 May 2009 and accepted 14 October 2009.

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Objective To evaluate the significance of GAD antibodies (GADA) and family history for type 1 or type 2 diabetes (FH_{T1}) in non-diabetic subjects.

Research design and methods GADA were analysed in 4976 non-diabetic relatives of type 2 diabetic patients or control subjects from Finland. Altogether 289 (5.9%) were GADA+. 253 GADA+ and 2511 GADA- 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 Those within highest quartile of GADA+ (GADA_{high};) had more often 1st 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 non-diabetic (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. 134/2511 (5.3%), P<0.00001]. GADA_{high} conferred a 4.9-fold increased risk of diabetes (95% CI 2.8 – 8.5) as compared with GADA-. Seroconversion to positive during the follow-up was associated with 6.5-fold (2.8 – 15.2) and 1st degree FH_{T1} with 2.2-fold (1.2-4.1) risk of diabetes. Only three subjects developed type 1 diabetes, others had a non-insulin dependent phenotype one 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 LADA. GADA positivity predicts diabetes independently of family history of diabetes and this risk was further increased with high GADA concentrations.

LADA (Latent Autoimmune Diabetes in Adults) was introduced nearly two decades ago to separate a GAD antibody (GADA) positive subgroup of adult patients initially diagnosed with non-insulin dependent diabetes or 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 about 25% in subjects younger than 35 years, and between 4-13% in those older than 35 years at diagnosis in populations of European origin (3-9). In follow-up studies, a progressive defect in insulin secretion has been observed in about 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 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, 14-17). 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 (18). Moreover, some data support that type 1 and type 2 diabetes cluster in same families (18-21), although this has been contradicted in a large UK study on parents of type 1 diabetic patients (22).

In children, progression to diabetes has been associated with high antibody levels and early development of multiple autoantibodies, while those with later appearance of antibodies had a slower progression (23-26). We have previously hypothesized that GADA 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 non-diabetic subjects (27). If that would be the case, GADA should also be a predictor of future diabetes in adults. This was not supported by two studies on general population (17, 28) but a Swedish study reported a 6-fold increased risk for diabetes in GADA+ subjects (16).

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

SUBJECTS 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 diabetes from all over Finland and type 1 diabetic patients from Western Finland since 1994 (29, 30). The study was subsequently extended to other parts of Finland and southern Sweden. The non-diabetic subjects were invited for follow-up examinations approximately every three years (30). GADA data were available for 4976 non-diabetic subjects over 20 years of age at the baseline examination: 4208 relatives diabetic patients including 92 patients with only FH_{T1} and 768 control subjects without family history of diabetes (spouses of the diabetic patients). Altogether 289 non-diabetic subjects were GADA positive (GADA+). Follow-up data were available for 253 (87.5%) of the 289 GADA+ and 2511 (53.6%) of the 4687 GADA- subjects during a median [Interquartile range, IQR] follow-up time of 9.3 [5.3] and 8.0 [5.5] years, respectively (Figure 1). IA2ab measurements were available for 249 of the 253 GADA+ subjects

and for 2049 of the 2511 GADA- subjects, who participated in the follow-up.

The GADA concentrations of the non-diabetic subjects were compared with those of patients previously diagnosed with type 1 (193 males (M)/200 females (F), median age 38.3 [15.9] years, age at diagnosis 19.0 [19.0] years) or type 2 diabetes (1496 M/ 1636 F, age 63.4 [17.7] years, age at diagnosis 55.0 [18.0] years). 191/393 (47.8%) of the type 1 diabetic patients and 260/3231 (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] yrs respectively.

Information on family history of type 1 or type 2 diabetes in 1st- 3rd 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 analysed.

Anthropometric and metabolic measurements. As explained in detail elsewhere (29) we measured the subjects' weight, height, waist and hip circumference, fat-free mass (Futrex, Gaithersburg, MD) and blood pressure (mean of two recordings). Body mass index (BMI) was calculated as weight (kg) divided by height (kg/m²). The subjects participated in a 75g oral glucose tolerance test (OGTT) after a 12-hr 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 anti-diabetic 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 lower than 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 minutes for the measurement of plasma glucose (PG) and serum insulin. Insulin resistance was estimated as the Homeostasis Model Assessment index ($HOMA_{IR} = \text{fasting serum insulin} * \text{fasting PG} / 22.5$) 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 (DI) was used to adjust insulin secretion for the degree of insulin resistance ($\text{insulinogenic index}/HOMA_{IR}$).

A structured questionnaire was used to collect data on other diseases, medication and life-style.

Assays. GADA and IA2ab were determined by a radiobinding assay using ³⁵S-labelled recombinant human GAD65 or IA-2ic produced by coupled *in vitro* transcription-translation as described earlier (14). The result was expressed as relative units (RU) until the year 2000 (for GADA) and as international units/ml (IU/ml) after the introduction of the WHO International Standard. The GADA results expressed as RU or IU/ml had a linear correlation up to a concentration of 250 IU/ml. Levels exceeding 5 RU or 32 IU/ml were considered positive for GADA and levels exceeding 5 IU/ml for IA2ab. 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 a sensitivity of 72% and a specificity of 100% (year 2005).

We measured the concentration of PG 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, UK) 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 analyser (Hoffman LaRoche, Basel, Switzerland) and LDL cholesterol concentration was calculated using the Friedewald formula.

Statistical analyses. All statistical analyses were performed using SPSS statistical software version 13 (SPSS, Chicago, IL). Data are expressed as frequencies, mean (standard deviation; SD) or median [interquartile range; IQR] in case of non-normally distributed values. Mann-Whitney test were used to compare group means and Chi Squared test (Pearson) 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 analysed 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 as some subjects became positive during the follow-up. Two-sided p -values lower than 0.05 were considered statistically significant.

RESULTS

Altogether 236 (4.7%) non-diabetic subjects were GADA positive at the baseline visit and 53 converted to positive during the follow-up

(Figure 1). 3.9% (11/281) of the GADA+ subjects were also IA2ab positive compared with only one of the GADA- subjects ($p < 0.0001$). The GADA+ and GADA- groups had similar age [50.6 (22.0) vs. 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%). 15.7% and 15.4% of the GADA+ and GADA- subjects, respectively, were controls without family history of diabetes.

The GADA concentrations of the non-diabetic subjects were compared with those of diabetic patients. Among all GADA positive subjects in the Botnia Study, the type 1 diabetic patients had the highest median antibody concentrations (239 [1865] 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] yrs) and GADA+ non-diabetic subjects (46 [48] IU/ml) ($P < 0.00001$, $df=2$). However, the GADA concentrations of these three groups overlapped and 13.9% of the GADA+ non-diabetic subjects had concentrations exceeding the median of the type 1 diabetic subjects. In further analyses, the GADA+ non-diabetic subjects were stratified into quartiles, and those having GADA within the highest quartile (GADA_{high}; >89 IU/ml) were compared with those having lower concentrations (GADA_{low/med}) or no antibodies (GADA-).

GADA-positivity and type 1 diabetes clustered in families (Figure 2). Compared with the GADA- subjects, particularly the GADA_{high} subjects had significantly more often 1st degree relatives with type 1 diabetes (29.2% vs. 7.9%, $P < 0.00001$) as well as GADA+ relatives (50% vs. 23%, $P < 0.00001$): both non-diabetic GADA+ relatives (26.4% vs. 13.3%, $P = 0.010$) and GADA+ relatives

diagnosed with type 2 diabetes (LADA; 21.3% vs. 13.7%, $P=0.002$) (Figure 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 more often GADA+ relatives (33% vs. 23%, $P=0.002$).

Overall, the clinical characteristics of the GADA positive and negative subjects did not much differ 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 indices, than subjects with lower GADA concentrations or no GADA (Table 1).

Development of diabetes The GADA+ subjects developed diabetes significantly more often than the GADA- subjects [36/253 (14.2 %) vs. 134/2511 (5.3%), $P<0.00001$] and higher GADA concentrations were associated with a higher risk (Figure 3). Surprisingly, type 1 diabetes was diagnosed in only three male subjects aged 31- 44 years. Two had been highly GADA positive 4.0 and 5.2 years earlier and the third was ICA and IA2ab positive at diagnosis. Altogether five 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+ prediabetic subjects had a reduced waist circumference (92.2 vs. 97.6 cm, $P=0.019$) and lower BMI (27.4 vs. 28.9 kg/m², $P=0.059$). We have previously shown that both FPG and BMI were strong predictors of diabetes (30), this applied also to the GADA positive 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/m²; $P=0.023$], but the groups had had similar weight gain. GADA+ patients had also 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 FPG (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 FPG 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) vs. 71.8 (7.8) years, $p=0.0001$] than those without any family history. There was no significant difference between those with FH_{T1} (7.9%) and FH_{T2} (6.2%). However, the majority of subjects with FH_{T1} had also type 2 diabetic relatives, so we could not analyse the effect of pure type 1 family history. At diagnosis of diabetes, patients with 1st degree FH_{T1} were markedly younger (43.2 [12.7] yrs) than those without any family history for diabetes (71.8 [13.2] yrs), ($P<0.005$, FH- vs. all other groups).

Thus, both GADA concentration and family history for diabetes affected the risk of diabetes. However, high GADA 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 1 of 4 control subjects with high GADA 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 those with family history for type 1 diabetes and those with family history for type 2 diabetes. Thus, high GADA concentrations implied a clearly increased risk of diabetes in both relatives and controls, while low or medium high levels implied an increased risk only in relatives of diabetic patients.

Having shown that age, sex, BMI, GADA 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 GADA as a time-dependent variable. The traditional risk factors age, sex, BMI and fasting glucose at baseline were independent determinants of risk (HR 1.03-2.42) and 1st 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 positive during the follow-up. Figure 2 shows the proportion of subjects surviving without diabetes during the follow-up according to GADA-positivity including age, gender 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 was highest in those with GADA in the highest quartile of positivity (23.3%; RR 4.9, 95% CI 2.80 – 8.51), intermediate in those having GADA within the three lower

quartiles (13.0%) and lowest in GADA negative 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 as 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 any GADA cut-off, 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 negative to positive during the follow-up conferred an increased risk of diabetes, but IA2-Ab were too rare in this population to have an impact

High GADA concentrations have been associated with lower C-peptide concentration in cross-sectional studies of LADA (14, 31, 32) as well as development of complete beta-cell failure in adults with newly diagnosed diabetes of any type (33), although data from the UKPDS did not support an association between GADA level and need of insulin ((34). Phenotypically only three of our patients, who developed diabetes, had type 1 diabetes, whereas all other GADA+ patients had a non-insulin dependent 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 GADA might be associated with a defect in insulin secretion. We have previously shown in non-diabetic 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 (27). Apparently, the insulin secretory defect associated with GADA is mild and can only be seen with a test that stresses the beta-cells maximally.

GADA positivity and type 1 diabetes clustered in families. In concert with the high

prevalence of type 1 diabetes in Finland (35), FH_{T1} was found in approximately 8% of GADA-negative and $GADA_{+low/med}$ subjects, while almost a 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, like HLA and PTPN22, explain of this clustering. With this background, the high frequency of GADA (4.7%) among the non-diabetic relatives and even control subjects without any family history for diabetes was not that surprising.

In a six-year follow-up study of this population, family history for type 2 diabetes together with BMI >30 kg/m² and fasting PG >5.5 mmol/l conferred a 3.7-fold risk of diabetes (30). We now hypothesized, that also family history for type 1 diabetes (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 (RR 2.2, CI 1.23-4.01). However, even when FH_{T1} was in the model, high GADA implied an even stronger risk (RR 4.9, CI 2.8 – 8.5). Further, there seemed to be a difference in incidence rates between the population controls without any family history for diabetes, whose diabetes risk only high GADA affected, and subjects with family history for type 1 or type 2 diabetes, whose diabetes risk was doubled also with low or medium high GADA. 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 (36, 37).

Although the low number of GADA+ population controls 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 GADA for diabetes in the general population, where no increased risk was found during a comparable 8-year follow-up (17, 28). Another difference between the studies was the number of GADA positive subjects, which was only 18 in the Italian Cremona Health Study (17) and 23 in the Swedish Västerbotten County Health Project. However, in another part of the Västerbotten Study, seven 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 2209 (3.9%) GADA- subjects ($p < 0.0001$). Only one of the seven was diagnosed with type 1 diabetes (16).

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.

ACKNOWLEDGEMENTS

The Botnia Research Group is acknowledged for recruiting and clinically studying the subjects. The study was supported by grants from the Academy of Finland, the Sigrid Juselius Foundation, the Finnish Diabetes Research Foundation, the Folkhalsan Research Foundation, the Finska Läkaresällskapet, the Novo Nordisk Foundation, the Swedish Cultural Foundation in Finland, the Ollqvist Foundation, Korsholm, Malax, Närpes and Vasa Health Care Centers and The Helsinki University Central Hospital.

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Table 1. Clinical characteristics of the non-diabetic subjects at baseline according to the strength of GADA positivity (GADA negative; GADA within the three lower quartiles of positivity, GADA^{+low/med}; GADA within the highest quartile, GADA^{+high}).

	GADA -	P- value	GADA ^{+low/med}	P- value	GADA ^{+high}	P-value (High vs. neg)
	4687					
N (M/F)	(2107/2580)		216 (101/115)		73 (33/40)	
NGT/IGT (%)	72/28		79/30		67/33	
Age at baseline (yr)	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)	
P-gluc 30' (mU/l)	8.5 (1.6)		8.6 (1.7)		8.6 (1.8)	
P-gluc 120' (mU/l)	6.3 (1.5)	0.002	6.1 (1.6)	0.04	6.4 (1.8)	
FS- insulin (mU/l)	4.7 [4]		4.7 [3.5]		4.7 [4.5]	
S-Insulin 30' (mU/l)	35.9 [37.8]		41.7 [35.5]	0.053	31.9 [31.9]	0.04
S-Insulin 120' (mU/l)	26.7 [33.2]	0.029	26.0 [31.0]		23.7 [16.7]	
FS-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)	
SBP (mmHg)	130.2 (18.6)		134.5 (20.2)		127.4 (19.8)	
DBP (mmHg)	79 (10.4)		80.4 (10.7)		78.6 (10.4)	
HBA1 _c (%)	5.3 (0.5)		5.4 (0.5)		5.3 (0.7)	
LDL cholesterol *	3.6 (1.0)		3.6 (1.1)		3.4 (1.0)	
HDL cholesterol *	1.4 (0.4)		1.4 (0.3)		1.4 (0.4)	
Triglycerides *	1.4 (0.9)		1.3 (0.7)		1.2 (0.6)	

Data are given as mean (SD) or median [IQR]. SBP, systolic blood pressure; DBP, diastolic blood pressure. 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.

* mmol/l

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

	GADA-		GADA+		P-value*	
	DM-	P-value	DM+	DM-		P-value
N	2377		134	216	36	
Age (yr)	54.6[20.6]	<0.0001	61.9[19.4]	56.9[20.9]	60.1[19]	
Follow-up time (yr) †	8.0[5.6]		7.7[5.4]	9.6[5.4]	6.3[4.9]	0.039
HBA1C (%)	5.5 (0.5)	<0.0001	6.3 (0.6)	5.7 (0.4)	6.7 (1.1)	0.004
FPG (mmol/l)	5.3 (0.6)	<0.0001	6.9 (1.0)	5.3 (0.6)	6.9 (0.9)	
P-gluc 30' (mmol/l)	8.5 (1.8)	<0.0001	11.5 (2.2)	8.4 (1.7)	11.5 (1.4)	
P-gluc 120' (mmol/l)	5.9 (1.7)	<0.0001	11.3 (2.9)	5.8 (1.7)	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
S-Insulin 30' (mU/l)	53.5 [43.5]	0.0003	50.5 [46.6]	53.4 [38.0]	36.8 [37.1]	
S-Insulin 120' (mU/l)	30.9 [37.2]	<0.0001	74.6 [91.9]	29.3 [33.8]	61.2 [74.7]	
fS-C-peptide (nmol/l)	0.5 (0.3)	<0.0001	0.9 (0.5)	0.5 (0.3)	0.7 (0.4)	
Insulinogenic index	14.6 [16.2]	<0.0001	8.1 [10.9]	15.6 [13.2]	6.4 [7.3]	
HOMA	1.7 [1.6]	<0.0001	4.0 [3.9]	1.7 [1.7]	2.1 [2.6]	0.005
Disposition index	8.8 [11.2]	<0.0001	2.3 [1.9]	8.8 [10.2]	2.9 [2.5]	
Waist (cm)	91.8 (12.5)	<0.0001	102.9 (12.6)	91.1 (11.5)	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)	
SBP (mmHg)	133.5 (19.6)	<0.0001	145.3 (22)	135.0 (19.2)	146.6 (27.4)	0.055
DBP (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 given as mean (SD) or median [IQR]. SBP, systolic blood pressure; DBP, diastolic blood pressure. 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 negative subjects who developed diabetes.

* Difference between GADA+DM+ and GADA-DM+

† Time until diagnosis of diabetes or until last visit.

FIGURE LEGENDS

Figure 1. Flow-chart showing the number of relatives and control subjects at baseline and during the follow-up according to GADA positivity and progression to diabetes.

Figure 2. The proportion of non-diabetic subjects having any GADA+ relatives (black columns), GADA+ non-diabetic relatives (striped columns), GADA+ type 2 diabetic relatives (LADA, white columns) or type 1 diabetic relatives (hatched columns) according to strength of GADA positivity.

P<0.0001, GADA+_{high} vs GADA- regarding subjects having any GADA+ relatives or T1 relatives;

P=0.002, GADA+_{high} vs. GADA- regarding subjects having GADA+ type 2 diabetic relatives;

P=0.01, GADA+_{high} vs. GADA- regarding subjects having non-diabetic GADA+ relatives.

Figure 3. Development of diabetes according to strength of GADA positivity: GADA 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 gender. The y-axis show the cumulative proportion of subjects without diabetes, the x-axis shows the follow-up time in years (P<0.00001, Cox proportional hazards model)

Figure 1

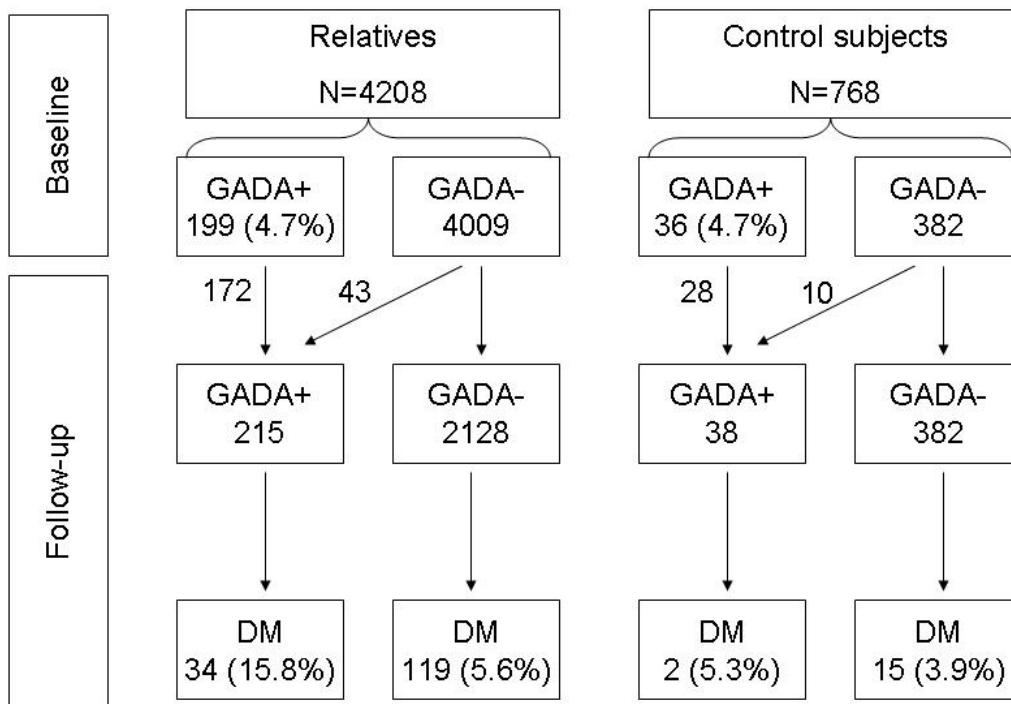


Figure 2

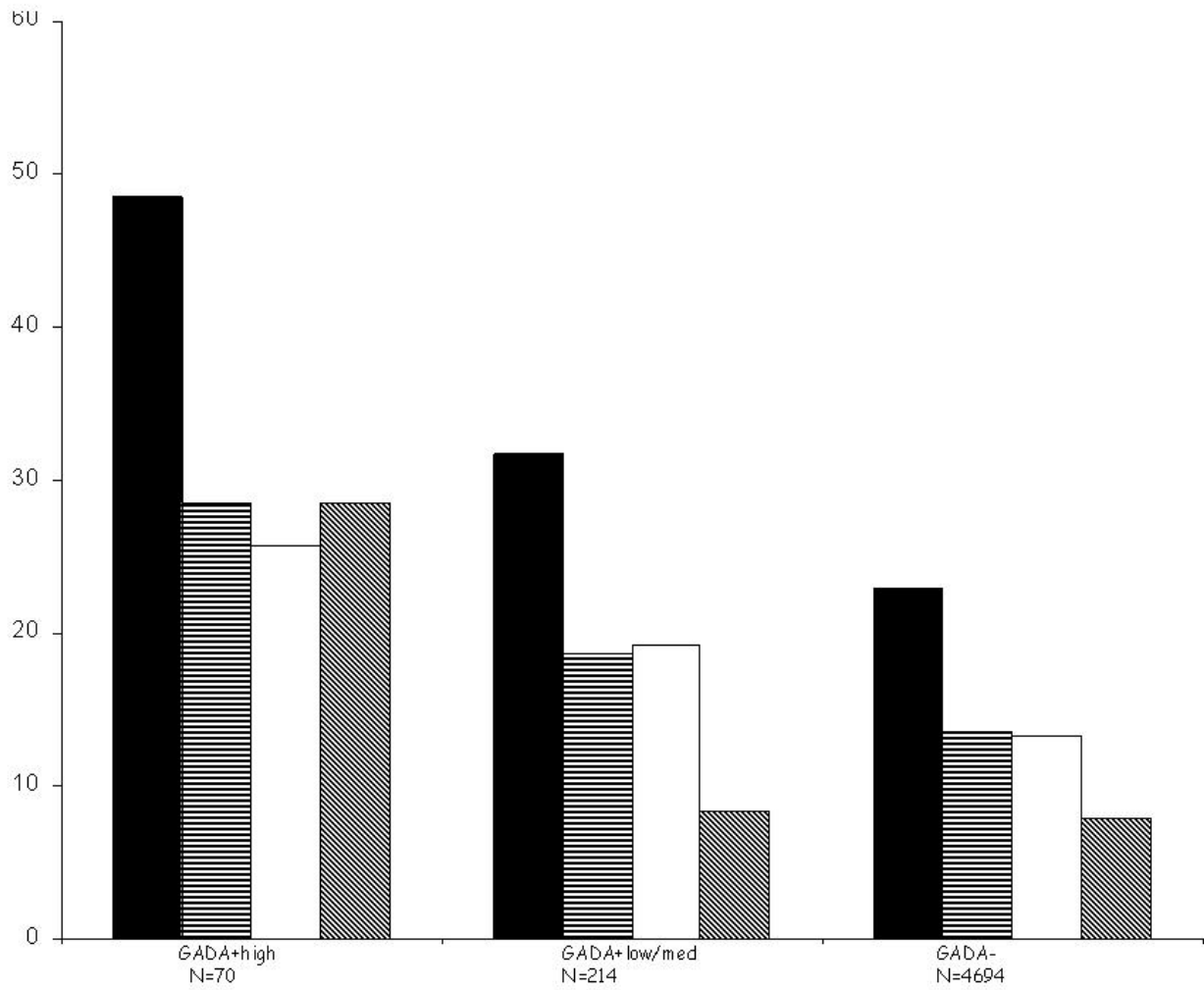


Figure 3

