Diabetes-Related Complications and Mortality in Patients With Young-Onset Latent Autoimmune Diabetes: A 14-Year Analysis of the Prospective Hong Kong Diabetes Register

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OBJECTIVE

Young-onset diabetes is heterogeneous in etiology and disease progression. We compared the incidence of diabetes-related complications and mortality in patients with young-onset type 2 diabetes with or without anti-GAD antibodies and patients with type 1 diabetes. We determined changes in glycemic control before and after commencement of insulin therapy stratified by antibody status.

RESEARCH DESIGN AND METHODS

Between 1994 and 2012, 1,504 consecutively enrolled patients with type 2 diabetes who had received a diagnosis at <40 years of age and had available anti-GAD antibody status and 251 patients with type 1 diabetes from the Hong Kong Diabetes Register were followed for incident cardiovascular disease (CVD), end-stage renal disease (ESRD), severe hypoglycemia, and all-cause mortality until June 2015. Information on insulin use and HbA_{1c} levels during follow-up was obtained.

RESULTS

Anti-GAD antibodies were positive in 8.1% of patients with type 2 diabetes (GAD^+). By multivariate Cox regression, patients with GAD^+ had a lower hazard of CVD (hazard ratio [HR] 0.43, $P = 0.048$), a higher hazard of severe hypoglycemia (HR 1.63, $P = 0.032$), and a similar hazard of ESRD and mortality compared with counterparts without anti-GAD antibodies (GAD^-). Compared with patients with type 1 diabetes, ESRD was more likely to develop (HR 2.91, $P = 0.043$) in patients with GAD^+, but no differences were detected in the hazards of severe hypoglycemia, CVD, and mortality. Among new insulin users ($n = 304$), patients with GAD^+ had larger reductions in HbA_{1c} than patients with GAD^-after 12 months of insulin use ($-2.30 \pm 3.80\%$ [25 ± 42 mmol/mol] vs $-0.72 \pm 1.86\%$ [8 ± 20 mmol/mol], $P = 0.05$).

CONCLUSIONS

Anti-GAD positivity identifies a group of patients with a different prognosis compared with patients without antibodies and those with type 1 diabetes. Patients with GAD^+ responded differently to insulin compared with patients with GAD^-.

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In Asia, up to one-fifth of adults with diabetes receive a diagnosis at <40 years of age (1). Identifying the type of diabetes is challenging in the younger group in whom diversity in the cause and phenotype is being increasingly recognized. Autoimmune diabetes refers to diabetes caused by autoimmune destruction of pancreatic β-cells, and the severity and rapidity of this process determine the clinical manifestation ranging from acute ketosis to slowly progressive hyperglycemia. The term "latent autoimmune diabetes in adults" (LADA) has been used to describe older patients (age of onset >35 years of age) with autoimmune tendency demonstrable by the presence of islet antibodies, whose presentation of nonketotic diabetes is otherwise indistinguishable from that of type 2 diabetes (2,3). From large cohorts of adolescents or young adults with type 2 diabetes, between 9.8% and 21.2% harbored islet antibodies, suggesting that latent autoimmune diabetes extends to the youth population (4–6). Early genetic studies showed that patients with latent autoimmune diabetes carry risk-conferring alleles in genes within the MHC that are also present in patients with type 1 diabetes, albeit at a lower frequency (7,8). More recently, risk genotypes in type 2 diabetes genes such as *TCF7L2* were found to be overrepresented among patients with positive islet antibodies when compared with normal control without diabetes, raising the notion that insulin deficiency in these individuals may occur through both autoimmune and nonautoimmune processes (9,10).

Previous studies using glucose clamps demonstrated that patients with phenotypic type 2 diabetes and positive islet antibodies are less insulin resistant but have a greater loss of insulin secretion than their counterparts without antibodies (11). Clinically, these individuals have fewer features of the metabolic syndrome but worse glycemic control, and are more likely to require insulin (12,13). Current guidelines advocate a personalized approach to diabetes management, and early insulin initiation is recommended in this subgroup of patients on the theoretical basis that perseverance with oral antihyperglycemic agents is unlikely to normalize blood glucose levels. The long-term outcome of patients with positive islet antibodies has been reported in several studies with conflicting results (13–17). Some identified reduced risks, while others observed similar risks of diabetic kidney disease, diabetic retinopathy, and cardiovascular disease (CVD) compared with patients without antibodies. Using a prospective database of Chinese with young-onset diabetes, defined arbitrarily as age at diabetes diagnosis of <40 years, we compared the incidence of diabetes-related complications and all-cause mortality among patients with clinical type 2 diabetes and positive islet antibodies, type 2 diabetes and negative islet antibodies, and type 1 diabetes. Additionally, we determined the changes in glycemic control before and after commencement of insulin treatment stratified by islet antibody status.

RESEARCH DESIGN AND METHODS

Patients

The Hong Kong Diabetes Register was established in 1994 at the Diabetes and Endocrine Centre, the Prince of Wales Hospital, Hong Kong Special Administrative Region. Patients with physician-diagnosed diabetes who attended the Diabetes and Endocrine Centre for comprehensive evaluation of diabetes complications were consecutively recruited. Referral sources included hospital-based specialist outpatient clinics and family medicine clinics. Blood samples for future biomedical research were collected from all patients at the time of study enrollment and stored at −20°C. For this study, stored serum samples from patients enrolled between 1994 and 2012 with clinical type 2 diabetes diagnosed before the age of 40 years were retrieved for determination of anti-GAD antibodies and C-peptide levels. The analyzable cohort included Chinese patients with type 2 diabetes with measured anti-GAD antibodies and all Chinese patients with type 1 diabetes, a subset of whom was also tested for anti-GAD positivity. Patients were classified to have type 1 diabetes if they presented with acute ketoacidosis or required continuous use of insulin within 1 year of diagnosis. The Declaration of Helsinki was adhered to, and written informed consent was obtained from patients at study enrollment for the use of their anonymized clinical data for research purposes. Ethics approval was received from the local Clinical Research Ethics Committee.

Baseline Clinical Assessment

Details of the clinical assessment were described previously (18). Patients attended the Diabetes and Endocrine Centre after an 8-h overnight fast and underwent structured evaluation including history taking of sociodemographic status, significant medical conditions, and medication use. Anthropometric measurements (weight, height, waist circumference) and vital signs (blood pressures, pulse) were recorded. Eyes were examined for visual acuity and the presence of diabetic retinopathy, with the latter examined using retinal photography and interpreted by trained endocrinologists. Lower limbs were assessed for peripheral arterial disease and peripheral sensory neuropathy.

Fasting blood samples were taken for the measurement of plasma glucose, glycated hemoglobin (HbA_{1c}), lipids (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides), renal function, and complete blood count. Spot urine was collected to determine the albumin-to-creatinine ratio (ACR). Glomerular filtration rate (GFR) was estimated using the abbreviated MDRD equation calibrated for the Chinese population as follows: estimated GFR = $186 \times (\text{serum creatinine } [\mu\text{mol/L}] \times 0.011)^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times 1.233$, where 1.233 is the adjusting coefficient for Chinese (19). Chronic kidney disease (CKD) was defined as estimated GFR <60 mL/min/1.73 m^2, and end-stage renal disease (ESRD) was defined as estimated GFR <15 mL/min/1.73 m^2. Microalbuminuria was defined as urine ACR of 2.5–30 mg/mmol in men or 3.0–30 mg/mmol in women, and macroalbuminuria as urine ACR >30 mg/mmol. Laboratory tests were performed in the Department of Chemical Pathology, the Prince of Wales Hospital, which was accredited by the National Association of Testing Authorities, Australia, and the Royal College of Pathologists of Australasia for medical testing.

Overweight was defined as BMI ≥25 to <30 kg/m^2 and obesity as BMI ≥30 kg/m^2, in accordance with the World Health Organization classification. We defined hypertension as systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥80 mmHg, or use of antihypertensive drugs; and dyslipidemia as LDL cholesterol ≥2.6 mmol/L or use of lipid-lowering drugs.

Measurement of Anti-GAD Antibody and C-Peptide Levels

Between 2016 and 2017, blood samples were retrieved for measurement of anti-GAD antibodies and C-peptide levels. Anti-GAD antibodies were measured using the GAD Autoantibody ELISA kit (RSR Limited). The lower detection limit of the kit was 0.57 units/mL. The intra-assay and interassay coefficients of variation range from 3.5–7.3% to 5.2–6.4% for anti-GAD antibody levels 5.7–97 units/mL, respectively. C-peptide was measured by ELISA using the commercial C-Peptide ELISA kit (Mercodia), which has been calibrated against the International Reference Reagent for C-peptide, C-peptide 84/510 (1st International Reference Reagent). The lower detection limit of the kit was 25 pmol/L. The intra-assay and interassay coefficients of variation range from 2.9–4.8% to 0.6–4.8% for C-peptide levels of 304–1,803 pmol/L, respectively. Both anti-GAD antibodies and C-peptide levels were measured at the Diabetes Genome Laboratory, Li Ka Shing Institute of Health Sciences, the Chinese University of Hong Kong, Prince of Wales Hospital (Hong Kong Special Administrative Region, People's Republic of China).

Outcome Identification and Definition

The Hospital Authority (HA) is a statutory body that governs all public hospitals and outpatient clinics and provides 95% of the total hospital bed-days and 80% of the outpatient visits in Hong Kong. Admission records, laboratory results, and prescriptions are stored in the HA Central Computer System, which identifies patients by their unique Hong Kong Identity Card number, which is compulsory for all Hong Kong residents. We retrieved hospital discharge diagnoses coded according to the ICD-9 of all patients in this cohort from the day of their enrollment until 31 May 2015. Diagnosis codes listed as principal diagnosis on discharge summaries were used to identify clinical outcome for this study (18). As outcome capture was based on hospitalization records, clinically silent events (e.g., silent myocardial infarction) would not be identified. We extracted all measurements of serum creatinine during the observation time to ascertain renal events. Information on death obtained from the Hong Kong Death Registry of the Immigration Department was linked to the HA Central Computer System. We also acquired serial HbA_{1c} measurements and insulin prescription data for all patients during the observation period. Incident insulin use was defined as the first insulin prescription of at least 28 days and excluding use of insulin during an inpatient period.

Statistical Analysis

Analysis was performed using the R statistical software. Baseline clinical characteristics of patients with type 1 diabetes and type 2 diabetes with or without anti-GAD antibodies were compared using descriptive statistics. Multivariate Cox logistic regression analysis was performed to derive hazard ratios (HRs) and 95% CIs of anti-GAD–positive type 2 diabetes (GAD^+) versus anti-GAD–negative type 2 diabetes (GAD^-) or type 1 diabetes for incident CVD, ESRD, severe hypoglycemia, and all-cause mortality. Regression models were adjusted for sex, age, disease duration, year of assessment, smoking status, BMI, systolic blood pressures, HbA_{1c}, LDL cholesterol, triglyceride, HDL cholesterol, urine ACR, estimated GFR, retinopathy, history of CVD (in the models for ESRD, severe hypoglycemia and all-cause mortality), as well as baseline use of insulin and other medications, including renin-angiotensin system inhibitors, lipid-lowering drugs, and antihypertensive drugs. Only patients without a history of CVD or ESRD were included in the analysis of the incidence of the respective event.

Next, we determined the prevalent and incident use of insulin, as well as the time to insulin use in patients with GAD^+ and GAD^-. The changes in HbA_{1c} before and after initiation of insulin therapy were examined in patients who were not prevalent users of insulin at baseline but commenced insulin during the follow-up period. Here, the differences between the most recent HbA_{1c} levels acquired prior to the first prescription of insulin and at 6 months and 12 months after insulin prescription were compared between the two groups.

Continuous variables are expressed as the mean $\pm$ SD or as median (interquartile range [IQR]), and categorical variables are expressed as percentages. χ^2 test was used for between-group comparisons of categorical variables, *t* test for normally distributed continuous variables, and Kruskal-Wallis test for continuous variables with skewed distribution. All statistical analyses were two tailed with statistical significance defined at $P < 0.05$.

RESULTS

Patients

Between 1994 and 2012, 4,005 patients had young-onset diabetes, among whom 251 had type 1 diabetes, 3,708 had clinical type 2 diabetes, and 46 had diabetes of unknown type and were excluded. Anti-GAD antibodies were measured in 1,504 patients with clinical type 2 diabetes and 95 patients with type 1 diabetes. The analyzable cohort included a total of 1,755 patients, with a mean age of 41.0 $\pm$ 11.7 years, a mean age at diagnosis of 31.6 $\pm$ 8.0 years, and a median disease duration of 7.0 years (IQR 1.0, 15.0 years).

Baseline Clinical Characteristics

Anti-GAD antibodies were detected in 8.1% of patients (122 of 1,504 patients) with clinical type 2 diabetes and 51.6% of patients (49 of 95 patients) with type 1 diabetes. Among patients with type 2 diabetes, the frequency of positive anti-GAD antibodies was 5.8% in those who were overweight and 13.2% in those who had normal weight. The proportion of patients with positive anti-GAD antibodies did not vary across the enrollment period (Supplementary Fig. 1).

Patients with GAD^+ were similar in age and age at diabetes diagnosis to patients with GAD^- and were older than patients with type 1 diabetes (Table 1). Frequency of self-reported family history of diabetes was similar among patients with GAD^+ and GAD^-, but was significantly lower in those with type 1 diabetes. Overweight and obesity were the most prevalent traits in patients with GAD^- and the least prevalent in patients with type 1 diabetes. The GAD^+ group was intermediate in the control of metabolic risk factors including blood pressure, triglyceride, and HDL cholesterol levels. Still, in the GAD^+ group, over half were overweight or obese and between 54% and 66% had either hypertension or dyslipidemia. Levels of HbA_{1c} were comparable between patients with GAD^+ and patients with type 1 diabetes, and were lower in patients with GAD^-. Among 216 patients with newly diagnosed type 2 diabetes at study enrollment, HbA_{1c} levels

Table 1—Baseline characteristics of 1,755 patients with type 1 diabetes, GAD+, and GAD$^-$

	Type 1 diabetes (n = 251)	GAD$^+$ (n = 122)	GAD$^-$ (n = 1,382)	P value*	P value†	P value‡
Age at enrollment, years	28.9 ± 12.4	41.7 ± 10.3	43.2 ± 10.3	<0.001	0.142	<0.001
Male, %	47.8	36.9	44.9	0.040	0.089	0.388
Ex- or current smoker, %	21.8	23.8	25.2	0.665	0.734	0.254
Age at diagnosis, years	20.4 ± 10.8	33.1 ± 5.4	33.5 ± 5.5	<0.001	0.228	<0.001
Duration of diabetes, years	8.0 (2.0, 12.0)	6.0 (1.0, 13.0)	7.0 (1.0, 15.0)	0.658	0.320	0.401
Family history of diabetes, %	24.3	56.6	56.1	<0.001	0.931	<0.001
BMI, kg/m^2	21.8 ± 3.7	24.5 ± 5.4	26.0 ± 4.7	<0.001	0.003	<0.001
Waist (women), cm	70.9 ± 9.2	78.9 ± 11.2	83.5 ± 11.1	<0.001	0.001	<0.001
Waist (men), cm	77.0 ± 11.2	85.1 ± 13.4	89.7 ± 11.3	0.001	0.028	<0.001
Systolic blood pressure, mmHg	115.5 ± 15.4	125.3 ± 20.6	127.2 ± 17.0	<0.001	0.332	<0.001
Diastolic blood pressure, mmHg	68.0 ± 9.6	73.9 ± 12.1	74.9 ± 10.2	<0.001	0.348	<0.001
HbA_{1c}, %	8.5 ± 2.1	8.4 ± 2.4	7.8 ± 1.9	0.746	0.014	<0.001
FPG, mmol/L	10.1 ± 5.5	9.6 ± 4.6	9.0 ± 3.7	0.369	0.116	0.001
Total cholesterol, mmol/L	4.9 ± 0.9	5.1 ± 1.2	5.1 ± 1.1	0.108	0.986	0.002
Triglyceride, mmol/L	0.8 (0.5, 1.0)	1.0 (0.7, 1.5)	1.4 (1.0, 2.2)	<0.001	<0.001	<0.001
HDL cholesterol, mmol/L	1.7 ± 0.5	1.5 ± 0.5	1.3 ± 0.4	0.001	<0.001	<0.001
LDL cholesterol, mmol/L	2.8 ± 0.8	3.1 ± 1.5	3.0 ± 0.9	0.043	0.439	0.001
Triglyceride/HDL ratio	0.5 (0.3, 0.7)	0.7 (0.4, 1.2)	1.2 (0.7, 2.0)	<0.001	<0.001	<0.001
Hemoglobin, g/dL	14.0 ± 1.4	13.9 ± 1.8	13.9 ± 0.8	0.550	0.920	0.431
Estimated GFR, mL/min/1.73 m^2	111.0 ± 21.9	97.3 ± 21.4	96.8 ± 23.1	<0.001	0.794	<0.001
Urine ACR, mg/mmol	1.0 (0.5, 2.3)	1.5 (0.8, 7.6)	1.7 (0.7, 8.2)	0.001	0.997	<0.001
Anti-GAD detectable, %	51.6	100	0	<0.001	NA	<0.001
C-peptide, pmol/L	47.9 (47.9, 47.9)	136.0 (47.9, 445.0)	220.6 (47.9, 566.0)	<0.001	0.063	<0.001
Overweight, %	21.1	38.2	54.8	0.012	0.001	<0.001
Obese, %	0.0	14.6	18.1	<0.001	0.407	<0.001
Hypertension, %	25.5	54.1	58.0	<0.001	0.403	<0.001
Dyslipidemia, %	57.8	66.1	72.0	0.126	0.168	<0.001
Microalbuminuria, %	15.2	26.3	24.3	0.012	0.631	0.002
Macroalbuminuria, %	4.9	12.7	13.8	0.008	0.736	<0.001
CKD, %	1.6	6.6	8.5	0.023	0.464	<0.001
ESRD, %	0.0	0.0	1.0	NA	0.616	0.239
Retinopathy, %	17.9	16.2	25.8	0.700	0.026	0.011
Sensory neuropathy, %	9.6	11.5	13.7	0.566	0.498	0.076
Coronary heart disease, %	0.4	3.3	4.3	0.041	0.601	0.002
Congestive heart failure, %	0.0	0.0	1.1	NA	0.626	0.147
Stroke, %	0.0	0.0	1.6	NA	0.250	0.037
PAD, %	4.8	4.1	3.5	0.767	0.797	0.342
Medications, %						
Oral antidiabetic drugs	6.6	62.5	64.8	<0.001	0.627	<0.001
Insulin	100.0	39.3	28.4	<0.001	0.011	<0.001
Antihypertensive drugs	8.8	29.5	28.5	<0.001	0.809	<0.001
RAS inhibitors	7.9	25.2	21.1	<0.001	0.313	<0.001
Lipid-lowering drugs	5.6	9.0	14.5	0.213	0.095	<0.001

Data are mean ± SD or median (IQR) unless otherwise specified. FPG, fasting plasma glucose; NA, not applicable; PAD, peripheral arterial disease; RAS, renin-angiotensin system. *Comparison between patients with GAD$^+$ and patients with type 1 diabetes. †Comparison between patients with GAD$^+$ and GAD$^-$. ‡Comparison between patients with GAD$^-$ and patients with type 1 diabetes.

were significantly higher in the GAD$^+$ group than in the GAD$^-$ group (11.0 ± 3.6% vs. 7.8 ± 2.1%, P = 0.002). For comparable disease duration, frequencies of albuminuria and CKD were higher in patients with clinical type 2 diabetes than in those with type 1 diabetes, irrespective of anti-GAD status. C-peptide levels were the lowest in patients with type 1 diabetes and similar between the GAD$^+$ and GAD$^-$ groups.

Incidence of Diabetes-Related Complications and All-Cause Mortality

Over a median follow-up period of 14 years (IQR 10.5, 18.5 years), new events of CVD developed in 196 patients (11.2%), of ESRD in 199 patients (11.3%), and of severe hypoglycemia in 277 patients (15.8%), and 192 patients (10.9%) died. The incidence of CVD and all-cause mortality were the highest in patients with GAD$^-$ and were comparable between patients with GAD$^+$ and those with

type 1 diabetes (Table 2). The incidence of ESRD did not differ between the GAD^+ and GAD^- groups but was lower in the group with type 1 diabetes. Severe hypoglycemia occurred at similar frequencies among patients with GAD^+ and those with type 1 diabetes, whereas patients with GAD^- were less likely to experience hypoglycemic events.

Using multivariate Cox proportional regression, we found that patients with GAD^+ had a lower hazard of CVD (HR 0.44 [95% CI 0.21, 0.94], $P = 0.034$]) and higher hazard of severe hypoglycemia (HR 2.11 [95% CI 1.44, 3.09], $P < 0.001$]) than their GAD^- counterparts, adjusted for age, sex, diabetes duration, and year of assessment (Table 3). The associations remained robust (CVD: HR 0.43 [95% CI 0.19, 0.99], $P = 0.048$; severe hypoglycemia: HR 1.63 [95% CI 1.04, 2.54], $P = 0.032$) upon further adjustment for glycemic and metabolic control, comorbidities, and medication use including insulin. The inclusion of baseline use of sulfonylurea in the model did not alter the relationships (CVD: HR 0.41 [95% CI 0.18, 0.95], $P = 0.038$; severe hypoglycemia: HR 1.70 [95% CI 1.09, 2.65], $P = 0.020$). Anti-GAD positivity was not associated with ESRD or all-cause mortality in patients with type 2 diabetes.

Relative to patients with type 1 diabetes, patients with GAD^+ were more likely to progress to ESRD (HR 2.91 [95% CI 1.03, 8.23], $P = 0.043$), adjusted for clinical variables (Table 3). No difference in the hazards of CVD, all-cause mortality, and severe hypoglycemia in patients with GAD^+ versus patients with type 1 diabetes was detected.

Incident Insulin Use and Glycemic Response in Patients with Clinical Type 2 Diabetes

At baseline, patients with GAD^+ were more likely to be treated with insulin than patients with GAD^- (39.3% vs. 28.4%, $P = 0.011$). During follow-up, more of the patients with GAD^+ who were nonusers of insulin at baseline initiated insulin therapy compared with patients with GAD^- (41.9% vs. 27.6%, $P = 0.009$). The median time to insulin therapy initiation was 2.3 years (IQR 0.7, 7.0 years) and 5.8 years (IQR 1.9, 8.8 years) in the GAD^+ and GAD^- groups, respectively ($P = 0.009$). In the GAD^+ group, the median time to insulin therapy initiation was numerically shorter in those who were of normal weight compared with those who were overweight or obese, but the difference did not reach statistical significance (1.6 years [IQR 0.4, 3.2 years] vs. 6.5 years [IQR 1.5, 8.5], $P = 0.157$). In patients with GAD^-, BMI was also not a differentiating factor for time to commencement of insulin therapy.

Among incident insulin users ($n = 304$), changes in glycemic control following insulin therapy initiation were compared between patients with GAD^+ and GAD^-. Levels of HbA_{1c} prior to insulin therapy commencement were higher in the GAD^+ group than in the GAD^- group ($10.7 \pm 3.1\%$ [93 ± 34 mmol/mol] vs. $9.3 \pm 1.9\%$ [78 ± 21 mmol/mol], $P = 0.020$). At 6 months (median 185 days [IQR 159, 210 days]), there was no difference in the change in HbA_{1c} level from baseline between the GAD^+ and GAD^- groups ($-1.76 \pm 3.47\%$ [19 ± 38 mmol/mol] vs. $-0.75 \pm 1.75\%$ [8 ± 19 mmol/mol], $P = 0.15$ adjusted for baseline HbA_{1c} level). At 12 months (median 358 days [IQR 333, 386 days]), the GAD^+ group achieved a significantly larger reduction in HbA_{1c} than the GAD^- group ($-2.30 \pm 3.80\%$ [25 ± 42 mmol/mol] vs. $-0.72 \pm 1.86\%$ [8 ± 20 mmol/mol], $P = 0.05$ adjusted for baseline HbA_{1c} level) (Fig. 1).

CONCLUSIONS

To our knowledge, this is the largest cohort of patients with young-onset diabetes with detailed baseline clinical characterization and long follow-up duration in whom anti-GAD status is available for prognostication. We report three major observations. First, patients with GAD^+ who were deemed to have latent autoimmune diabetes had a high prevalence of overweight and related metabolic abnormalities including hypertension and dyslipidemia, in contrast with patients with classical type 1 diabetes in whom abnormal metabolic features were less frequent. Second, CVD was less likely to develop in patients with GAD^+ compared with their GAD^- counterparts. On the other hand, their propensity for ESRD was similar to that of patients with GAD^- and higher than patients with type 1 diabetes, independent of clinical risk factors. Anti-GAD positivity therefore identifies a group of patients with a different prognosis. Third, glycemic response to insulin initiation was greater in patients with GAD^+ than in patients with GAD^-. This supports the timely institution of insulin therapy in patients with latent autoimmune diabetes in whom oral antihyperglycemic drugs may not be effective.

Table 2—Incidence rate of diabetes-related complications and all-cause mortality among 1,755 patients with clinical type 1 diabetes, GAD^+, and GAD^-

	Type 1 diabetes		GAD^+		GAD^-				
	Number of patients with event	Incidence rate, per 1,000 person-years	Number of patients with event	Incidence rate, per 1,000 person-years	Number of patients with event	Incidence rate, per 1,000 person-years	*P* value*	*P* value†	*P* value‡
CVD	9	2.49 (1.32, 4.35)	7	4.17 (2.06, 7.78)	180	10.45 (9.03, 12.03)	0.304	0.017	<0.001
ESRD	9	2.37 (1.26, 4.15)	14	8.04 (4.82, 12.76)	176	9.39 (8.10, 10.82)	0.004	0.576	<0.001
Severe hypoglycemia	57	18.67 (14.42, 223.81)	31	21.11 (14.91, 29.17)	189	10.70 (9.28, 12.27)	0.581	<0.001	<0.001
All-cause mortality	14	3.65 (2.19, 5.80)	10	5.56 (3.05, 9.49)	168	8.58 (7.38, 9.93)	0.312	0.182	0.002

*Comparison between patients with GAD^+ and patients with type 1 diabetes. †Comparison between patients with GAD^+ and GAD^-. ‡Comparison between patients with GAD^- and patients with type 1 diabetes.

Table 3—Positive anti-GAD antibodies versus (*A*) negative anti-GAD antibodies and (*B*) type 1 diabetes in relation to the hazard of incident complications and all-cause mortality using multivariate Cox proportional regression

	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
A. GAD^+ vs. GAD^-						
CVD	0.44 (0.21, 0.94)	0.034	0.44 (0.20, 0.95)	0.037	0.43 (0.19, 0.99)	0.048
ESRD	0.96 (0.55, 1.65)	0.875	1.02 (0.57, 1.82)	0.947	0.67 (0.34, 1.31)	0.239
Severe hypoglycemia	2.11 (1.44, 3.09)	<0.001	2.12 (1.43, 3.13)	<0.001	1.63 (1.04, 2.54)	0.032
All-cause mortality	0.72 (0.38, 1.37)	0.317	0.76 (0.39, 1.45)	0.398	0.57 (0.26, 1.23)	0.152
B. GAD^+ vs. type 1 diabetes						
CVD	1.29 (0.46, 3.59)	0.627	0.84 (0.29, 2.45)	0.746	0.79 (0.25, 2.43)	0.676
ESRD	3.72 (1.53, 9.06)	0.004	2.83 (1.09, 7.38)	0.033	2.91 (1.03, 8.23)	0.043
Severe hypoglycemia	0.96 (0.59, 1.59)	0.887	0.92 (0.55, 1.54)	0.747	1.06 (0.60, 1.89)	0.839
All-cause mortality	1.01 (0.43, 2.36)	0.981	0.94 (0.38, 2.33)	0.891	0.82 (0.29, 2.29)	0.698

Model 1, adjusted for age, sex, diabetes duration, and year of assessment. Model 2, adjusted for smoking, BMI, systolic blood pressures, HbA_{1c}, LDL cholesterol, HDL cholesterol, log triglyceride, and variables in Model 1. Model 3, adjusted for log urine ACR; estimated GFR; baseline retinopathy; baseline history of CVD (for ESRD, severe hypoglycemia, all-cause mortality); baseline use of insulin, renin-angiotensin system inhibitors, lipid-lowering drugs, blood pressure–lowering drugs, and antiplatelet drugs; and variables in Model 2.

Metabolic Profile by Anti-GAD Antibody Status

The presence of islet antibodies in patients with clinical type 2 diabetes signifies an underlying autoimmune process in the initiation and progression of β-cell failure. Previous studies (7,12,20,21) reported a lower frequency of the metabolic syndrome in patients with autoantibodies than those without. We also found that patients in the GAD^+ group were leaner and had a more favorable lipid profile than their GAD^- counterparts, but remained metabolically more adverse relative to patients with type 1 diabetes. Importantly, over half of the patients with GAD^+ were overweight or obese. The reliance on a lean phenotype as a differentiating factor to screen for autoimmune diabetes would lead to missed detection of up to half of the patients with GAD^+. It is noteworthy that the proportion of patients with a family history of diabetes was similar between patients with and without anti-GAD antibodies but was significantly lower in those with type 1 diabetes. These observations support the emerging recognition that diabetes characterized by detectable islet antibodies and slowly progressive hyperglycemia is a hybrid of immune-mediated effects and genetic predisposition for β-cell dysfunction, exacerbated by obesity, which is increasingly common in the general population (9,10).

Glycemic Control and Response to Insulin Therapy

For similar disease duration, patients with GAD^+ had worse glycemic control and insulin use was higher at baseline and during follow-up than in patients with GAD^-. In our cohort, the median time to insulin initiation was ∼2 years in the GAD^+ group, and after 20 years of diabetes, 65% of those patients had started insulin therapy compared with 48% of patients in the GAD^- group. Among incident insulin users, we assessed glycemic response following commencement of insulin therapy and observed a greater decline in HbA_{1c} levels at 12 months in patients with anti-GAD antibodies than in those without, even after adjustment for preinsulin HbA_{1c} levels. Our findings contrast with those from an early analysis of the UK Prospective Diabetes Study (UKPDS) cohort, which found that islet antibody status did not modify HbA_{1c} trajectory following insulin initiation (22). The between-study difference in inclusion age of the recruited patients (UKPDS 25–65 years; present study 18–40 years) may partly explain the discrepancy. It is possible that patients in whom autoimmune diabetes develops at an older age have less severe β-cell dysfunction and therefore show less pronounced glycemic response to insulin therapy. Overall, our results favor the preferential use of insulin above non-insulin agents to maintain glycemic control in this subgroup who have a predominance of insulin insufficiency over insulin resistance. Notwithstanding, our results cannot be taken to infer long-term benefits, and whether early institution of insulin will be translated to reduction in the risks of vascular

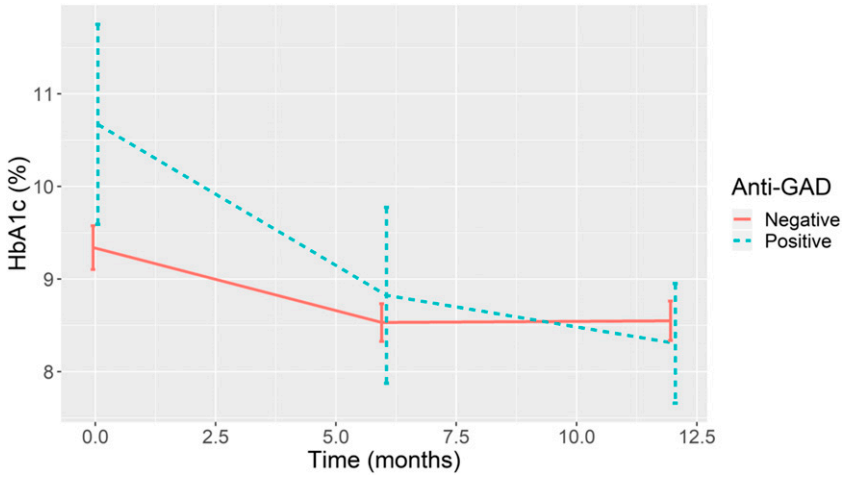

Figure 1—Changes in HbA_{1c} level from baseline to 6 and 12 months after insulin initiation in incident insulin users with and without anti-GAD antibodies. Red, negative anti-GAD antibodies (n = 273); blue, positive anti-GAD antibodies (n = 31).

complications in these patients is yet to be determined.

Diabetes-Related Complications and All-Cause Mortality

Among patients with clinical type 2 diabetes, we detected no difference in the hazard of all-cause mortality between patients with GAD^+ and those with GAD^-. The incidence of CVD was lower in the GAD^+ group, and the risk differential remained significant even after adjustment for clinical risk factors. One possible explanation is that GAD+ patients who might have faster progression of hyperglycemia, henceforth more likely to be symptomatic, were identified earlier in their disease trajectory as compared with GAD^- patients who had a more protracted course and remained undiagnosed for a longer time. This is supported by the significantly higher mean HbA_{1c} level in patients with GAD^+ compared with patients with GAD^- at diabetes diagnosis (11.0 $\pm$ 3.6% vs. 7.8 $\pm$ 2.1%). Longer exposure to untreated diabetes and other metabolic risk factors in the GAD^- group during the undiagnosed period might contribute to a subsequently higher incidence of CVD.

Three previous studies (14,15,23) also investigated the mortality of patients with LADA. Each reported no difference in mortality between patients with LADA and those with type 2 diabetes, although the sample sizes were small in all the studies. The Botnia study (15) compared 90 patients with autoimmune diabetes and 929 patients with type 2 diabetes and found that both all-cause and cardiovascular mortality did not differ between the two groups after 6 years of follow-up. The HUNT2 study, which comprised 208 patients with autoimmune diabetes and 2,425 patients with type 2 diabetes who were followed for 9 years, showed that the hazards for all-cause and cardiovascular mortality were similar, adjusted for age and diabetes duration (23). The Fremantle Diabetes Study (14) was the only prospective study that evaluated incident CVD and observed similar incidences of coronary artery disease and cerebrovascular disease among 45 patients with LADA and 1,210 patients with type 2 diabetes and negative anti-GAD antibodies over 9 years of surveillance. We note the wide age difference between the present cohort and the cohorts from earlier studies, which limits the comparability of findings across studies. The age disparity may also account for the discordant conclusions between our study (mean age 41 years) and the Fremantle Diabetes Study (mean age 63 years in patients with LADA and 64 years in patients with type 2 diabetes). Older individuals have greater screening opportunities, and as such, any potential difference in the propensity for CVD between patients with GAD^+ and GAD^- related to disparate exposure to undiagnosed diabetes might become less apparent compared with younger people. Moreover, older patients with autoimmune diabetes might have acquired more risk factors as part of aging, and their subsequent risks of CVD increased to approach those of patients with type 2 diabetes.

We found comparable prevalence of CKD and albuminuria at baseline and similar incidence of ESRD across the GAD^+ and GAD^- groups. No former studies have reported the progression to ESRD, although in several cross-sectional studies the frequencies of albuminuria were the same in patients with LADA as in those with type 2 diabetes (15,16,24). The similar propensity for ESRD even before adjusting for metabolic risk factors indicates that patients with GAD^+ were not protected from renal complications despite an apparently healthier metabolic profile. Of note, patients with type 1 diabetes had a lower risk of renal complications compared with patients with GAD^+. This may be explained in part by differences in care processes, as patients with type 1 diabetes were more likely to attend specialist clinics and to receive regular nurse review, which in turn could lead to better outcomes.

Compared with patients with GAD^-, patients with GAD^+ were more prone to severe hypoglycemia, with an unadjusted HR of 2.1, which was attenuated to 1.7 after adjustment for the use of insulin and sulfonylurea. Indeed, patients with GAD^+ shared risks of severe hypoglycemia similar to patients with type 1 diabetes, and it may be that, similar to type 1 diabetes, autoimmune destruction also involves α-cells in patients with GAD^+, leading to impaired counterregulatory responses. In a nested case-control analysis of the ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial cohort, positivity for islet antibodies increased the odds of the composite of severe hypoglycemia and failure to achieve HbA_{1c} $<$6.0% by 4-fold to 16-fold (25). Anti-GAD positivity is therefore an important risk marker for significant hypoglycemia. In this light, knowledge of islet antibodies status may be valuable in goal setting and in guiding antihyperglycemic therapy.

Prevalence of Anti-GAD Positivity in Young-Onset Diabetes

Interethnic differences in the prevalence of islet antibody positivity have been suggested previously, with higher rates reported in the Caucasian population and lower rates among Asians including Chinese (7,20,26,27). The prevalence of 8.1% in the current study is comparable to figures from two major series of Caucasian patients with youth-onset diabetes but is lower than the results from the SEARCH for Diabetes in Youth study and the UKPDS (4–6). In the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study (5), 9.8% of 1,206 adolescents 10–17 years of age with phenotypic type 2 diabetes and obesity had detectable anti-GAD and/or anti-IA2 antibodies. Contrarily, among 151 adolescents 10–19 years of age with clinical type 2 diabetes and available anti-GAD status registered in the SEARCH study, anti-GAD antibody was present in up to 21.2% (6). From the UKPDS (28), 21% of 157 patients with type 2 diabetes who received a diagnosis between 25 and 34 years of age were positive for anti-GAD antibodies. Recently, the LADA China Study Group (20) initiated a nationwide study to ascertain the prevalence of LADA and found that 5.9% of 4,880 adults (mean age 51 years) with nonketotic diabetes harbored anti-GAD antibodies. The older age distribution of the LADA China Study may account for the lower frequency of anti-GAD positivity compared with the current study, as the prevalence of anti-GAD positivity tends to decline with increasing age at diagnosis (7,28). As the majority of patients from the current study were recruited from hospital-based clinics and were more likely to be insulin users than patients from general practices, the frequency of anti-GAD positivity reported herein may be an overestimate of the true prevalence among patients from a community setting. The lack of

standardization in methods used to measure anti-GAD antibodies would also limit the comparability of the prevalence of anti-GAD positivity across studies.

Limitations

We acknowledge the following limitations. First, only anti-GAD antibodies were tested in our cohort and we might have missed identifying patients with autoimmune tendency who carry other islet antibodies. Given that antibodies to GAD are the most prevalent and were previously reported to identify between 67% and 96% of patients with autoimmune diabetes, the number of missed individuals would be small and unlikely to affect the results of the analysis appreciably (26,29,30). Second, as there was a lag time of 6 years between diagnosis and sample collection for the measurement of anti-GAD antibodies, it is possible that we have misclassified patients who had antibodies initially but later seroconverted. On the other hand, anti-GAD antibodies have been shown to persist in the circulation for up to 12 years (31), and as such, the probability of misclassification is deemed to be low. Third, patients in this study were recruited over a 19-year period from 1994 to 2012, and improvement in care and a resultant decline in rates of the development of diabetes complications have been previously shown (32). We have adjusted for the year of assessment to minimize the impact of time dependency in outcomes. Fourth, this research was conducted in patients with young-onset diabetes, and the results may not be generalizable to patients with usual onset age. Last, the small number of GAD^+ patients limited the power of the study to determine the incidence of major clinical events accurately, as evidenced by the wide CI around the estimates, and a larger cohort is required to confirm these findings.

Conclusion

In summary, patients with GAD^+ are protected from CVD, but their risks for renal diseases are similar to those of their GAD^- counterparts. Anti-GAD positivity predicts better glycemic response to insulin therapy, although the greater susceptibility to severe hypoglycemia warrants close glycemic monitoring and high-level vigilance during treatment escalation in these patients. Our results support testing for islet antibodies irrespective of metabolic phenotype for risk stratification and informing antihyperglycemic management.

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