Latent Autoimmune Diabetes in Adults With Low-Titer GAD Antibodies: Similar Disease Progression With Type 2 Diabetes
A Nationwide, Multicenter Prospective Study (LADA China Study 3)

Lingjiao Liu,[1] Xia Li,[1] Yufei Xiang,[1] Gan Huang,[1] Jian Lin,[1] Lin Yang,[1] Yunjuan Zhao,[1] Zhifang Yang,[1] Can Hou,[1] Yijun Li,[1] Jie Liu,[2] Dalong Zhu,[3] R. David Leslie,[4] Xiangbing Wang,[1] and Zhiguang Zhou,[1] on behalf of the LADA China Study Group

OBJECTIVE

This study investigated the relationship between GAD autoantibody (GADA) titers and changing of β-cell function in patients with latent autoimmune diabetes in adults (LADA).

RESEARCH DESIGN AND METHODS

This 3-year prospective study enrolled 95 subjects from 15 Chinese cities including 25 high-titer (GADA ≥180 units/mL) LADA patients, 42 low-titer (GADA <180 units/mL) LADA patients, and 28 type 2 diabetic patients, the latter two groups as controls of similar age, sex, and BMI. Clinical characteristics were determined annually, including glycosylated hemoglobin (HbA$_{1c}$), fasting C-peptide (FCP), and 2-h postprandial C-peptide (PCP).

RESULTS

Despite similar initial FCP and PCP, FCP and PCP both decreased more in subjects with high GADA titer (FCP from mean 0.49 nmol/L at entry to 0.13 nmol/L at the third year; $P < 0.05$) than with low GADA titer (FCP from mean 0.48 to 0.38 nmol/L) and type 2 diabetes (FCP from mean 0.47 to 0.36 nmol/L); the latter two groups being similar. After 3 years, residual β-cell function (FCP >0.2 nmol/L) was detected in only 42% with an initial high GADA titer compared with 90% with a low GADA titer and 97% with type 2 diabetes ($P < 0.01$ for both). GADA positivity at the third year persisted more in subjects with initially high GADA (92%) than with low GADA (26%) titers ($P < 0.01$).

CONCLUSIONS

In selected LADA patients, initial GADA titers identified subjects with different degrees of persistent autoimmunity and disease progression. LADA patients with a low GADA titer had metabolic phenotypes and loss of β-cell function similar to type 2 diabetic patients.

[1]Institute of Metabolism and Endocrinology, The Second Xiangya Hospital and the Diabetes Center, Metabolic Syndrome Research Center, Key Laboratory of Diabetes Immunology, Ministry of Education, Central South University, National Clinical Research Center for Metabolic Diseases, Changsha, Hunan, China
[2]Department of Endocrinology and Metabolism, Shanxi Provincial People's Hospital, Taiyuan, China
[3]Department of Endocrinology and Metabolism, Nanjing University affiliated Nanjing Drum Tower Hospital, Nanjing, China
[4]Department of Diabetes and Metabolic Medicine, Blizard Institute, London, U.K.

Corresponding author: Zhiguang Zhou, zhouzg@hotmail.com.

Latent autoimmune diabetes in adults (LADA), an adult-onset form of autoimmune diabetes, shares clinical and immunogenetic characteristics with either type 1 diabetes or type 2 diabetes (1–3). In contrast to type 2 diabetes, a larger proportion of LADA patients progress to insulin treatment, and current therapeutic strategies include the early use of insulin and avoidance of sulfonylurea drugs (4–7). Despite insulin therapy, individuals with LADA tend to have worse glycemic control than patients with type 2 diabetes (8). At present, LADA is diagnosed by the presence of at least one islet autoantibody, most commonly GAD autoantibody (GADA), and insulin-independence for at least 6 months postdiagnosis (9,10).

Several cross-sectional studies have shown that GADA titers are associated with the phenotypic heterogeneity of clinical features in LADA patients (11–16). The apparent bimodal distribution of GADA titers can be used to classify LADA into two subgroups with distinct clinical, autoimmune, and genetic features (13–15). Studies of Chinese (16) and Caucasians (14) both showed that the characteristics of patients with low GADA titers were similar to those of antibody-negative type 2 diabetic patients, including sex and age at diagnosis, BMI, HbA$_{1c}$, β-cell function reserve, the metabolic syndrome, and insulin treatment. Therefore, whether a single autoantibody positive at low titers is sufficient evidence for autoimmune diabetes remains controversial (17,18). We therefore selected well-matched patients from the multicenter LADA China Study to investigate the relationship between GADA titers and the β-cell destruction rate. The aim of this prospective study was to explore the natural history of pancreatic β-cell function in LADA patients with different GADA titers.

RESEARCH DESIGN AND METHODS

Subjects

Patients were recruited from 25 centers in 15 major cities in China from a prospective study from January 2006 to December 2011. The study enrolled three groups of patients: a high-GADA titer LADA group, a low-GADA titer LADA group, and a type 2 diabetic group. LADA was defined according to the Immunology of Diabetes Society 2004 definition, based on the diagnosis of diabetes at age ≥30 years old, independence of insulin therapy for at least 6 months, and GADA positivity (10). GADA

levels ≥180 units/mL were defined as "high GADA level," whereas those between 18 and 180 units/mL were defined as "low GADA level" (16). The inclusion criteria were 1) diagnosis of diabetes (World Health Organization [WHO] 1999 criteria) at age ≥30 years, 2) duration of diabetes <5 years, and 3) patients with residual islet function (fasting C-peptide [FCP] ≥0.2 nmol/L or 2-h postprandial C-peptide [PCP] ≥ 0.4 nmol/L). The additional inclusion criteria for the low-GADA titer group and type 2 diabetic group were that patients should be similar for age, sex, and BMI with those in the high-GADA titer group. The exclusion criteria were 1) history of any malignancy or other severe diseases, 2) unable to finish the 3-year follow-up, or 3) poor compliance or refusal to participate. This study was conducted in accordance with the Declaration of Helsinki and was approved by local ethics committees. All patients signed an informed consent.

A total of 5,324 newly diagnosed phenotypic type 2 diabetic patients were tested for GADA, and 287 were screened out as LADA. Among those 287 patients with LADA, 48 were disqualified by inclusion criteria, 98 refused to participate, and 35 had been included in other trials. Of the 67 patients included in the current study, 25 had high GADA titers and 42 had low GADA titers. Enrolled as control subjects were 28 age-, sex-, and BMI-similar

GADA-negative type 2 diabetic patients (Fig. 1). All patients were recommended to have early administration of insulin or oral drugs (rosiglitazone or metformin) according to their specific disease conditions. No other hypoglycemic agents, such as sulfonylurea, were used during the study period.

In the high-GADA titer LADA group, 20 patients received insulin therapy (of whom 18 were given Insulin Aspart 30 or Novolin 30R, and 2 were given insulin glargine), 6 received metformin, and 15 received rosiglitazone. In the low-GADA titer LADA group, 22 patients received insulin therapy (of whom 14 were given Insulin Aspart 30 or Novolin 30R, and 8 were given insulin glargine), 20 received metformin, and 19 received rosiglitazone. In the type 2 diabetic group, 6 patients received insulin therapy (all using Insulin Aspart 30 or Novolin 30R), 22 received metformin, and 10 received rosiglitazone.

The study lasted for 36 months, and the follow-up visits took place at 0, 12, 24, and 36 months. On each annual visit, tests were started before 9:00 A.M. Long-acting insulin was withheld before the visit day. Short-acting insulin and oral antidiabetic drugs were not permitted on the visit day. After an overnight fast, blood samples were collected from each subject before and 2 h after a standard 500-kcal mixed-meal tolerance test (53.8% of calories as
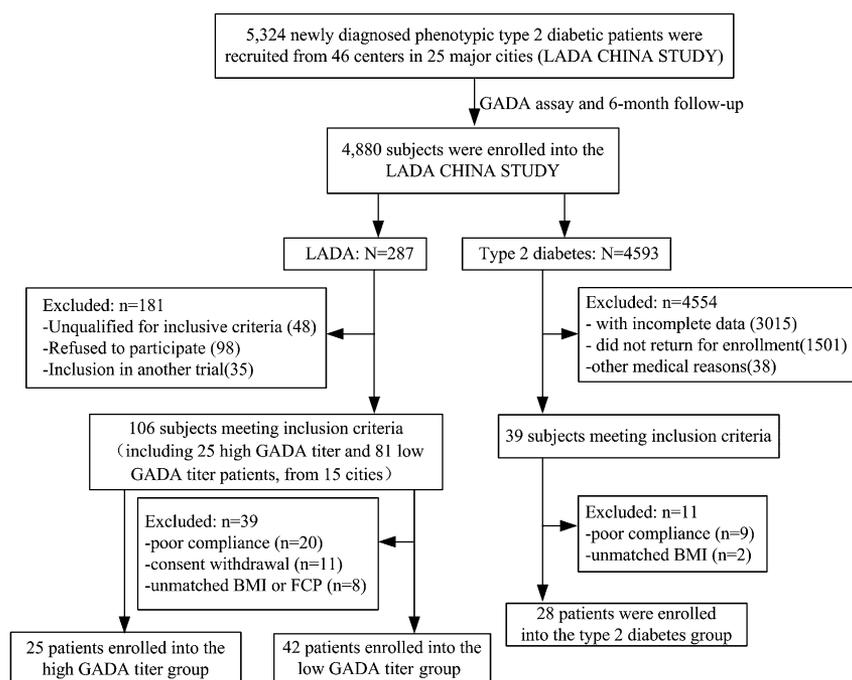

Figure 1—Flowchart shows the process of patients' enrollment.

carbohydrate, 25.6% as fat, and 9.6% as protein) (19). Serum glucose and C-peptide levels were measured before and 120 min after the mixed meal. Biochemical indicators were determined at the study sites by standard methods. Glycosylated hemoglobin (HbA_{1c}), C-peptide level, and GADA testing were measured at the core laboratory of the Second Xiangya Hospital. Body height, weight, waist and hip circumference, and blood pressure were measured with a standardized procedure. BMI and waist-to-hip ratio (WHR) were calculated. Among all patients, 22 of 25 high-GADA LADA patients consented to the genetic study, as did 34 of 42 and patients in the low-GADA LADA group and 16 of 28 type 2 diabetic patients.

C-Peptide and HbA_{1c} Assays

Serum C-peptide levels were measured by a chemiluminescence method using the Adiva Centaur Systemakit (Siemens, Munich, Germany). The inter- and intra-assay variation coefficients were 3.7–4.1% and 1.0–3.3%, respectively. HbA_{1c} was measured by automated liquid chromatography (VARIANT-II Hemoglobin Testing System; Bio-Rad Laboratories, Hercules, CA). Insulin resistance (IR) was measured with HOMA2-IR, the computerized version of HOMA (20).

GADA Assays

GADA was analyzed by radioligand assay in duplicate. The GADA titer of 18 units/mL or higher was defined as positive and confirmed by repeated assay. The sensitivity and specificity were 82% and 98%, respectively. Intra- and interassay coefficients of variation were 8.9% and 11.2%, respectively. The assay has been validated by Islet Autoantibody Standardization Program 2012 and sponsored by the Immunology of Diabetes Society.

HLA Genotyping

Genomic DNA was extracted from anti-coagulated peripheral blood using a phenol-chloroform method. HLA-DQA1 and -DQB1 genotypes were defined by DNA analysis using PCR to amplify exon 2 of both DQA1 and DQB1 genes, followed by standard DNA sequencing-based typing (21). The definition of HLA protective haplotypes and HLA susceptibility haplotypes was according to that in LADA China Study reported by Zhou et al. (16).

Statistical Analysis

Statistical analysis was performed with SPSS 16.0 software. Data are presented as mean ± SD or as indicated. A one-way ANOVA was used to examine the baseline data and the changes in BMI, waist and hip circumference, HbA_{1c}, and C-peptide among the groups. A paired t test was

used for a comparison of the C-peptide changes from the baseline value within the same group. A χ^2 test was used to compare categorical variables. Two-sided statistical tests were performed, and a P value of < 0.05 was considered significant.

RESULTS

The 3-year follow-up was completed by 24 of 25 patients in the high-titer group, 39 of 42 in the low-titer group, and 27 of 28 with type 2 diabetes. The subjects' baseline characteristics are reported in Table 1. There were no significant differences in age at diagnosis and BMI (by design) or in HbA_{1c}, HLA genotype, HOMA2-IR, and β-cell function (including FCP, PCP, and ΔCP [ΔCP = FCP − PCP]) among the three groups (P > 0.05), nor did the insulin daily dose differ among the three groups (Table 1). The diabetes-protective haplotypes in Chinese (including DQA1*0102-DQB1*0601, DQA1*0102-DQB1*0602, and DQA1*0601-DQB1*0301) in the three groups were 18.2% (4 of 22), 17.6% (6 of 34), and 18.8% (3 of 16), respectively. The frequency of Chinese diabetes-susceptibility haplotypes (including DQA1*03-DQB1*0303, DQA1*03-DQB1*0401, DQA1*05-DQB1*0201) in all the three groups was 50%. The frequencies of diabetes-protective haplotypes and

Table 1—Baseline data of LADA and type 2 diabetic patients

	Type 2 diabetes $n = 28$	Low GADA titer LADA $n = 42$	High GADA titer LADA $n = 25$
Age (years)	53.6 ± 10.1	52.2 ± 9.0	50.6 ± 11.7
Sex			
Male	18	29	15
Female	10	13	10
Duration (years)	2.2 ± 0.7	1.8 ± 0.9	2.0 ± 1.3
BMI (kg/m^2)	24.4 ± 2.9	24.6 ± 2.5	23.5 ± 2.6
Metformin (yes/no), n	22/6	20/22*	6/19*
Rosiglitazone (yes/no), n	10/18	19/23	15/10
Metformin and rosiglitazone (yes/no), n	9/19	11/31	3/22
Insulin (yes/no), n	6/22	22/20*	20/5*
Insulin dose (units/kg/day)	0.34	0.34	0.35
HbA_{1c} (%)	6.4 ± 1.2	6.8 ± 1.0	6.9 ± 1.1
HbA_{1c} (mmol/mol)	46 ± 13	51 ± 11	52 ± 12
HOMA2-IR	1.1 (0.5, 2.3)	1.2 (0.6, 3.4)	1.3 (0.5, 5.3)
FCP (nmol/L)	0.47 (0.22, 0.90)	0.48 (0.25, 1.04)	0.49 (0.20, 1.07)
PCP (nmol/L)	1.74 (0.42, 3.64)	1.80 (0.48, 3.55)	1.55 (0.73, 3.67)
ΔCP (PCP − FCP) (nmol/L)	1.22 (0.14, 2.74)	1.27 (0.00, 2.44)	1.24 (0.11, 3.28)
HLA protective haplotypes, % (n/N)	18.2 (4/22)	17.6 (6/34)	18.8 (3/16)
HLA susceptibility haplotypes, % (n/N)	50 (8/16)	50 (17/34)	50 (11/22)

Data are shown as mean ± SD, median (minimum, maximum), or as indicated. *$P < 0.05$ compared with type 2 diabetic group.

diabetes-susceptibility haplotypes were similar among the three groups (Table 1). In type 2 diabetic patients, less insulin therapy and more metformin were used ($P < 0.05$, Table 1).

During the 3 years, there were no significant differences of BMI, WHR, and HbA$_{1c}$ level among the three groups (Table 2). FCP, PCP, and ΔCP decreased significantly in the high-GADA titer group, with modest changes in the low-GADA titer group and type 2 diabetic group (Fig. 2). The PCP levels (minimum, maximum) decreased significantly from baseline 1.55 (0.73, 3.67) nmol/L to 1.16 (0.19, 3.33) nmol/L; 0.75 (0.04, 4.01) nmol/L, and 0.51 (0.01, 2.71) nmol/L at the first, second, and third year in the high-GADA titer group respectively. In contrast, low-GADA titer group patients showed no significant changed from baseline 1.80 (0.48, 3.55) nmol/L to 1.40 (0.11, 2.88) nmol/L during the 3-year follow-up. In the type 2 diabetic group, the PCP levels also had no significant change from baseline 1.74 (0.42, 3.64) nmol/L to 1.17 (0.20, 3.37) nmol/L by the third year.

The average rate of decrease of FCP was 18.7% per year in the high-GADA titer group, 6.5% per year in the low-GADA titer group, and 4.8% per year in the type 2 diabetic group (Supplementary Fig. 1). The rate of decline of FCP and PCP per year was significantly higher in the high-GADA titer group ($P < 0.05$ for both) than in the low-GADA titer group and type 2 diabetic patients.

Because FCP >0.2 nmol/L was defined as residual β-cell function (22,23), we then further evaluated the percentage of individuals with FCP >0.2 nmol/L over time among the three groups. The patients with residual β-cell function decreased from 100% at baseline to 72% at the first year, to 48% at the second year, and to 42% at the third year in high-GADA group. In contrast, residual β-cell functions by the third year were maintained in more patients in the other two groups (90% with low GADA titers and 96% with type 2 diabetes; $P < 0.01$ for both compared with the high-GADA titer group) (Fig. 2). Metformin could protect human pancreatic islets from the lipotoxicity (24,25). That early insulin treatment could protect β-cell function of LADA patients was also reported (26,27). At entry, more patients in the

high- and low-GADA titer LADA groups, compared with the type 2 diabetic group, were taking insulin, and fewer patients were taking metformin. At the third year, the difference in the proportion of patients with insulin therapy between low-GADA titer and type 2 diabetic groups was not significant (11 of 27 in the type 2 diabetic group and 29 of 39 in low-GADA titer group, as reported in Supplementary Table 1). Despite greater use of insulin and less use of metformin in the former, the rate of decline of β-cell function did not differ significantly between the low-GADA titer group and the type 2 diabetic group after adjustment for the different use of drugs (by ANCOVA, $P > 0.05$).

We analyzed the GADA level after the 3-year follow-up, as summarized in Supplementary Table 2. GADA positivity persisted after 3 years in 92% with an initial high GADA titer but only 26% with a low GADA titer ($P < 0.01$).

In the high-GADA titer group, 83.4% of subjects (20 of 24) remained with high GADA titers, whereas only 8.3% of subjects (2 of 24) became low GADA titer, and 8.3% (2 of 24) became GADA negative. Although 74.4% of subjects (29 of 39) in the low-GADA titer group became GADA negative, only 20.5% (8 of 39) remained low-titer GADA positive, whereas 5.1% (2 of 39) became high-GADA titer positive.

We further analyzed the clinical features and proportions of changes according to GADA titers among LADA patients (Supplementary Table 3). Despite the lack of significant difference in sex, age, duration, BMI, HOMA-IR, and HbA$_{1c}$ between the GADA high-titer and low-titer groups, the PCP and ΔCP were both lower in the subjects with β-cell failure (defined as FCP <0.2 nmol/L, opposite to "with residual β-cell function"; $P < 0.05$). In addition, compared with patients without β-cell failure, the patients with β-cell failure had a higher GADA titer at entry and at the end of the third year (both $P < 0.01$), and a larger proportion of patients with higher GADA titer also showed persistent GADA positivity or continuous high GADA titers (both $P < 0.05$).

CONCLUSIONS

GADA titers are associated with the heterogeneity of clinical features and β-cell function in LADA patients. Our present

Table 2—General data changes among the three groups

	Type 2 diabetes			Low GADA titer			High GADA titer		
	12 months	24 months	36 months	12 months	24 months	36 months	12 months	24 months	36 months
BMI (kg/m^2)	24.8 ± 2.3	21.7 ± 8.5	22.8 ± 7.1	24.9 ± 2.8	25.2 ± 2.9	24.2 ± 4.9	24.1 ± 3.3	23.9 ± 3.2	23.8 ± 3.4
WHR	0.89 ± 0.06	0.88 ± 0.06	0.90 ± 0.05	0.91 ± 0.07	0.91 ± 0.07	0.91 ± 0.07	0.86 ± 0.07	0.88 ± 0.08	0.87 ± 0.07
HbA$_{1c}$, %	6.7 ± 1.0	6.7 ± 0.9	6.9 ± 1.0*	6.9 ± 0.9	6.8 ± 0.9	7.0 ± 1.1	6.9 ± 1.3	6.7 ± 1.2	7.2 ± 1.7
HbA$_{1c}$ (mmol/mol)	50 ± 12	50 ± 9	52 ± 12*	51 ± 9	51 ± 10	53 ± 12	52 ± 10	50 ± 11	55 ± 17
FCP (nmol/L)	0.43 (0.15, 0.98)	0.35 (0.19, 0.83)	0.36 (0.16, 0.77)*	0.50 (0.13, 1.29)	0.34 (0.11, 1.01)*	0.38 (0.04, 0.96)*	0.34 (0.01, 1.57)*	0.23 (0.01, 0.77)*#	0.13 (0.01, 0.90)*#
PCP (nmol/L)	1.39 (0.44, 4.03)	1.45 (0.45, 3.63)	1.17 (0.2, 3.37)*	1.56 (0.22, 3.64)	1.30 (0.18, 3.35)*	1.40 (0.11, 2.88)*	1.16 (0.19, 3.33)*	0.75 (0.04, 4.01)*	0.51 (0.01, 2.71)*#

Data are shown as mean ± SD or median (minimum, maximum). *$P < 0.05$ compared with baseline in each group. #$P < 0.05$ compared with type 2 diabetic group.
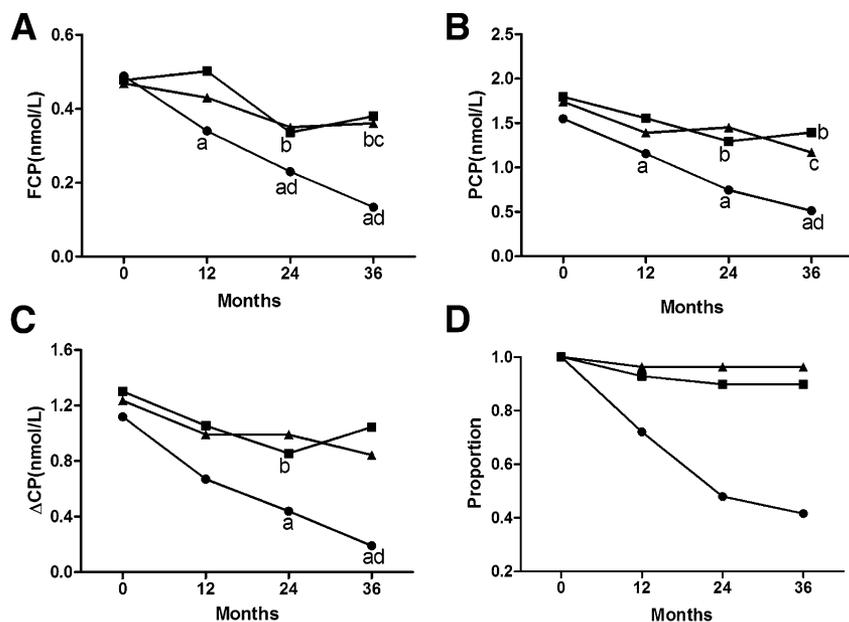
Figure 2—The levels of FCP (*A*), PCP (*B*), and ΔCP (*C*) concentrations in the high-titer GADA, low-titer GADA, and type 2 diabetic groups. ●, high-titer group; ■, low-titer group; ▲, type 2 diabetic group. High-titer group: [a]$P < 0.05$ compared with baseline, [d]$P < 0.05$ compared with type 2 diabetic group; low-titer group: [b]$P < 0.05$ compared with baseline; type 2 diabetic group: [c]$P < 0.05$ compared with baseline. *D*: Proportion of individuals with FCP >0.2 nmol/L over time.

results indicate that selected patients with a low GADA titer were metabolically similar to those GADA-negative type 2 diabetic patients. In this prospective study of Chinese diabetic patients with high and low GADA titers, we found a significant distinction between the two groups, with a more marked decline in FCP and PCP after 3 years of follow-up. In addition, high titer GADA tended to remain positive with high GADA titer in contrast to low titer GADA and was less evanescent. These results mirror previous smaller European studies, which showed the more aggressive nature of the disease, allied to GADA persistence, in those with "high levels" of GADA (28) and "high autoantibody affinity" (29). In this latter study, GADA affinity correlated strongly with GADA titer, and low affinity GADA was associated with prolonged preservation of FCP, as was low titer GADA in our present study. In LADA there is a broad range of progression in β-cell function, GADA titer, and GADA affinity. These three features have been shown to be positively associated both now in Chinese as well as previously in Europeans (29). It follows that there is an association between GADA and the destructive autoimmune effect.

These observations raise an important question about how LADA with low GADA titer differs from antibody-negative type 2 diabetes. Certainly, in metabolic terms, this present study found no difference in rates of disease progression between low-titer GADA patients and patients with type 2 diabetes. A recent small, but detailed, European study of β-cell function and insulin sensitivity also found no differences between LADA and type 2 diabetes (30). Also, in our present study, 74.4% of low-GADA titer patients became GADA negative versus only 8.3% for high-GADA titer patients, so low GADA titer is an unstable state that reflects a limited degree of autoimmune-mediated destruction, unrelated to progression to type 1 diabetes (29) or, indeed, only reflects the systemic inflammation characteristic of type 2 diabetes (31,32). It was reported that GADA could result from distinct immunization events, in which those children with high-affinity GADA and/or epitope specificity are at high risk of type 1 diabetes (33). Certainly, we have now found a similar pattern in Chinese adults in whom high GADA titers are maintained for a few years after diagnosis of diabetes associated with a decline in β-cell function, with similar results

reported in previous European studies (28,34).

Although our results clearly showed that low-GADA titer LADA had similar disease progression as type 2 diabetes, we cannot conclude that low-GADA titer LADA and type 2 diabetes can be equated, as previously proposed (18). First, in our previous larger study (16) we found that the total HLA DQ susceptibility haplotypes were higher in the low-GADA titer group compared with type 2 diabetes, whereas the total protective haplotypes were similar between the two groups. Second, low-titer GADA autoimmunity is associated with a markedly increased prevalence of autoimmune thyroid disease, especially among European men, and in contrast to type 2 diabetes (17). It will be important in further studies to dissect ethnic variation. According to the LADA China study, the frequency of GADA in China was similar to that in Europe, but with low GADA titer in 74% of the former compared with 26% of the latter (14,16).

Our current study has several strengths. First, it was a novel multicenter study performed in China as part of LADA China. Second, it was a prospective study that, for the first time, specifically sought distinctions between high- and low-GADA titer groups in terms of decline in β-cell function. Third, we excluded a series of confounding factors by ensuring similarity for them among the three groups, including age at onset of diabetes, disease duration, and BMI, but also with similar baseline glycemic control, residual β-cell function, and HLA-DQ genotypes. Because the low-GADA titer patients did not show an accelerated decline in β-cell function during the study period and were metabolically similar to patients with type 2 diabetes, it follows that it might be possible to treat them in the same way as type 2 diabetic patients.

The potential limitations of the current study are that the number of subjects was relatively small and the observation time was relatively short. Also, all patients in the low-GADA titer and type 2 diabetic groups were selected to be initially similar with those in the high-GADA titer group, which means that these patients could not truly represent all low-GADA titer or type 2 diabetic patients. A longer duration of study and a larger-scale

prospective study with subjects showing a wider range of clinical features is needed to confirm our observations.

References

1. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM. Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? Diabetes 2005;54 (Suppl. 2):S62–S67

2. Andersen MK, Lundgren V, Turunen JA, et al. Latent autoimmune diabetes in adults differs genetically from classical type 1 diabetes diagnosed after the age of 35 years. Diabetes Care 2010;33:2062–2064

3. Grant SF, Hakonarson H, Schwartz S. Can the genetics of type 1 and type 2 diabetes shed light on the genetics of latent autoimmune diabetes in adults? Endocr Rev 2010;31:183–193

4. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993;42:359–362

5. Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. Diabetes Care 2001;24:1460–1467

6. Brophy S, Davies H, Mannan S, Brunt H, Williams R. Interventions for latent autoimmune diabetes (LADA) in adults. Cochrane Database Syst Rev 2011:CD006165

7. Maruyama T, Tanaka S, Shimada A, et al. Insulin intervention in slowly progressive insulin-dependent (type 1) diabetes mellitus. J Clin Endocrinol Metab 2008;93:2115–2121

8. Andersen CD, Bennet L, Nyström L, et al. Worse glycaemic control in LADA patients than in those with type 2 diabetes, despite a longer time on insulin therapy. Diabetologia 2013;56:252–258

9. Cernea S, Buzzetti R, Pozzilli P. β-cell protection and therapy for latent autoimmune diabetes in adults. Diabetes Care 2009;32(Suppl. 2):S246–S252

10. Fourlanos S, Dotta F, Greenbaum CJ, et al. Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005;48:2206–2212

11. Jin P, Huang G, Lin J, et al. High titre of antiglutamic acid decarboxylase autoantibody is a strong predictor of the development of thyroid autoimmunity in patients with type 1 diabetes and latent autoimmune diabetes in adults. Clin Endocrinol (Oxf) 2011;74:587–592

12. Maruyama T, Nakagawa T, Kasuga A, Murata M. Heterogeneity among patients with latent autoimmune diabetes in adults. Diabetes Metab Res Rev 2011;27:971–974

13. Buzzetti R, Di Pietro S, Giaccari A, et al.; Non Insulin Requiring Autoimmune Diabetes Study Group. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 2007;30:932–938

14. Hawa MI, Kolb H, Schloot N, et al.; Action LADA consortium. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. Diabetes Care 2013;36:908–913

15. Lohmann T, Kellner K, Verlohren HJ, et al. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). Diabetologia 2001;44:1005–1010

16. Zhou Z, Xiang Y, Ji L, et al.; LADA China Study Group. Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. Diabetes 2013;62:543–550

17. Mahadeb YP, Gruson D, Buysschaert M, Hermans MP. What are the characteristics of phenotypic type 2 diabetic patients with low-titer GAD65 antibodies? Acta Diabetol 2014;51:103–111

18. Redondo MJ. LADA: time for a new definition. Diabetes 2013;62:339–340

19. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, et al.; Type 1 Diabetes Trial Net Research Group; European C-Peptide Trial Study Group. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 2008;31:1966–1971

20. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–1495

21. Wang JP, Zhou ZG, Lin J, et al. Islet autoantibodies are associated with HLA-DQ genotypes in Han Chinese patients with type 1 diabetes and their relatives. Tissue Antigens 2007;70:369–375

22. The DCCT Research Group. The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. Diabetes 1986;35:530–545

23. Quattrin T, Bélanger A, Bohannon NJ, Schwartz SL; Exubera Phase III Study Group. Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin therapy in patients with type 1 diabetes: results of a 6-month, randomized, comparative trial. Diabetes Care 2004;27:2622–2627

24. Lupi R, Del Guerra S, Fierabracci V, et al. Lipotoxicity in human pancreatic islets and the protective effect of metformin. Diabetes 2002;51(Suppl. 1):S134–S137

25. Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F. Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes 2000;49:735–740

26. Thunander M, Thorgeirsson H, Törn C, Petersson C, Landin-Olsson M. β-cell function and metabolic control in latent autoimmune diabetes in adults with early insulin versus conventional treatment: a 3-year follow-up. Eur J Endocrinol 2011;164:239–247

27. Maruyama T, Shimada A, Kanatsuka A, et al. Multicenter prevention trial of slowly progressive type 1 diabetes with small dose of insulin (the Tokyo study): preliminary report. Ann N Y Acad Sci 2003;1005:362–369

28. Niskanen LK, Tuomi T, Karjalainen J, Groop LC, Uusitupa MI. GAD antibodies in NIDDM. Ten-year follow-up from the diagnosis. Diabetes Care 1995;18:1557–1565

29. Krause S, Landherr U, Agardh CD, et al. GAD autoantibody affinity in adult patients with latent autoimmune diabetes, the study participants of a GAD65 vaccination trial. Diabetes Care 2014;37:1675–1680

30. Juhl CB, Bradley U, Holst JJ, Leslie RD, Yderstraede KB, Hunter S; Action LADA Consortium. Similar weight-adjusted insulin secretion and insulin sensitivity in short-duration late autoimmune diabetes of adulthood (LADA) and Type 2 diabetes: action LADA 8. Diabet Med 2014;31:941–945

31. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011;11:98–107

32. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004;27:813–823

33. Mayr A, Schlosser M, Grober N, et al. GAD autoantibody affinity and epitope specificity identify distinct immunization profiles in children at risk for type 1 diabetes. Diabetes 2007;56:1527–1533

34. Kasuga A, Maruyama T, Nakamoto S, Ozawa Y, Suzuki Y, Saruta T. High-titer autoantibodies against glutamic acid decarboxylase plus autoantibodies against insulin and IA-2 predicts insulin requirement in adult diabetic patients. J Autoimmun 1999;12:131–135