The heterogeneity of islet autoantibodies and the progression of islet failure in type 1 diabetic patients

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Type 1 diabetes mellitus is heterogeneous in many facets. The patients suffered from type 1 diabetes present several levels of islet function as well as variable number and type of islet-specific autoantibodies. This study was to investigate prevalence and heterogeneity of the islet autoantibodies and clinical phenotypes of type 1 diabetes mellitus; and also discussed the process of islet failure and its risk factors in Chinese type 1 diabetic patients. A total of 1,291 type 1 diabetic patients were enrolled in this study. Demographic information was collected. Laboratory tests including mixed-meal tolerance test, human leukocyte antigen alleles, hemoglobinA1c, lipids, thyroid function and islet autoantibodies were conducted. The frequency of islet-specific auto-antibody in newly diagnosed T1DM patients (duration shorter than half year) was 73% in East China. According to binary logistic regressions, autoantibody positivity, longer duration and lower Body Mass Index were the risk factors of islet failure. As the disease developed, autoantibodies against glutamic acid decarboxylase declined as well as the other two autoantibodies against zinc transporter 8 and islet antigen 2. The decrease of autoantibodies was positively correlated with aggressive beta cell destruction. Autoantibodies can facilitate the identification of classic T1DM from other subtypes and predict the progression of islet failure. As there were obvious heterogeneity in autoantibodies and clinical manifestation in different phenotypes of the disease, we should take more factors into consideration when identifying type 1 diabetes mellitus.

autoantibodies, heterogeneity, islet failure, type 1 diabetes

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a T-cell-mediated autoimmune disease in which insulin-producing beta cells in pancreatic islets of Langerhans are selectively destroyed, leading to insulin deficiency and dysregulation of glucose metabolism (Atkinson and Maclaren, 1994; Eisenbarth, 1986; Gillespie, 2006; Zhang et al., 2008). The incidence of diabetes highly varies in different countries, and this is probably related to genetic and environmental factors, such as nutrition or lifestyle (Patterson et al., 2001). It is estimated that 30%–50% of the genetic risk for type 1 diabetes can be attributed to the human leukocyte antigen alleles (HLA) region (Noble et al., 1996). The strongest genetic association for type 1 diabetes is HLA class II genes, while HLA class I alleles also influence susceptibility to T1DM and humoral autoimmunity. Autoantibodies against insulin (IAA), islet cell (ICA), glutamate decarboxylase (GADA), islet antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) might appear in patients months or even years prior to diagnosis of T1DM. The increase of the persistent islet-specific autoantibodies was positively correlated to the probability of developing disease (Bingley et al., 2006; Gale, 1994; Orban et al., 2009; Siljander et al., 2009; Verge et al., 1996; Ziegler, 2004). The identification and study of these autoantibodies associated with T1DM (Baekkeskov et al., 1990; Bonifacio et al., 1995; Bottazzo et al., 1974; Palmer et al., 1983; Wenzlau et al., 2007) have emphasized their roles as biomarkers in diagnosis (Wasserfall and Atkinson, 2006), prognosis, patient treatment stratification (Christie et al., 2002; Hagopian et al., 2011), tolerating therapies as well as providing insights into pathophysiology of the disease (Ludvigsson et al., 2008). Consequently, with highly sensitive laboratory assays, the autoantibodies positivity among European T1DM patients at diagnosis was nearly 98% (Wenzlau et al., 2007). However, the autoantibody assays were often negative in Asian T1DM patients. It might due to the unsensitive or unspecific assays, or testing far from diagnosis as antibody titers diminish, yet-to-be-identified auto-antigens, or foremost heterogeneity of islet

autoantibodies in Chinese population (Lu et al., 2012; Wang et al., 2007; Zhou et al., 2013).

There are many facets of diabetes with great heterogeneity. T1DM indeed represents a heterogeneous disease which manifests remarkable diversity in pathogenic processes, genetics and phenotypic characteristics. The diagnosis for T1DM in most epidemiological research depends on clinical characteristics, which are affected by phenotypic variation. Lower incidence of T1DM in China was reported and whether the wide variation disturbed the clinical diagnosis, especially in adult-onset type 1 diabetes, still not be elucidated. Therefore, we analyzed the heterogeneity of islet-specific autoantibodies; clinical characteristics and the progression of islet failure in Chinese cohort with 1,291 T1DM patients. We expect this will help the design of epidemiological study for type 1 diabetes in China and the customized medication in the future.

RESULTS

Combined detection of enzyme linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) improve the prevalence of islet autoantibodies in Chinese T1DM patients

The positivity of GADA, ZnT8A, and IA2A were 53.7%, 40.7% and 33.8% respectively among 1,206 patients (85 subjects of the whole sample had not been tested, Figure 1A). The autoantibody positivity of newly diagnosed diabetic patients (duration shorter than half a year) was 72.6%, which didn't show significant difference from the total sample (71.5%, $P=0.230$). Meanwhile, three antibodies (GADA, ICA, and IA2A) were detected by ELISA in 432 patients of whole subject and the frequency was 56.7%, 24.1% and 26.6%, respectively (Figure 1B). Apparently, RIA showed better sensitivity and efficacy than ELISA

(71.5% vs. 67.8% for at least 1 autoantibody positive, $P<0.001$, Figure S1 in Supporting Information). When taking these two methods combined, the sensitivity and efficacy were further improved ($P<0.001$, Figure 1C).

Islet function and islet-specific autoantibody positivity declined faster in classic T1DM compared with latent autoimmune diabetes of adult (LADA) patients

As shown in Figure 2, the prevalence of GADA, ZnT8A, IA2A decreased significantly in classic T1DM patients suffered longer than five years ($P=0.026$, $P=0.026$ and $P=0.005$ respectively, Figure 2A). Obviously, long-standing classic T1DM had lower fasting and stimulated C-peptide compared to those suffered shorter than five years ($P<0.001$ and $P=0.002$, Figure 2B). However, among LADA patients, neither the frequency of autoantibodies (ZnT8A, GADA and IA2A; $P=0.165$, $P=0.554$ and $P=0.712$ respectively, Figure S2A in Supporting Information) nor the fasting and stimulated C-peptide changed depending on the duration of diabetes ($P=0.342$ and $P=0.336$, Figure S2B in Supporting Information).

Comparison between autoantibodies-positive and auto-antibodies-negative patients

More female and short-duration patients were found in the autoantibodies-positive group, in comparison with the autoantibodies-negative group (Table 1, $P=0.042$ and $P=0.018$). Most importantly, both fasting C-peptide levels and area under the curve (AUC) of C-peptide were significantly lower in autoantibodies-positive patients ($P<0.001$ and $P<0.001$). Notably, GADA-positive patients were shown to have higher levels of high-density lipoprotein (HDL) ($P=0.005$), and tended to be leaner ($P=0.002$). Higher frequency of HLA gene haplotype HLA-A*11:01-DRB1*09:01 and HLA-A*11:01-DRB1*03:01 was found in GADA-

Table 1 Comparison between autoantibodies- positive and autoantibodies-negative patients[a]

Variable	ZnT8A		GADA		IA2A		*P* value		
	+	−	+	−	+	−	ZnT8A	GADA	IA-2A
n	486	707	640	551	408	798	−	−	−
Female-*n* (%)	254 (53.0)	328 (47.0)	332 (52.8)	251 (45.9)	212 (53.0)	372 (47.7)	0.042[**]	0.018[**]	0.085[**]
Age (years)	30.3±18.9	30.9±17.7	30.4±19.3	31.4±16.9	28.8±20.4	31.8±16.9	0.585[*]	0.339[*]	0.013[*]
Duration (months)	25.5±57.7	37.8±71.4	28.4±61.0	39.3±73.4	23.9±58.1	37.6±70.5	0.004[*]	0.016[*]	0.002[*]
Age of onset (years)	28.1±19.1	27.5±16.9	27.9±19.1	27.8±16.3	26.9±20.3	28.3±16.3	0.594[*]	0.887[*]	0.250[*]
BMI (kg m^{-2})	20.7±4.5	21.1±4.0	20.6±4.2	21.4±4.1	20.6±4.9	21.1±3.7	0.188[*]	0.002[*]	0.108[*]
MAP (mmHg)	89.3±12.2	90.9±12.3	88.4±11.8	92.7±12.3	89.1±12.2	90.8±12.2	0.042[*]	0.000[*]	0.034[*]
HDL (mmol L^{-1})	1.3±0.5	1.3±0.6	1.4±0.7	1.2±0.4	1.3±0.7	1.3±0.5	0.921[*]	0.005[*]	0.152[*]
HbA1C (%)	10.4±3.2	10.8±3.4	10.4±3.2	10.8±3.5	10.2±3.2	10.9±3.4	0.091[*]	0.163[*]	0.007[*]
FCP (pmol L^{-1})	183±163	250±224	180±164	278±235	180±157	245±221	<0.001[*]	<0.001[*]	<0.001[*]
AUC of C-peptide (pmol mL^{-2} min^{-2})	87.7±67.3	124.4±115.6	82.2±76.1	149.7±119.0	91.9±86.9	119.6±107.7	<0.001[*]	<0.001[*]	0.006[*]
HLA-A*11:01-DRB1*09:01 -*n* (%)	7 (8.1)	12 (6.7)	16 (10.3)	3 (2.7)	14 (12.0)	8 (4.5)	0.680[**]	0.018[**]	0.022[**]
HLA-A*11:01-DRB1*03:01 -*n* (%)	5 (5.7)	12 (6.6)	14 (8.9)	3 (2.6)	8 (8.9)	9 (4.9)	0.798[**]	0.035[**]	0.238[**]

a) Data are expressed as *n* (%) or mean ± SD. The statistical significance is determined by *, independent samples *t* test, **, χ^2 test. *P* value is indicated. FCP, fasting C-peptide.
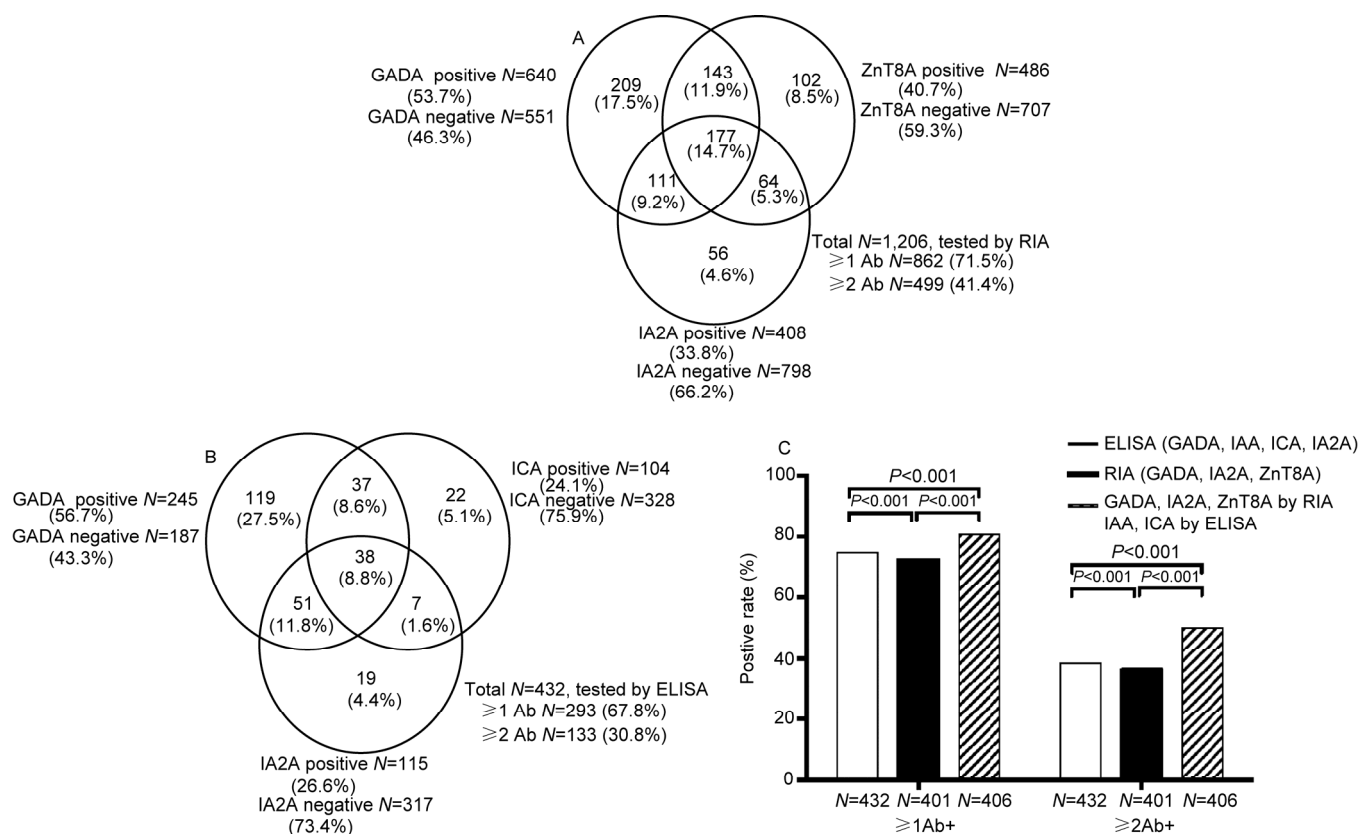
Figure 1 A and B, Positive rate of islet autoantibodies detected by RIA and ELISA respectively. C, Different positive rate for at least one islet antibody calculated by ELISA, RIA or combined ELISA and RIA. white bars=GADA, IAA, ICA, IA2A by ELISA; black bars=GADA, IA2A, ZnT8A by RIA; loxotic bars=GADA, IA2A, ZnT8A by RIA and IAA, ICA by ELISA.

positive patients (P=0.018 and P=0.035 respectively). Disappointedly, differences in clinical manifestation were not observed between autoantibodies-positive and autoantibodies-negative patients.

The frequency of autoantibodies was negatively correlated with islet function

Among 1,291 subjects, the autoantibodies of 1,232 subjects were determined by RIP or ELISA. According to Table 2, type 1 diabetes was heterogeneous. In autoantibody-positive group, LADA patients, compared with those classic T1DM patients, were older (P<0.001, Table 2), with higher body mass index (BMI) (P<0.001, Table 2) and had better islet function (P<0.001, Figure 3), higher levels of mean arterial pressure (MAP) (P<0.001, Table 2), low density lipoprotein (LDL) (P=0.001, Table 2) and lower hemoglobinA1c (bA1C) (P<0.001, Table 2). In addition to these parameters, they had lower incidence of viral infection (P=0.009, Table 2) and diabetic ketoacidosis (DKA) (P<0.001, Table 2). The frequency of ZnT8A, GADA, IA2A and ICA in LADA patients was between the 1Ab$^+$ and the 2Ab$^+$ group of classic T1DM patients (P<0.001, Table 2), whereas the frequency of IAA was as high as the 2Ab$^+$ group and significantly higher than the 1Ab$^+$ group (P<0.001, Table 2). Even among the classic T1DM patients, the clinical characteris-

tics are significantly divergent. The patients in Ab$^-$ group had islet function as good as in LADA patients (P=0.881, Figure 3). They had neither metabolic nor immune factors while patients in the 2Ab$^+$ group, showed the highest frequency of autoantibodies, the youngest age of onset (P<0.001, Table 2), the lowest islet function (P<0.001, Table 2), the lowest BMI (P<0.001, Table 2), the lowest MAP (P<0.001, Table 2) and LDL (P<0.001, Table 2).

Autoantibodies especially GADA were the risk factors of islet function

Compared to patients with better islet function, those with worse islet function were leaner (P<0.001, Table 3) and had a longer duration (P<0.001, Table 3), with more autoantibodies (P<0.001, Table 3) and higher frequency of autoantibodies (P<0.001, Table 3). Further analysis of logistic regression demonstrated that the duration and the autoantibodies were the risk factors to islet failure (Table 3, Table S1 in Supporting Information) while BMI was the protective factor (OR=0.796, P<0.001, Table 3). The positive rate of ZnT8A, IA2A and GADA were negatively correlated with level of fasting C-peptide (P<0.001, P<0.001, P<0.001, respectively, Table S3 in Supporting Information). However, no significant difference of the prevalence of ZnT8A was observed in the level of AUC of C-peptide

**Table 2**   Correlation between different phenotypes in T1DM patients[a]

Variable	2Ab$^+$	1Ab$^+$	Ab$^-$	LADA	*P* value
n	437	325	257	213	
Female-*n* (%)	224 (52.2)	150 (46.6)	112 (44.1)	115 (54.5)	0.059[*]
Age (year)	23.2±15.3	28.1±15.7	29.9±15.9	52.4±13.5	<0.001[*]
Duration (month)	24.0±57.6	41.3±72.9	38.2±79.4	32.5±49.8	0.007[*]
Age of onset (year)	20.3±13.5	23.5±14.5	26.5±14.2	51.1±13.9	<0.001[*]
BMI (kg m^{-2})	19.4±3.9	20.5±3.9	21.3±3.9	23.7±3.9	<0.001[*]
MAP (mmHg)	86.7±10.6	89.5±11.0	92.5±11.0	94.9±12.4	<0.001[*]
HDL (mmol L^{-1})	1.4±0.8	1.3±0.4	1.2±0.4	1.3±0.3	0.029[*]
LDL (mmol L^{-1})	2.6±0.9	2.8±1.2	2.8±1.1	2.9±0.9	0.010[*]
RBP (mg L^{-1})	30.8±16.7	29.4±18.6	31.6±14.8	43.2±23.8	<0.001[*]
BUN (mmol L^{-1})	21.6±203.2	6.4±18.0	4.7±2.0	6.0±2.6	0.608[*]
Cr (μmol L^{-1})	56.4±32.3	58.0±50.5	55.0±30.8	70.3±43.1	0.071[*]
Virus Infection -*n* (%)	47 (16.7)	33 (18.9)	29 (18.8)	3 (3.6)	0.009[**]
Ketosis Acid -*n* (%)	195 (55.1)	123 (55.7)	104 (58.1)	25 (26.6)	<0.001[**]
HbA1C (%)	11.2±2.9	11.2±3.2	11.5±3.7	8.5±2.9	<0.001[*]
ZnT8A -*n* (%)	305 (71.9)	78 (24.8)	0 (0)	103 (49.8)	<0.001[**]
GADA -*n* (%)	365 (84.9)	135 (43.3)	0 (0)	140 (67.3)	<0.001[**]
IA-2A -*n* (%)	269 (62.6)	42 (13.2)	0 (0)	97 (46.6)	<0.001[**]
ICA -*n* (%)	116 (39.1)	24 (11.3)	0 (0)	33 (29.5)	<0.001[**]
IAA -*n* (%)	85 (29.2)	20 (9.6)	0 (0)	39 (34.8)	<0.001[**]
HLA-A*30:01-DRB1*07:01 -*n* (%)	7 (6.0)	12 (16.7)	5 (9.1)	2 (4.8)	0.065[**]
HLA-A*24:02-DRB1*09:01 -*n* (%)	11 (9.8)	5 (7.0)	5 (9.1)	8 (21.1)	0.134[**]
HLA-A*11:01-DRB1*09:01 -*n* (%)	13 (11.6)	4 (5.6)	0 (0)	2 (5.4)	0.043[**]
HLA-A*11:01-DRB1*03:01 -*n* (%)	10 (8.8)	6 (8.2)	1 (1.8)	0 (0)	0.096[**]

a) Data are expressed as *n*(%) or mean±SD. *P* value, * is the result of non-parametric test or ANOVA test, ** is the result of χ^2 test. RBP, retinol-binding protein. BUN, blood urine nitrogen. Cr, creatinine.

Table 3   Correlation between different fasting C-peptide levels in T1DM[a]

| Variable | Levels of fasting C-P (pmol L^{-1}) | | | | *P* value | | |
	<70	70–170	170–320	>320	P^1	OR (95%CI)	P^2
n	215	212	208	203			
Age (years)	28.9 (26.4–31.4)	25.2 (23.0–27.4)	26.2 (24.0–28.5)	29.9 (27.8–32.0)	0.013[*]	Not tested	
Female-*n*(%)	112 (52.6%)	103 (48.8%)	84 (40.8%)	79 (39.1%)	0.015[**]	Not tested	
Age of onset (years)	23.0 (20.8–25.2)	23.2 (21.2–25.3)	23.9 (21.8–26.1)	28.2 (26.2–30.3)	0.002[*]	0.972 (0.958–0.987)	<0.001
Duration (months)	57.0 (43.8–70.2)	17.9 (10.8–25.3)	18.3 (11.8–24.9)	17.0 (11.5–22.5)	<0.001[*]	1.147 (1.082–1.216)	<0.001
BMI (kg m^{-2})	19.7 (19.1–20.2)	19.2 (18.7–19.7)	19.9 (19.3–20.5)	22.3 (21.7–23.0)	<0.001[*]	0.796 (0.743–0.852)	<0.001
LDL (mmol L^{-1})	2.6 (2.4–2.8)	2.7 (2.5–2.9)	2.8 (2.6–3.0)	3.0 (2.7–3.2)	0.171[*]	0.802 (0.575–1.119)	0.195
Cr (μmol L^{-1})	59.8 (50.0–69.6)	56.0 (50.4–61.6)	54.7 (49.9–58.4)	63.7 (55.4–72.0)	0.369[*]	0.997 (0.989–1.005)	0.452
BUN (mmol L^{-1})	8.3 (2.3–14.2)	28.2 (15.1–41.7)	8.4 (1.3–15.5)	5.0 (4.4–5.5)	0.553[*]	1.055 (0.929–1.198)	0.410
ZnT8 positive -*n* (%)	73 (37.6%)	93 (48.2%)	72 (38.3%)	44 (23.5%)	<0.001[**]	2.39 (1.449–3.942)	0.001
GAD positive -*n* (%)	119 (61.0%)	127 (65.5%)	111 (58.7%)	64 (34.8%)	<0.001[**]	2.505 (1.57–3.996)	<0.001
IA2 positive -*n* (%)	65 (32.8%)	77 (39.1%)	74 (38.7%)	32 (17.0%)	<0.001[**]	2.851 (1.656–4.91)	<0.001
Number of positive Ab≥1 -*n* (%)	160 (80.4%)	160 (80.0%)	139 (70.9%)	95 (50.0%)	<0.001[**]	3.058 (1.781–5.251)	<0.001
Number of positive Ab≥2 -*n* (%)	76 (38.2%)	96 (48.0%)	81 (41.3%)	35 (18.4%)	<0.001[**]	3.22 (2.0–5.185)	<0.001

a) Data are expressed as mean (95% CI range) or *n* (%). P^1, * is the result of non-parametric test or ANOVA test. ** is the result of χ^2 test; P^2 is the result of multiple factor logistic regression. For multiple factor logistic regression, analyses of BMI, LDL, Cr, BUN, ZnT8, GADA, IA-2, Number of positive Ab≥1 and Number of positive Ab≥2 were adjusted for Age of onset and duration.
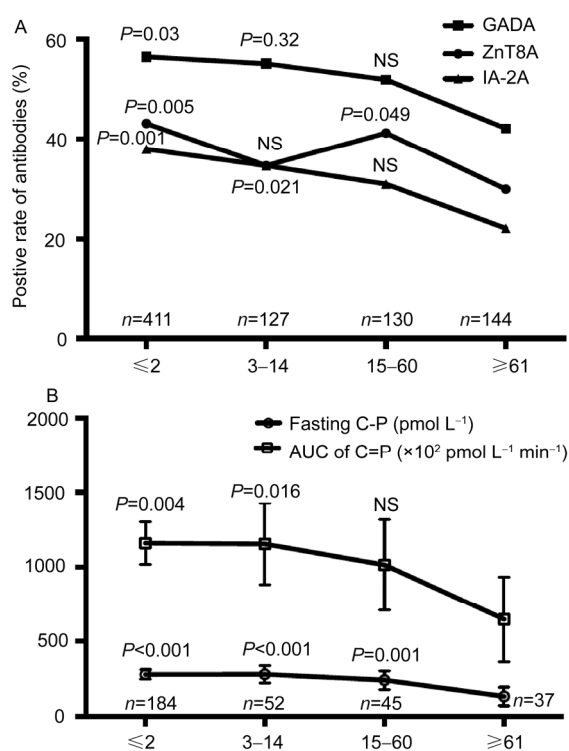
Figure 2 A, Heterogeneity of positive rate (%) of islet autoantibodies among different durations. B, Heterogeneity of islet function among different durations. black square=GADA; black circle=ZnT8A; black triangle=IA-2A; white square=AUC of C-peptide (×10² pmol L⁻¹ min⁻¹); white circle=Fasting C-peptide (pmol L⁻¹); bars=95% CI range. *P* value was indicated. (vs. the group with duration longer than 61 months). C-P, C-peptide; IA2A, islet antigen-2 autoantibody; NS, no statistical significance.
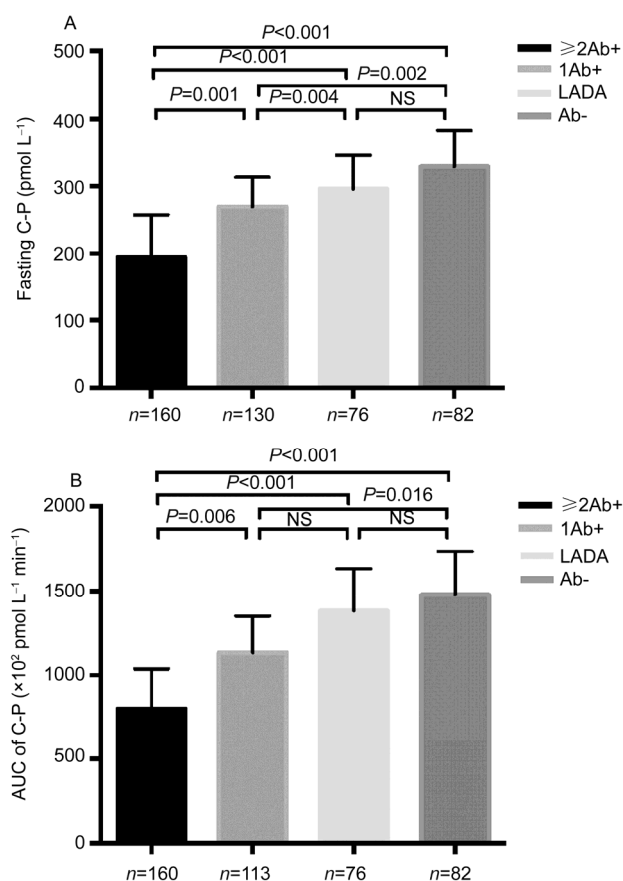
Figure 3 A, Heterogeneity of fasting C-peptide (pmol L⁻¹) among different clinical phenotypes. B, Heterogeneity of AUC of C-peptide (×10² pmol L⁻¹ min⁻¹) among different clinical phenotypes. bars=95% CI range. *P* value was indicated. Ab, antibody. NS, no statistical significance.

(*P*=0.097, Table S1 in Supporting Information).

Taken the clinical characteristics into consideration, we further evaluated the correlation factors of fasting C-peptide in different clinical phenotypes using Binary Logistic regression model (Table 4). It suggested that in 2Ab⁺, the patients with longer duration (OR=1.037, *P*=0.035, Table 4), haplotype of HLA-A*11:01-DRB1*09:01(OR=6.376, *P*=0.001, Table 4) were prone to have faster islet failing process. As for 1Ab⁺, GADA positivity was the risk factor of islet function (OR=2.678, *P*=0.032, Table 4), while ICA positivity was the protective factor (OR=0.188, *P*=0.046, Table 4). In Ab⁻ group, those with lower BMI (*P*=0.008, Table 4) were inclined to have worse islet function. Among the LADA patients, the risk factors were longer duration (OR=1.014, *P*=0.022, Table 4) and GADA positivity (OR=5.726, *P*=0.004, Table 4).

DISCUSSION

It was well known T1DM-associated autoantibodies against islet-specific autoantigens were not always detectable in all T1DM patients, however the prevalence of islet autoantibodies in Asian population was significantly lower than that

in Caucasian population. It might due to (i) unreliable assays with low sensitivity or specificity, (ii) longer duration with lower titer of antibodies, (iii) yet-to-be-identified auto-antigens, or foremost (iv) heterogeneity of islet autoantibodies among Chinese group (Ludvigsson et al., 2008). We detected the islet autoantibodies with the standardized assay, RIA, which may be both sensitive and efficacious. When considering the detective stability of IAA and ICA, we used ELISA instead of RIA. Combining these two methods together, the testing power was further improved. It suggested that the more autoantibodies detected by standard methods, the better results we could get. To investigate whether the frequency of autoantibodies declines along with the interval between disease onset and laboratory test, we further analyzed the samples from patients with durations shorter than six months, the results showed no significantly difference (73.0% vs. 71.5%, *P*=0.230). Above all, the yet-to-be-identified auto-antigens and the heterogeneity of islet autoantibodies among Chinese might have crucial impact on the race diversity of the prevalence of antibodies.

In the present cohort, there was no significant decrease of positivity for ZnT8A, IA2A and GADA within five years after diagnosis. This was in line with the observed pattern

Table 4　Correlation factors analysis of fasting C-peptide in different T1DM phenotypes[a]

Variable	Wald	OR (95%CI)	*P* value
LADA			
Duration	5.214	1.014 (1.002–1.027)	0.022
GADA	8.225	5.726 (1.738–18.871)	0.004
ZnT8	0.013	0.942 (0.339–2.616)	0.539
ICA	0.008	1.05 (0.369–2.989)	0.927
Ab$^-$-T1D			
Duration	3.068	1.008 (0.999–1.018)	0.080
BMI	7.026	0.819 (0.076–0.949)	0.008
DKA	2.977	0.441 (0.175–1.114)	0.083
1Ab$^+$T1D			
Duration	0.392	1.002 (0.997–1.007)	0.531
GADA	4.587	2.678 (1.087–6.596)	0.032
ZnT8	2.255	2.41 (0.764–7.6)	0.133
ICA	3.983	0.188 (0.036–0.97)	0.046
DKA	0.194	0.835 (0.373–1.865)	0.660
2Ab$^+$T1D			
Duration	4.429	1.037 (1.003–1.074)	0.035
HLA-A*11:01-DRB1*09:01	6.641	6.376 (1.558–26.087)	0.010
GADA	0.034	0.869 (0.197–3.824)	0.853
ZnT8	1.083	1.654 (0.641–4.268)	0.298
ICA	0.153	0.827 (0.319–2.413)	0.695

a) Data are expressed as mean (95% CI range). *P* value is the result of Binary Logistic regression. For LADA, the analyses of GADA, ZnT8, and ICA were adjusted for duration; For Ab$^-$T1D, the analyses of BMI and DKA were adjusted for duration; For 1Ab$^+$T1D, the analyses of GADA, ZnT8, ICA and DKA were adjusted for duration; For 2Ab$^+$T1D, the analyses of GADA, ZnT8, ICA and *HLA*-A*11:01-DRB1*09:01 were adjusted for duration.

(Nielsen et al., 2011). Among classic T1D patients, their autoantibodies declined significantly during five years after they were diagnosed. The mean age at which a patient was diagnosed with diabetes was 27.6±17.7 years, which was similar to the observation in the report, Tridgell et al. (Tridgell, 2011). They found that for individuals who were diagnosed with diabetes at age 14 or older, the prevalence for GADA changed little during the first five years after diagnosis, as we demonstrated. Whereas for IA2A, they found that the change in antibody frequency was similar despite age of diagnosis during the next eight years from onset of disease, which was different from our findings. In this study we had further demonstrated that autoantibody diminishing and islet failure in classic type 1 diabetic were faster than LADA.

To explore the heterogeneity of clinical phenotypes in Chinese T1DM, we compared our cohort with other T1DM studies. In contrast to the Sweden Study (Ludvigsson et al., 2013), the classic T1DM patients with autoantibody positivity in China had better islet function and higher frequency of GADA. Moreover, we found that HLA-A*11:01-DRB1*09:01was associated with 2Ab$^+$ patients.

Slight ethnic differences can be found in LADA patients between our data and the studies in the West (Hawa et al., 2013). For example, the age of onset is older and the level of LDL was higher in the East. BMI is the major difference between the Westerners and Asians.

Although one of the hallmarks of T1DM is the presence of one or several autoantibodies, it is well recognized that there is a subset of presumed T1DM patients who are auto-antibody-negative at diagnosis. The proportion of cases presented with DKA and the mean hemoglobin A1C (HbA1C) levels was higher in these autoantibody-negative subjects than that in USA (Gerard-Gonzalez et al., 2013). Meanwhile, the beta-cell function was similar to that of LADA without the probably metabolic protective factors, which might due to the absence of autoantibodies or the moderately destructive effect of yet-to-be identified autoantibodies.

Although the autoantibody was a risk factor to the loss of islet function, there were still some differences. While fasting C-peptide was closely correlated to glucagon-stimulated C-peptide, AUC seemed to be a more stable marker to indicate the stimulated islet function than fasting islet function (Gjessing et al., 1987; Besser et al., 2013). The different prevalence of GADA, ZnT8A, IA2A between fasting C-peptide and AUC could be explained by that GADA was associated with stimulated islet function, while ZNT8A, IA2A were associated with fasting islet function. This observation was in line with previous report, the patients with GADA had a more rapid decline in residual beta-cell function, while GADA negative patients were more likely to exhibit a remission phase (Mortensen et al., 2010).

There were further limitations to this study. First, the strongest genetic association for T1DM is HLA CLASS II alleles, especially HLA-DQA1 and DQB1. However, we only tested HLA-A and HLA-DR alleles under limited conditions in our laboratory, so we will further genotype HLA-DQA1 and DQB1 in the follow-up analysis. Besides, the GADA, IA2A and ZnT8A were not quantified with titer.

Second, not all patients conducted a 3-hour mixed-meal tolerance test at baseline. Indeed, they took a 2-hour mixed-meal tolerance test instead.

It was the first time to report the prevalence of type 1 diabetes in East China. It included 1,291 T1D patients from 22 hospitals in Jiangsu Province. We assessed GADA, IA2A and ZnT8A with RIA, and demonstrated their association with heterogeneity of islet failure and clinical phenotypes. Patients with one or several antibodies positivity reflected that they were suffered autoimmune attack to their pancreas. And sometimes it might occur before detection. As a result, their loss of beta cell function was significantly greater than those without autoantibodies.

MATERIALS AND METHODS

Subjects

Sera samples were obtained from 1,291 (49.3% female) Chinese T1DM patients who were identified and examined at 22 centers from 2009 to 2015. Individuals with T1DM were diagnosed according to the criteria of World Health Organization (WHO) and The American Diabetes Association (ADA). Definite T1DM requires that at least one of the following is present: (i) age less than 10 year at diagnosis; (ii) positive pancreatic autoantibodies at any time (GADA, IA-2A, ICA, or ZnT8A) or positive anti-insulin autoantibody at diagnosis only (within 10 d of starting insulin); or (iii) the presence of two or more of the following clinical indicators suggestive of T1DM: (1) age at diagnosis less than 40 year; (2) nonobese at diagnosis according to body mass index (< 95th percentile pediatric and < 30 kg m^{-2} adult); (3) diabetic ketoacidosis (DKA) at any time; (4) plasma C-peptide level below 0.8 ng mL^{-1} (with blood glucose > 80 mg dL^{-1} if available) at any time; and (5) family history of T1DM in a first-degree relative (parent, sibling, or child) (Beck et al., 2012). The mean age at onset and the mean duration of diabetes was 27.6±17.7 years (mean±SD) and 33.2±69.2 months, respectively. To further explore the impact of the autoimmune and clinical factors, we divided these patients into the following four subgroups: (i) classic T1DM patients with at least two autoantibodies positive (2Ab$^+$); (ii) classic T1DM patients with just one autoantibody positive (1Ab$^+$); (iii) classic T1DM patients with autoantibody negative (Ab$^-$); (iv) latent autoimmune diabetes of adult (LADA). The diagnosis of LADA is based on three criteria: (1) diagnosed over the age of 30 years; (2) the presence of circulating islet autoantibodies (GADA, IA-2A, ICA, or ZnT8A); and (3) lack of requirement of insulin for at least six months after diagnosis (Fourlanos et al., 2005). A written form was filled in for each patient including name, age, sex, age at onset, duration of diabetes, anthropometric data, clinical manifestation, birth weight, way of delivery, feeding pattern, family history of diabetes, known complications and other illnesses. Characteristics of study participants in the current study are given in Table 2. All subjects gave informed consent, and the protocol was approved by the local Ethics Committee and was carried out in agreement with the Declaration of Helsinki as revised in 2000.

Laboratory data

The hospital-based laboratory services (including blood glucose, HbA1c, lipids, C-peptide, insulin, islet autoantibodies) were available. Patients attended the centers in a fasted state and blood was taken into plain tubes. Lipids and glucose levels were measured on an automatic enzymatic analyzer (Beckman Coulter, USA), while islet autoantibodies (including ICA and IAA) were determined by ELISA (Euroimmun Medizinische Labordiagnostika AG, Germany; Biomerica, USA).

A mixed-meal tolerance test was performed after a time interval of treatment with insulin until the fasting blood glucose lower than 10.0 mmol L^{-1}. Plasma levels of glucose, insulin and C-peptide were measured at baseline and 30, 60, 120 and 180 min after mixed-meal consumption. Plasma insulin and C-peptide concentrations were measured by chemiluminescence (Roche Diagnostics, Switzerland). The 3-h C-peptide area under the curve (AUC) was calculated using the trapezoidal rule over the 3-h period (0–180 min).

Blood taken into the plain tubes were centrifuged at 350×*g* and stored frozen at −80°C. Later, RIA was used for detecting ZnT8A, GADA and IA2A as previously described (Gu et al., 2011). Briefly, human 35S-labeled recombinant antigens were produced in an *in vitro*-coupled transcription and translation system with SP6 (GAD, IA2) or T7 (ZnT8A) RNA polymerase and nuclease-treated rabbit reticulocyte lysate (Promega, USA). Sera (5 μL) were incubated with 35 S-antigens (⩾20 000 of TCA-precipitable radioactivity). After an overnight incubation at 4°C, antibody-bound 35 S-antigens were separated from unbound antigen by precipitation with Protein A Sepharose (GE Amersham Biosciences, USA). The immuno-precipitated radioactivity was counted on a Wallac Microbeta Liquid Scintillation Counter (Perkin Elmer Life and Analytical Sciences, USA).

Antibody levels were expressed as a relative immuno-precipitation index, which is defined as (sample-negative control)/(positive control−negative control). The cut-off for positivity for ZnT8A, GADA and IA2A was defined as a value above 0.015, 0.048 and 0.018 respectively, based on the 99th percentile of 102, 315 and 218 healthy control subjects (non-diabetic individuals without known autoimmune disease and no family history of diabetes). The GADA, IA2A, ZnT8 assays achieved a laboratory-defined sensitivity of 64%, 64% and 36%, with 97.8%, 100% and 97.8% specificity, respectively in Islet Antibody Standardization Program of 2013.

Statistical Analysis

Statistical analysis was performed using the SPSS program (version 21.0; SPSS, USA). Values are reported as means±SEM or means±SD. For categorical variables, differences were compared with the χ^2 or Fisher's exact test if the expected number of subjects in any cell was less than five. For continuous variables with normal distribution of the values, differences were tested using the Student's *t*-test or ANOVA test, while nonparametric tests were used for skewed data. Binary logistic regression and multivariate logistic regression analyses were used to explore the relationship between islet autoantibodies and islet function in patients with type 1 diabetes. *P* values <0.05 (two-tailed significance) were considered statistically significant.

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SUPPORTING INFORMATION

Figure S1   Different positive rate for at least one islet antibody calculated by ELISA or RIA.

Figure S2   A, Heterogeneity of positive rate (%) of islet autoantibodies among different durations in LADA patients. B, Heterogeneity of islet function among different durations in LADA patients.

Table S1   Correlation factors analysis of AUC of C-peptide levels in T1DM

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