

RESEARCH

Prevalence of antibodies targeting ubiquitin-conjugating enzyme 2L3 and eukaryote translation elongation factor 1 α 1 in Chinese Han and American Caucasian populations with type 1 diabetes

Li Qian^{1,2,*}, Yuxiao Zhu^{1,*}, Yan Luo¹, Mu Zhang¹, Liping Yu³, Yu Liu^{1,†} and Tao Yang^{1,2,†}¹Department of Endocrinology, Sir Run Run Hospital, Nanjing Medical University, Nanjing, Jiangsu, China²Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China³Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, Colorado, USACorrespondence should be addressed to T Yang or Y Liu: yangt@njmu.edu.cn or drluiyu@njmu.edu.cn

* (L Qian and Y Zhu contributed equally to this work as first authors)

† (T Yang and Y Liu contributed equally to this work as corresponding authors)

Abstract

We assessed the prevalence of two novel islet autoantibodies, those targeting ubiquitin-conjugating enzyme 2L3 (UBE2L3) and eukaryote translation elongation factor 1 α 1 (eEF1A1), in type 1 diabetes mellitus (T1DM) to evaluate their utility in T1DM diagnosis with comparison to other islet autoantibodies. We also aimed to determine whether age and ethnicity impacted their diagnostic value. Electrochemiluminescence assay was used to detect UBE2L3-Ab and eEF1A1-Ab in 193 Chinese Han and 570 American Caucasian subjects with T1DM, and 282 Chinese Han and 199 American Caucasian controls. In Chinese and American cohorts, the UBE2L3-Ab cut-off indices were 0.039 and 0.038, and the eEF1A1-Ab cut-off indices were 0.048 and 0.050, respectively. The prevalence of UBE2L3-Ab was significantly higher in the Chinese (9.33%) and American (3.86%) subjects with T1DM than in the controls ($P < 0.05$). The prevalence of UBE2L3-Ab in T1DM was significantly higher in Chinese than in American ($P < 0.05$). Albeit not statistically significant, the prevalence of UBE2L3-Ab in T1DM was slightly higher in children than in adults of both ethnicities. The differences in eEF1A1-Ab levels between subjects with T1DM and controls were not significant. Meanwhile, all American subjects with UBE2L3-Ab also harbored glutamic acid decarboxylase autoantibody (GADA) or insulin autoantibody (IAA). In contrast, 2.07% of the Chinese subjects with UBE2L3-Ab positive were previously classified as autoantibody-negative based on GADA and IAA. So the prevalence of UBE2L3-Ab in T1DM patients was significantly higher than in controls and was variable according to ethnicity as well as tended to be higher in children than adults. However, UBE2L3-Ab and eEF1A1-Ab may not be reliable diagnostic biomarkers for T1DM.

Key Words

- ▶ autoantibodies
- ▶ UBE2L3
- ▶ eEF1A1
- ▶ type 1 diabetes

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Introduction

Type 1 diabetes mellitus (T1DM) is well-defined as one of the most common and lifelong autoimmune diseases characterized by pancreatic β -cell destruction

(1, 2). Several autoantibodies, such as glutamic acid decarboxylase autoantibody (GADA), insulin autoantibody (IAA), insulinoma-associated protein-2

autoantibody (IA-2A), and zinc transporter-8 autoantibody (ZnT8A), are currently used as the most reliable serological biomarkers for the prediction and diagnosis of T1DM (3, 4, 5). Recently, two novel islet autoantibodies targeting ubiquitin-conjugating enzyme 2L3 (UBE2L3-Ab) and eukaryote translation elongation factor 1 α 1 (eEF1A1-Ab) were identified in the Korean population using a high-density protein microarray (6). The prevalence of UBE2L3-Ab and eEF1A1-Ab was 35.8 and 29.5%, respectively, in Korean T1DM by solid-phase ELISA (6). Of note, 40.9% of T1DM patients who lack GADA, the most prevalent autoantibody in T1DM, had UBE2L3-Ab and/or eEF1A1-Ab. The prevalence of these autoantibodies in other ethnicities is unclear. Given that solid-phase ELISA is not sufficiently sensitive for the detection of islet autoantibodies, we used an extensively validated electrochemiluminescence (ECL) assay methodology with high sensitivity and specificity (7) to evaluate the prevalence of UBE2L3-Ab and eEF1A1-Ab both in Chinese Han and American Caucasian subjects with T1DM in comparison with healthy controls.

Research design and methods

The quantity and characteristics of samples and patients

The ECL assay was used to detect UBE2L3-Ab and eEF1A1-Ab levels in serum samples from 2 independent subjects: 193 subjects with T1DM and 282 healthy controls recruited in Sir Run Run Hospital, Nanjing Medical University, Nanjing, China, and 570 subjects with T1DM and 199 healthy controls recruited in Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, Colorado, USA. The healthy controls were randomly divided into 2 subgroups, including 100 controls in China and 100 controls in the USA to establish cutoff values and 182 controls in China and 99 controls in the USA to compare the rate of autoantibodies positive between controls and subjects with T1DM. The mean ages of Chinese Han and American Caucasian subjects with T1DM were 26.2 ± 14.7 and 19.8 ± 15.1 years, respectively, whereas the mean ages of Chinese Han and American Caucasian controls (182 and 99 subjects, respectively) were 32.7 ± 15.6 and 18.9 ± 9.7 years, respectively. The inclusion criteria were as follows: clinical diagnosis of T1DM based on the 1999 World Health Organization criteria; insulin dependence at diagnosis; and positivity for at least one circulating islet autoantibodies, including GAD, IAA, IA-2, or ZnT8, based on RIA. To exclude the effect of exogenous

insulin, all patients were tested for IAA prior to insulin administration. All subjects with T1DM recruited in the present study had a disease duration within 1 year. In both cohorts, the inclusion criteria for healthy controls were as follows: fasting plasma glucose concentration < 6.1 mmol/L and 2-h postprandial glucose concentration < 7.8 mmol/L; no history of diabetes and autoimmune disease; and no first-degree relatives with diabetes mellitus.

All participants provided written informed consent. The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Ethics Committee of Nanjing Medical University and the University of Colorado School of Medicine. The laboratory tests and ECL assays were conducted in China and the USA for the Chinese Han and American Caucasian cohorts, respectively. The protocol and the materials for the ECL assay, including the antigens, were provided by the same vendor in both laboratories.

Anthropometric measurements, including height and weight, were performed by physicians. BMI was calculated by dividing weight in kilograms by height in meters, squared. Plasma glucose was assessed by the glucose oxidase method with the normal range of 3.9–6.1 mmol/L. HbA1c was measured by automated liquid chromatography (VARIANT II Hemoglobin Testing System, Bio-Rad), and the normal range was 4.0–6.0%.

ECL assay

The format of ECL-UBE2L3 and eEF1A1 assay was adapted from ECL-GADA assay (8). Briefly, 4 μ L of serum premixed with 16 μ L of PBS buffer was incubated with 20 μ L of antigen buffer containing both a sulfo-tag and biotin-labeled antigen proteins (either UBE2L3 or eEF1A1), and the mixture was shaken at room temperature on a low setting for 1–2 h, followed by incubation at 4°C overnight. On the same day, 150 μ L of 3% Blocker A (Meso Scale Diagnostics (MSD), Gaithersburg, MD, USA) were added to each well to block the streptavidin-coated plate (MSD) overnight at 4°C. On the second day, the blocked plate was washed three times with PBST buffer (PBS containing 0.05% Tween-20), followed by adding 30 μ L serum/antigen mixture to each well. After shaking at room temperature for 1 h on a low-speed setting, the plate was rewashed with PBST 3 times to remove unbound antigen proteins (either UBE2L3 or eEF1A1). Finally, the read buffer was added, and the plate was counted on an MSD Sector Imager 2400. Antibody values are shown as counts per second (CPS). The results are expressed as an index calculated using the following equation: index

value = (CPS (sample) – CPS (negative control)) / (CPS (monoclonal antibody) – CPS (negative control)).

Statistical analysis

All analyses were performed using SPSS software (version 21.0). Comparisons were performed by *t*-test or ANOVA. GraphPad Prism 5.0 software (GraphPad Software) was used for all graphical representations. Normally distributed data are shown as mean ± S.D., while variables with a skewed distribution are reported as median (25th–75th percentile) and log-transformed to approximate normality before analysis. Significance was accepted when $P < 0.05$.

Results

Clinical characteristics of T1DM patients and healthy controls in both ethnic cohorts

The mean age and BMI were comparable between the subjects with T1DM and controls in both Chinese Han and American Caucasian cohorts ($P > 0.05$). However, the fasting blood glucose and HbA1c levels were significantly higher and the C-peptide levels were significantly lower in the subjects with T1DM than in the controls ($P < 0.05$). In both ethnic cohorts, several subjects with T1DM had autoimmune thyroid disease (AITD) or celiac disease (CD), although without significance ($P > 0.05$). However, no autoimmune disease was found in healthy groups (Table 1).

The prevalence of UBE2L3-Ab and eEF1A1-Ab

With assay specificity 99% percentile of 100 healthy controls in Chinese Han and American Caucasian population, the cut-off indices for UBE2L3-Ab were 0.039 and 0.038, and the cut-off indices for eEF1A1-Ab were 0.048 and 0.050, respectively. The intra-assay coefficients of variation (CVs) and inter-assay CVs for UBE2L3-Ab

and eEF1A1-Ab ranged from 1.12 to 3.77% and from 2.71 to 7.91%, respectively. The UBE2L3-Ab was present in 9.33% (18/193) of patients with T1DM and in 0.55% (1/182; $P < 0.05$) of the healthy controls in Chinese Han population ($P < 0.005$). Conversely, the UBE2L3-Ab was present in 3.86% (22/570) of the subjects with T1DM and in 0.00% (0/99) of the controls in the American Caucasian cohort ($P < 0.005$). Meanwhile, the eEF1A1-Ab was detected in 0.52% (1/193) of the subjects with T1DM and in 0.00% (0/182) of the controls in Chinese Han cohort ($P > 0.05$). Finally, the eEF1A1-Ab was detected in 2.81% (16/570) of the subjects with T1DM and 2.02% (2/99) of the controls in American Caucasians ($P > 0.05$). As shown in Fig. 1 and Table 1, the prevalence of UBE2L3-Ab was significantly higher in the subjects with T1DM than in the controls. However, the prevalence of eEF1A1-Ab did not differ between the subjects with T1DM and the controls in either cohort.

The subjects with T1DM were divided into the following 2 groups, according to age at diagnosis: children (<18 years) and adults (≥ 18 years). The prevalence of UBE2L3-Ab was 10.96% (8/73) in children and 8.33% (10/120) in adults in the Chinese Han cohort, whereas the prevalence of UBE2L3-Ab was 4.46% (9/202) in children and 3.53% (13/368) in adults in the American Caucasian cohort. The prevalence of UBE2L3-Ab tended to be higher in children than in adults in both cohorts, albeit without statistical significance ($P > 0.05$ for all).

Comparison of the clinical characteristics of the subjects with T1DM based on the UBE2L3-Ab status

The subjects with T1DM were further divided into two subgroups according to the results of UBE2L3-Ab, namely UBE2L3-Ab positive (UBE2L3-Ab⁺) group and UBE2L3-Ab negative (UBE2L3-Ab⁻) group. As shown in

Table 1 Comparison of the clinical features of patients in both ethnic groups.

	American		China	
	Control	T1DM	Control	T1DM
Subjects number of sera	99	570	182	193
Gender (M:F)	43/46	251/319	93/89	83/110
Age (year)	18.9 ± 9.7	19.8 ± 15.1	32.7 ± 15.6	26.2 ± 14.7
BMI (kg/m ²)	22.93 ± 2.93	22.45 ± 3.78	21.17 ± 3.46	20.61 ± 4.98
Fasting blood glucose (mmol/L)	-	-	4.47 ± 0.50	13.96 ± 7.42
Fasting C-peptide (nmol/L)	-	-	0.47 ± 0.35	0.12 ± 0.16
HbA1c (%)	-	-	4.192 ± 1.71	10.53 ± 3.19
AITD	0	35 (6.14%)	0	17 (8.8%)
CD	0	32 (5.61%)	0	9 (4.66%)
Number of UBE2L3-Ab ⁺	0	22	1	18
Number of eEF1A1-Ab ⁺	2	16	0	1

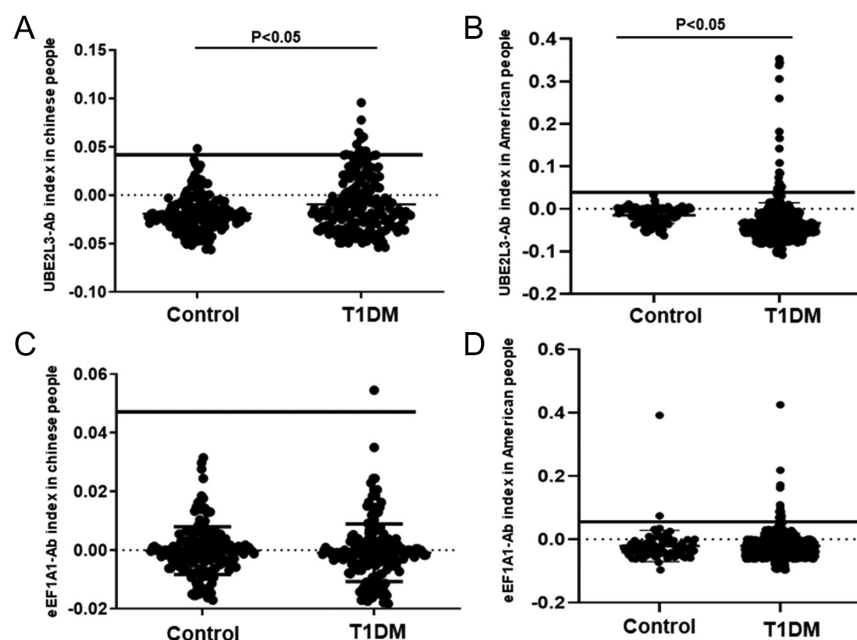


Figure 1
The prevalence of UBE2L3-Ab and eEF1A1-Ab in Chinese Han and American Caucasian people.

Table 2, BMI, age, and fasting C-peptide concentrations were not significantly different between the subjects with UBE2L3-Ab⁺ and subjects with UBE2L3-Ab⁻ ($P > 0.05$). The number of subjects with comorbid AITD or CD tends to be higher in those with UBE2L3-Ab⁺ than those with UBE2L3-Ab⁻, although the difference was not statistically significant ($P > 0.05$). In addition, the prevalence of GADA and/or IAA had no difference between UBE2L3-Ab⁺ and UBE2L3-Ab⁻ subgroups ($P > 0.05$).

Association of UBE2L3-Ab with GADA and IAA

Among the UBE2L3-Ab positive population, 21.05% (4/18) were positive for both GADA and IAA, 52.63% (10/18) were positive for either GADA or IAA, and 26.32% (4/18) were positive only for UBE2L3-Ab in the Chinese Han population. Thus, the proportion of subjects with T1DM who exhibited no evidence of autoimmunity declined from 24.35% (47/193) to 22.27% (43/193) when

UBE2L3-Ab status was added to the status for GADA and IAA. However, the subjects harboring UBE2L3-Ab overlapped 100% with those harboring GADA and IAA, indicating that UBE2L3-Ab did not exhibit diagnostic value in the American Caucasian cohort (Fig. 2).

Discussion

Islet autoantibodies play an essential role in the prediction and diagnosis of T1DM. The combination of four major autoantibodies (GADA, IAA, IA-2A, and ZnT8A) can diagnose 75–85% of the patients with T1DM (9). However, up to 15–25% of the patients with the clinical diagnosis of T1DM are negative for all currently known islet autoantibodies. Thus, the identification of novel islet autoantibodies and the development of accuracy detective assay are two main approaches that can improve the identification of patients with T1DM.

Table 2 Comparison of the clinical features of T1DM patients who were UBE2L3-Ab⁺ or UBE2L3-Ab⁻ in both ethnic groups.

	American		China	
	UBE2L3-Ab ⁺	UBE2L3-Ab ⁻	UBE2L3-Ab ⁺	UBE2L3-Ab ⁻
Subjects number of sera	22	548	18	175
Gender (M:F)	10/12	241/307	8/10	75/100
Age (year)	19.17 ± 13.36	19.98 ± 15.33	25.99 ± 14.15	26.30 ± 14.68
BMI (kg/m ²)	22.19 ± 3.16	22.56 ± 3.81	20.25 ± 4.27	20.73 ± 4.89
Fasting blood glucose (mmol/L)	-	-	13.75 ± 6.49	14.01 ± 7.40
Fasting C-peptide (nmol/L)	-	-	0.10 ± 0.14	0.13 ± 0.17
HbA1c (%)	-	-	4.29 ± 1.66	10.54 ± 3.17
AITD	3 (13.63%)	32 (5.84%)	3 (15.79%)	14 (8.04%)
CD	2 (9.09%)	30 (5.47%)	2 (10.52%)	7 (4.02%)

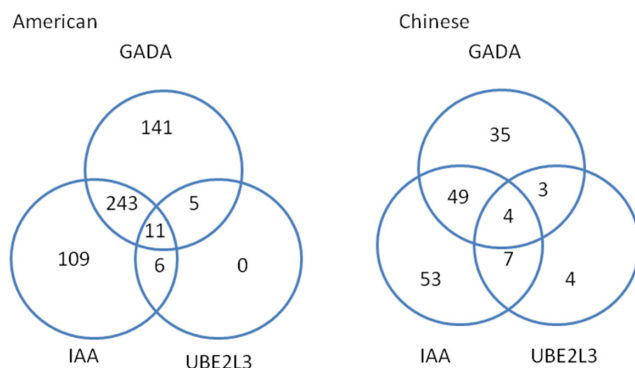


Figure 2
The Venn diagram shows the prevalence of UBE2L3-Ab association with GADA and IAA in American and Chinese cohorts.

Recent studies in Korea have reported UBE2L3-Ab and eEF1A-Ab as valid diagnostic markers for T1DM using solid-phase ELISA (6, 10). In the present study, we utilized a novel ECL assay to detect UBE2L3-Ab and eEF1A-Ab in Chinese Han and American Caucasian cohorts. Our analyses confirmed that the prevalence of UBE2L3-Ab was significantly higher in the subjects with T1DM than in healthy controls, although the eEF1A-Ab prevalence was not significantly different between subjects with T1DM and controls. However, in the present study, the prevalence of UBE2L3-Ab and eEF1A-Ab was lower in both the American Caucasian and Chinese Han cohorts compared to the recently reported Korean population. One of the previous studies (10) also reported that the prevalence of UBE2L3-Ab increased with decreasing age of disease onset and was only detected in people younger than 40 years. Consistent with the results of that study, we found that the prevalence of UBE2L3-Ab tended to be higher in children than in adults, although the difference was not statistically significant. However, two main factors are likely to explain these differences: first, solid-phase ELISA was used to detect UBE2L3-Ab and eEF1A-Ab in the Korean cohorts, whereas the present study utilized ECL for the detection of these two autoantibodies in the Chinese Han and American Caucasian cohorts. In contrast to ELISA, the ECL assay allows for a larger dynamic range with improved signal-to-noise ratio and exhibits excellent sensitivity and specificity for analytes with very low concentration. Several studies (7, 8, 11, 12, 13) have proved that ECL assays could discriminate high-affinity, high-risk diabetes-specific autoantibodies from low-affinity, low-risk autoantibodies and greatly improve sensitivity and disease specificity. Moreover, ECL assays, which capture all of the immunoglobulin classes (IgG, IgM, IgA, and IgE), are more sensitive than ELISA, which captures only

IgG. More importantly, previous studies have suggested that most of the single islet autoantibody detected only by ELISA are low-affinity antibody, perhaps resulting from immunization with a cross-reactive molecule. Second, patient ethnicity, age at onset, and disease course should be considered in the comparison of the studies: the prevalence of UBE2L3-Ab was higher in the Korean and Chinese cohorts, both Asian populations than in the American Caucasian cohort. Meanwhile, all subjects with T1DM in the present study had a disease duration within 1 year, whereas the disease duration of subjects with T1DM in the previous study varied from 1 to 14 years

UBE2L3 is involved in protein translation (14) and expressed in β -cells and skin, stomach, colon, kidney, lung, and the adrenal gland (15, 16, 17). UBE2L3 polymorphism amplifies NF- κ B activation and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases. Thus, UBE2L3 is associated with the increased risk of such autoimmune diseases as Crohn's disease (18), systemic lupus erythematosus (19), and rheumatoid arthritis (20). Furthermore, UBE2L3-Ab has also been detected in fulminant T1DM and Graves' disease (6). The present study excluded subjects with fulminant T1DM and those with latent autoimmune diabetes in adults. There were no significant differences in the rate of AITD and CD in the subjects with T1DM between the two ethnic cohorts evaluated in the present study. Thus, the co-existence of other autoimmune diseases might not be an essential reason for the difference in the prevalence of UBE2L3-Ab between the two ethnic cohorts included in the present study.

To further examine the diagnostic value of UBE2L3-Ab, we analyzed the association of UBE2L3-Ab with GADA and IAA. Our results showed that only 5 (2.59%) Chinese Han subjects and none of the American Caucasian subjects harbored UBE2L3-Ab alone. We also found that almost all subjects who were positive for UBE2L3-Ab were also positive for IAA or GADA. The number of subjects with single UBE2L3-Ab positive was further diminished if ZnT8A and IA-2A were included in the analysis. Based on these findings, we hypothesize that UBE2L3-Ab may come from epitope spreading, which is a typical phenomenon occurring during T1DM and other autoimmune diseases. This partly explained the prevalence of UBE2L3-Ab is significantly lower in newly diagnosed T1DM patients in the present study than T1DM patients whose disease course varied from 0 to 14 years included in the previous study (6). UBE2L3-Ab positivity might suggest that immune damage is still in progress and UBE2L3 might be considered as a potential

antigen-specific target for future immunotherapy. However, additional studies with larger populations are necessary to elucidate the function of UBE2L3 in beta-cell and to determine whether UBE2L3-Ab can be an effective indicator for β -cell immunity and a potential therapeutic target for T1DM.

In the present study, the subjects were not followed to determine the progression of autoimmunity, which was a limitation. Future studies with detailed functional outcomes and longitudinal cohorts are necessary to further elucidate the mechanisms underlying islet autoimmune destruction.

In conclusion, the prevalence of UBE2L3-Ab, although significantly higher in subjects with T1DM than in healthy controls, was still lower than the prevalence of GADA or IAA. Furthermore, almost all subjects with UBE2L3-Ab also harbored IAA or GADA. The prevalence of UBE2L3-Ab tended to be higher in children than in adults in both the American Caucasian and Chinese Han cohorts, although the difference was not statistically significant. The prevalence of eEF1A1-Ab was not significantly different between the subjects with T1DM and controls in both ethnic cohorts. Therefore, we found no evidence supporting UBE2L3-Ab and eEF1A1-Ab as reliable diagnostic biomarkers for T1DM.

Declaration of interest

The authors declare that there is no duality of interest associated with this manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contribution statement

Conception and design: Li Qian, Yuxiao Zhu, Tao Yang, Yu Liu; acquisition, analysis, and interpretation of data: Li Qian, Mu Zhang, Yan Luo, Liping Yu; drafting the work and revising: Li Qian, Yuxiao Zhu, Mu Zhang, Yan Luo; final approval: Tao Yang, Yu Li.

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