JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Association of *RPS26* gene polymorphism with different types of diabetes in Chinese individuals

Rong Song, Lingxiang Xie, Jin Ding, Yan Chen, Hailan Zou, Haipeng Pang, Yiman Peng, Ying Xia, Zhiguo Xie, Xia Li, Yang Xiao, Zhiguang Zhou, Jingyi Hu*

National Clinical Research Center for Metabolic Diseases, Key Laboratory of Diabetes Immunology (Central South University), Ministry of Education, and Department of Metabolism and Endocrinology, The Second Xiangya Hospital of Central South University, Changsha, Hunan, China

Keywords
Chinese Han population, Diabetes mellitus, Ribosomal protein S26

***Correspondence**
Jingyi Hu
Tel.: +86-731-8529-2154
Fax: +86-731-8536-7220
E-mail address:
hujingyi0066@csu.edu.cn

J Diabetes Investig 2024; 15: 34–43

doi: 10.1111/jdi.14117

ABSTRACT

Aims/Introduction: Different types of diabetes show distinct genetic characteristics, but the specific genetic susceptibility factors remain unclear. Our study aimed to explore the associations between the ribosomal protein S26 (*RPS26*) gene rs1131017 polymorphisms and susceptibility to type 1 diabetes mellitus, latent autoimmune diabetes in adults (LADA) and type 2 diabetes mellitus in the Chinese Han population, and their correlations with clinical features.

Materials and Methods: Genotyping of the rs1131017 variant was carried out for 1,006 type 1 diabetes mellitus patients, 210 LADA patients, 642 type 2 diabetes mellitus patients and 2,099 control individuals.

Results: We found that the rs1131017 C allele was a risk locus for both type 1 diabetes mellitus and LADA (odds ratio [OR] 1.50, 95% confidence interval [CI] 1.33–1.69, $P < 0.001$; OR 1.31, 95% CI 1.04–1.64, $P = 0.021$, respectively). Nevertheless, this association was not found for type 2 diabetes mellitus. Carrying the C allele genotype was associated with a lower postprandial C-peptide for type 1 diabetes mellitus (OR 1.41, 95% CI 1.11–1.80, $P = 0.006$) and lower fasting C-peptide for LADA (OR 1.55, 95% CI 1.01–2.38, $P = 0.047$). Interestingly, a lower GC frequency was noted for LADA than for type 1 diabetes mellitus, regardless of classification based on age at diagnosis, C-peptide or glutamic acid decarboxylase antibody positivity.

Conclusions: The *RPS26* polymorphism was associated with susceptibility and clinical characteristics of type 1 diabetes mellitus and LADA in the Chinese population, but was not related to type 2 diabetes mellitus. Thus, it might serve as a novel biomarker for particular types of diabetes.

INTRODUCTION

Diabetes is on the rise globally, posing a significant threat to human health due to its high disability and mortality rates[1]. It shows substantial clinical heterogeneity, encompassing various subtypes such as type 1 diabetes mellitus, latent autoimmune diabetes in adults (LADA) and type 2 diabetes mellitus. Recent evidence suggests substantial genetic differences among these diabetes subtypes. Genome-wide association studies (GWASs) have identified some susceptibility loci for type 1 diabetes mellitus[2], LADA[3] and type 2 diabetes mellitus[4]. However, these risk loci only partially overlap[3]. Notably, these studies primarily focused on white populations, whereas current research indicates significant genetic distinctions between the Chinese and white populations[2,5,6]. Therefore, it is essential to investigate genetic susceptibility loci for different diabetes subtypes in the Chinese population.

In recent years, there has been a gradual increase in genetic research related to diabetes in the Chinese population. The first

Received 10 October 2023; revised 7 November 2023; accepted 16 November 2023

GWAS of type 1 diabetes mellitus in the Chinese population identified two new type 1 diabetes mellitus risk susceptibility loci, BTN3A1 rs4320356 and GATA3 rs3802604[7]. The rare DR9/DR9 genotype, which is uncommon among white people, has been identified as a distinctive risk genotype for LADA in the Chinese population[8]. In the case of type 2 diabetes mellitus, current research has identified 56 new type 2 diabetes mellitus loci associated with East Asian diabetes patients, such as GRM8/PAX4 rs1177371187[9]. However, the genetic characteristics of different types of diabetes patients in the Chinese population are still not well elucidated.

The ribosomal protein S26 (*RPS26*) gene, which encodes a ribosomal protein, is implicated in various cellular processes, such as translation, endoplasmic reticulum (ER) stress, and the assembly and stability of ribosomal subunits[10]. Interestingly, research has found a correlation between the downregulation of ribosomal mitochondrial proteins and an increase in insulin content within β-cells[11]. Furthermore, existing research has established connections between T lymphocytes and various diabetes subtypes[12–14]. A previous study reported that the removal of *RPS26* from T lymphocytes disrupts their homeostasis and development[15], suggesting its potential involvement in diabetes pathogenesis.

Actually, *RPS26* has been identified as a risk locus for type 1 diabetes mellitus in several GWASs[16,17], and its single-nucleotide polymorphisms (SNPs), rs2292239[17], rs11171739[18] and rs1701704[19], have been recognized as markers for type 1 diabetes mellitus. The rs1131017 variant, situated in the untranslated region of the *RPS26* gene, shows linkage disequilibrium with rs10876864, rs11171739 and rs773125 (all $R^2 > 0.80$)[20]. Previous GWASs have associated these variants with vitiligo[21], type 1 diabetes mellitus in white people[18] and rheumatoid arthritis[22], respectively. Furthermore, rs1131017 has been identified as a lead SNP in a trans-acting regulatory region within T lymphocytes[20], affecting not only *RPS26* expression[23], but also the expression of genes associated with T lymphocyte activation and autoimmune disorders[24]. Although existing research suggests that the expression of the *RPS26* gene might not be the molecular trait directly responsible for susceptibility to type 1 diabetes mellitus in white people[23], the correlation between the rs1131017 polymorphism in the *RPS26* gene and susceptibility to type 1 diabetes mellitus and other diabetes subtypes in the Chinese population remains unexplored, and warrants further investigation.

Thus, in light of the heterogeneity among diverse types of diabetes and genetic variations across multiple ethnic groups, our research specifically focused on type 1 diabetes mellitus, LADA and type 2 diabetes mellitus patients in the comparatively understudied Chinese Han population. We aimed to elucidate the potential correlation between the rs1131017 polymorphism of the *RPS26* gene and susceptibility to different types of diabetes, as well as their clinical characteristics in the Chinese Han population.

MATERIALS AND METHODS

Participants

Our study included a total of 3,957 Chinese Han participants recruited from the Second Xiangya Hospital of Central South University, Changsha, Hunan, China, including 1,006 type 1 diabetes mellitus patients, 210 LADA patients, 642 type 2 diabetes mellitus patients and 2,099 control individuals. The inclusion criteria for each participant group are described here. All diagnoses for diabetes were required to adhere to the 1999 World Health Organization diabetes diagnosis criteria. Type 1 diabetes mellitus patients needed to meet specific criteria, including insulin dependence in the first 6 months and the presence of at least one designated islet autoantibody: glutamic acid decarboxylase antibody (GADA), protein tyrosine phosphatase antibody (IA-2A) and zinc transporter 8 antibody (ZnT8A). LADA patients were required to show insulin independence during the initial 6 months, and the presence of at least one of the GADA, IA-2A and ZnT8A antibodies at onset, and the age at diagnosis needed to exceed 18 years[25]. Type 2 diabetes mellitus patients also needed to show negative results for all three islet autoantibodies at onset[26]. Controls were defined as individuals without diabetes or a family history of diabetes within the same geographic area who had normal oral glucose tolerance test results[25]. The present study was approved by the Ethics Committee of the Second Xiangya Hospital of the Central South University, and was carried out according to the Declaration of Helsinki. All participants who were fully aware of this research signed an informed consent form.

Clinical features and biochemical measurements

In the present analysis of clinical characteristics, we divided type 1 diabetes mellitus patients into early-onset and late-onset groups based on the age of onset thresholds of 15 years[27], 18 years[25] and 20 years[28,29]. No age-based classification was applied for LADA and type 2 diabetes mellitus patients. Islet β-cell function was assessed using fasting C-peptide (FCP) and 2-h postprandial C-peptide (PCP) values. For autoimmune type 1 diabetes mellitus, FCP ≥200 pmol/L or PCP ≥400 pmol/L showed preserved β-cell function[30]. The FCP cutoff value of ≥200 pmol/L was also applied to define adequate β-cell function in previous studies of type 2 diabetes mellitus[31]. Thus, we chose the cutoff value of PCP ≥400 pmol/L to align with the previous studies. FCP, PCP and glycated hemoglobin levels were measured by automated liquid chromatography and chemiluminescence methods. Autoantibodies against pancreatic islets, including GADA, IA-2A and ZnT8A, were detected by radioligand binding assay[32].

SNP selection and genotyping

The main basis for selecting rs1131017 in *RPS26* was its reported association with other autoimmune diseases and its minor allele frequencies values >0.05 in Asian populations. Genomic DNA was extracted from peripheral blood using the

Genenode Genomic DNA Extraction Kit (Genenode Biotech Co. Ltd., Beijing, China), and stored at −80°C. Genotyping was carried out using the Illumina Asian Screening Array (San Diego, CA, USA).

Statistical analysis

All data are expressed either as the mean ± standard deviation (SD) or as the number of cases and constituent ratio. Differences in general information among type 1 diabetes mellitus, LADA, type 2 diabetes mellitus patients and controls were compared by different methods, including Student's *t*-test and ANOVA test for normal distribution, the Mann–Whitney *U*-test and Kruskal–Wallis *H*-test for non-normal distribution, and the χ^2-test for qualitative data. The frequencies of genotypes and alleles, and the genotype–phenotype associations were analyzed using χ^2-test and logistic regression, with odds ratio (OR) and *P*-values calculated. All statistical analyses were carried out using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). Two-sided *P*-values <0.05 were considered statistically significant.

RESULTS

Clinical and biochemical characteristics of cases and controls

DNA samples from 1,006 type 1 diabetes mellitus patients, 210 LADA patients, 642 type 2 diabetes mellitus patients and 2,099 control individuals were included in the present analysis. Clinical and biochemical characteristics are shown in Table 1. The four groups were matched in terms of sex (male/female). Both LADA and type 2 diabetes mellitus patients had a later age of onset than type 1 diabetes mellitus patients (both $P < 0.001$). Consistent with previous research, type 1 diabetes mellitus patients appeared to have the leanest body mass index, whereas type 2 diabetes mellitus patients had the highest body mass index values ($P < 0.001$). The lowest level of glycated hemoglobin was found in LADA patients, whereas type 2 diabetes mellitus patients had the highest level ($P < 0.001$). FCP and PCP, indicative of insulin secretion, were both lower in patients with type 1 diabetes mellitus than in those with LADA and type 2 diabetes mellitus (all $P < 0.001$). Compared with those in type 1 diabetes mellitus patients, the proportion of GADA positivity was higher, and the proportion of IA-2A and ZnT8A positivity was lower in LADA patients (all $P < 0.001$).

Frequency distributions of genotypes and alleles

We investigated the genotypic and allelic frequency distributions across various subgroups of participants by combined genetic models, including dominant, recessive, overdominant and additive models. The results are presented in Table 2. The genotypes and allele frequencies of type 2 diabetes mellitus patients were comparable to those of control individuals under all four genetic models. The results showed that compared with the G allele, the minor allele C increased the risk for type 1 diabetes mellitus and LADA (OR 1.50, 95% CI 1.33–1.69, $P < 0.001$; OR 1.31, 95% CI 1.04–1.64, $P = 0.021$, respectively). The CC genotype showed a more pronounced predisposition to type 1 diabetes mellitus than G allele carriers in all four genetic models (all $P < 0.05$). This pattern was replicated for LADA under the additive and recessive models (OR 1.30, 95% CI 1.04–1.62, $P = 0.022$; OR 2.01, 95% CI 1.23–3.28, $P = 0.005$, respectively). Furthermore, the C allele and CC genotype frequencies in type 1 diabetes mellitus patients surpassed those in type 2 diabetes mellitus patients across all genetic models (C allele: OR 1.51, 95% CI 1.28–1.77, $P < 0.001$; CC genotype: all $P < 0.05$). A similar trend was observed in LADA patients under the additive model (OR 1.30, 95% CI 1.02–1.67, $P = 0.036$) and the recessive model (OR 2.12, 95%

Table 1 | Clinical and biochemical characteristics of cases and controls

	Controls	T1DM	LADA	T2DM	*P*-value
Sample size	2,099	1,006	210	642	–
Sex (male/female)	1206/893	540/466**	120/90	384/258***	NS
Onset age (years)	–	24 ± 15	49 ± 13***	39 ± 4****.***	<0.001*
BMI (kg/m^2)	22.90 ± 3.11	19.09 ± 3.61**	22.38 ± 3.53***	24.97 ± 3.71**.****.***	<0.001*
FPG (mmol/L)	5.29 (4.98–5.60)	8.30 (5.97–12.80)**	8.54 (6.60–12.46)**	8.60 (6.78–12.09)**	<0.001*
PPG (mmol/L)	5.55 (4.82–6.53)	15.60 (10.80–20.80)**	14.64 (10.72–19.52)**	15.04 (11.23–19.62)**	<0.001*
HbA1c (%)	–	9.80 (7.63–12.68)	7.10 (6.10–9.10)***	10.75 (8.18–12.65)****.***	<0.001*
FCP (pmol/L)	–	79.00 (29.00–166.91)	460.60 (188.22–831.39)***	455.80 (287.43–795.05)****.***	<0.001*
PCP (pmol/L)	–	148.83 (52.65–324.55)	1,063.30 (578.60–2,176.00)***	1,113.95 (536.70–2,106.45)****.***	<0.001*
GADA positivity (%)	–	89.50	97.6***	0.00****.***	<0.001*
IA-2A positivity (%)	–	50.39	14.95***	0.00****.***	<0.001*
ZnT8A positivity (%)	–	34.82	11.54***	0.00****.***	<0.001*

*$P < 0.05$ was considered significant. **$P < 0.05$ other groups versus controls. ***$P < 0.05$ other groups versus type 1 diabetes mellitus. ****$P < 0.05$ other groups versus latent autoimmune diabetes in adults. BMI, body mass index; FCP, fasting C-peptide; FPG, fasting plasma glucose; GADA, glutamic acid decarboxylase antibody; HbA1c, glycated hemoglobin; IA-2A, protein tyrosine phosphatase antibody; LADA, latent autoimmune diabetes in adults; PCP, 2-h postprandial C-peptide; PPG, 2-h postprandial plasma glucose; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; ZnT8A, zinc transporter 8 antibody.

Table 2 | Genotype and allele frequencies of rs1131017 between cases and controls in the Chinese Han population

rs1131017	Controls (n = 2,099)	T1DM (n = 1,006)	LADA (n = 210)	T2DM (N = 642)	P-value	OR (95% CI)					
						T1DM *vs* controls	LADA *vs* controls	T2DM *vs* controls	LADA *vs* T1DM	LADA *vs* T2DM	T1DM *vs* T2DM
Allele											
G	3,258 (77.6)	1,405 (69.8)	305 (72.6)	998 (77.7)	<0.001*	1.50 (1.33–1.69)*	1.31 (1.04–1.64)*	NS	NS	1.32 (1.02–1.69)*	1.51 (1.28–1.77)*
C	940 (22.4)	607 (30.2)	115 (27.4)	286 (22.3)							
Dominant model											
GG	1,269 (60.5)	489 (48.6)	116 (55.2)	388 (60.4)	<0.001*	1.62(1.39–1.88)*	NS	NS	NS	NS	1.62(1.32–1.97)*
GC + CC	830 (39.5)	517 (51.4)	94 (44.8)	254 (39.6)							
Recessive model											
GG + GC	1,989 (94.8)	916 (91.1)	189 (90.0)	610 (95.0)	<0.001*	1.78 (1.33–2.37)*	2.01 (1.23–3.28)*	NS	NS	2.12 (1.19–3.76)*	1.87 (1.24–2.84)*
CC	110 (5.2)	90 (8.9)	21 (10.0)	32 (5.0)							
Overdominant model											
GG + CC	1,379 (65.7)	579 (57.6)	137 (65.2)	420 (65.4)	<0.001*	1.41 (1.21–1.65)*	NS	NS	0.72 (0.53–0.99)*	NS	1.40 (1.14–1.71)*
GC	720 (34.3)	427 (42.4)	73 (34.8)	222 (34.6)							
Additive model											
GG encode as 0	1,269 (60.5)	489 (48.6)	116 (55.2)	388 (60.4)	<0.001*	1.49 (1.33–1.69)*	1.30 (1.04–1.62)*	NS	NS	1.30 (1.02–1.67)*	1.51 (1.28–1.78)*
GC encode as 1	720 (34.3)	427 (42.4)	73 (34.8)	222 (34.6)							
CC encode as 2	110 (5.2)	90 (8.9)	21 (10.0)	32 (5.0)							

*P < 0.05 was considered significant. CI, confidence interval; LADA, latent autoimmune diabetes in adults; NS, not significant; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

CI 1.19–3.76, $P = 0.010$). When combining the overdominant model, a notable disparity in genotype frequencies was observed between type 1 diabetes mellitus and LADA patients, with a decreased occurrence of the GC genotype in LADA patients (OR 0.72, 95% CI 0.53–0.99, $P = 0.040$). In summary, these findings suggest that rs1131017 was connected with susceptibility to both type 1 diabetes mellitus and LADA, independent of type 2 diabetes mellitus susceptibility. Additionally, the C allele was considered a stronger risk factor for type 1 diabetes mellitus and LADA relative to the G allele.

Associations of clinical features in diabetes patients with rs1131017 genotypes

In addition, a comprehensive analysis of the correlation between the rs1131017 polymorphism and various clinical features in diabetes patients was carried out, such as age at diagnosis (Table 3), islet β-cell function (Table 4) and islet autoantibodies (Table 5).

As listed in Table 3, for the subgroups defined by age at onset (≥18 years and <18 years), we observed consistent and significant association between the rs1131017 CC genotype and early-onset type 1 diabetes mellitus and late-onset type 1 diabetes mellitus under all four classic genetic models (all $P < 0.05$). No significant heterogeneity of effects was observed between these two subgroups. In the overdominant model, the frequency of the GC genotype was higher in late-onset type 1 diabetes mellitus patients than in LADA patients (OR 0.70, 95% CI 0.50–0.97, $P = 0.030$). When comparing late-onset type 1 diabetes mellitus patients with type 2 diabetes mellitus patients, it was found that under all four genetic models, the GC and CC frequencies were higher in late-onset type 1 diabetes mellitus patients than in type 2 diabetes mellitus patients (all $P < 0.05$). Additionally, the CC frequency was higher in LADA patients than in type 2 diabetes mellitus patients under the additive model (OR 1.30, 95% CI 1.02–1.67, $P = 0.036$) and the recessive model (OR 2.12, 95% CI 1.19–3.76, $P = 0.010$). Furthermore, the results for the classification of type 1 diabetes mellitus according to the cutoff points of 15 years and 20 years confirmed the aforementioned findings (Table S1).

FCP levels were measured to evaluate β-cell function in patients with diabetes (Table 4). The C allele showed a negative trend for preserved FCP (FCP ≥200 pmol/L) in LADA patients (additive model, OR 1.55, 95% CI 1.01–2.38, $P = 0.047$), and patients carrying the GG genotype were found to be at markedly less risk for low FCP levels compared with those carrying the C allele (dominant model, OR 1.95, 95% CI 1.08–3.54, $P = 0.028$). However, no linkage was observed between FCP levels and the rs1131017 polymorphism in either type 1 diabetes mellitus or type 2 diabetes mellitus patients. In individuals with high C-peptide levels (FCP ≥200 pmol/L), LADA patients showed a lower GC frequency than type 1 diabetes mellitus patients (OR 0.61, 95% CI 0.39–0.95, $P = 0.029$), but there was no significant difference between LADA and type 2 diabetes mellitus patients. In addition, PCP results were also assessed (Table S2). Contrary to the aforementioned findings, an association between PCP levels and the rs1131017 polymorphism in type 1 diabetes mellitus patients was noted. Type 1 diabetes mellitus patients carrying the C allele genotypes showed lower PCP levels (additive model, OR 1.41, 95% CI 1.11–1.80, $P = 0.006$), but a relationship of PCP in LADA and type 2 diabetes mellitus patients with this polymorphism was not found.

Equally important, as detailed in Table 5 regarding GADA status, under the overdominant model, the GC frequency was lower in LADA patients who tested positive for GADA than in

Table 3 | Age at diagnosis stratified by RPS26 rs1131017 genotypes in Chinese patients with diabetes

rs1131017	T1DM (n = 1,006)		OR (95% CI)					
	Onset-age < 18	Onset-age ≥ 18	T1DM subgroups	T1DM Onset-age <18 *vs* Controls	T1DM Onset-age ≥ 18 *vs* Controls	LADA *vs* T1DM Onset-age ≥ 18	T1DM Onset-age ≥ 18 *vs* T2DM	LADA *vs* T2DM
Dominant model								
GG	202 (48.3)	287 (48.8)	NS	1.63 (1.32–2.02)*	1.60 (1.33–1.93)*	NS	1.60 (1.28–2.01)*	NS
GC + CC	216 (51.7)	301 (51.2)						
Recessive model								
GG + GC	374 (89.5)	542 (92.2)	NS	2.13 (1.47–3.07)*	1.53 (1.07–2.19)*	NS	1.62 (1.02–2.58)*	2.12 (1.19–3.76)*
CC	44 (10.5)	46 (7.8)						
Overdominant model								
GG + CC	246 (58.9)	333 (56.6)	NS	1.34 (1.08–1.66)*	1.47 (1.22–1.77)*	0.70 (0.50–0.97)*	1.45 (1.15–1.82)*	NS
GC	172 (41.1)	255 (43.4)						
Additive model								
GG encode as 0	202 (48.3)	287 (48.8)	NS	1.55 (1.32–1.83)*	1.45 (1.25–1.68)*	NS	1.47 (1.22–1.77)*	1.30 (1.02–1.67)*
GC encode as 1	172 (41.1)	255 (43.4)						
CC encode as 2	44 (10.5)	46 (7.8)						

*$P < 0.05$ was considered significant. CI, confidence interval; LADA, latent autoimmune diabetes in adults; NS, not significant; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 4 | β-Cell function stratified by *RPS26* rs1131017 genotypes in Chinese patients with diabetes

rs1131017	T1DM (n = 1,006)			LADA (n = 210)			T2DM (n = 642)			OR (95% CI)	
	Lower C-peptide levels (FCP <200)	Higher C-peptide levels (FCP ≥200)	OR (95% CI)	Lower C-peptide levels (FCP <200)	Higher C-peptide levels (FCP ≥ 200)	OR (95% CI)	Lower C-peptide levels (FCP <200)	Higher C-peptide levels (FCP ≥200)	OR (95% CI)	LADA *vs* T1DM (FCP ≥200)	LADA *vs* T2DM (FCP ≥200)
Dominant model											
GG	386 (47.8)	103 (52.0)	NS	28 (43.8)	88 (60.3)	1.95 (1.08–3.54)*	33 (66.0)	349 (60.3)	NS	NS	NS
GC + CC	422 (52.2)	95(48.0)		36 (56.3)	58 (39.7)		17 (34.0)	230 (39.7)			
Recessive model											
GG + GC	729 (90.2)	187(94.4)	NS	56(87.5)	133(91.1)	NS	48(96.0)	550(95.0)	NS	NS	NS
CC	79 (9.8)	11(5.6)		8(12.5)	13(8.9)		2(4.0)	29(5.0)			
Overdominant model											
GG + CC	465 (57.5)	114 (57.6)	NS	36 (56.3)	101 (69.2)	NS	35 (70.0)	378 (65.3)	NS	0.61 (0.39–0.95)*	NS
GC	343 (42.5)	84 (42.4)		28 (43.8)	45 (30.8)		15 (30.0)	201 (34.7)			
Additive model											
GG encode as 0	386 (47.8)	103 (52.0)	NS	28 (43.8)	88 (60.3)	1.55 (1.01–2.38)*	33 (66.0)	349 (60.3)	NS	NS	NS
GC encode as 1	343 (42.5)	84 (42.4)		28 (43.8)	45 (30.8)		15 (30.0)	201 (34.7)			
CC encode as 2	79 (9.8)	11 (5.6)		8 (12.5)	13 (8.9)		2 (4.0)	29 (5.0)			

*P < 0.05 was considered significant. CI, confidence interval; FCP, fasting C-peptide; LADA, latent autoimmune diabetes in adults; NS, not significant; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 5 | Autoantibodies status stratified by RPS26 rs1131017 genotypes in Chinese patients with diabetes

rs1131017	T1DM (n = 1,006)			LADA (n = 210)			OR for LADA *vs* T1DM	
	GADA (–), n = 106	GADA (+), n = 900	OR (95% CI)	GADA (–), n = 5	GADA (+), n = 205	OR (95% CI)	GADA (–)	GADA (+)
Dominant model								
GG	60 (56.6)	429 (47.7)	NS	4 (80.0)	112 (54.6)	NS	NS	NS
GC + CC	46 (43.4)	471 (52.3)		1 (20.0)	93 (45.4)			
Recessive model								
GG + GC	99 (93.4)	817 (90.8)	NS	5 (100.0)	184 (89.8)	NS	NS	NS
CC	7(6.6)	83(9.2)		0(0.0)	21(10.2)			
Overdominant model								
GG + CC	67 (63.2)	512 (56.9)	NS	4 (80.0)	133 (64.9)	NS	NS	0.71 (0.52–0.98)*
GC	39 (36.8)	388 (43.1)		1 (20.0)	72 (35.1)			
Additive model								
GG encode as 0	60 (56.6)	429 (47.7)	NS	4 (80.0)	112 (54.6)	NS	NS	NS
GC encode as 1	39 (36.8)	388 (43.1)		1 (20.0)	72 (35.1)			
CC encode as 2	7 (6.6)	83 (9.2)		0 (0.0)	21 (10.2)			

*P < 0.05 was considered significant. CI, confidence interval; GADA, glutamic acid decarboxylase antibody; LADA, latent autoimmune diabetes in adults; NS, not significant; T1DM, type 1 diabetes mellitus.

type 1 diabetes mellitus patients (overdominant model, OR 0.71, 95% CI 0.52–0.98, P = 0.037). There was no correlation between the rs1131017 polymorphism and type 1 diabetes mellitus, LADA or GADA-negative patients. No significant differences between patient subgroups were observed with respect to IA-2A and ZnT8A (Table S3).

DISCUSSION

To our knowledge, the present study is the first to focus on the link between the *RPS26* gene polymorphism and type 1 diabetes mellitus, LADA, and type 2 diabetes mellitus in the Chinese Han population. We unequivocally confirm that rs1131017 in *RPS26* is not only a risk factor for type 1 diabetes mellitus, but also a novel susceptibility locus for LADA. However, this variant does not pose a risk for type 2 diabetes mellitus, which might help distinguish type 1 diabetes mellitus and LADA patients from clinically diagnosed type 2 diabetes mellitus. Another factor differentiating LADA from type 1 diabetes mellitus is the greater association of LADA with a lower GC genotype frequency.

Current studies on genetic susceptibility loci for diabetes predominantly focus on white populations. However, due to differences in genetic backgrounds among different ethnic groups, the applicability of these studies to other populations is limited. For instance, DR3 and DR4 are the most significant human leukocyte antigen haplotypes in white populations, but their prevalence is significantly lower in the Chinese population[8]. Furthermore, the 1858C > T of PTPN22, commonly observed in white patients with type 1 diabetes mellitus, is seldom found in Chinese populations and shows no association[33]. Regarding LADA, previous research has identified DR3/DR4 as a susceptibility genotype in white populations, whereas in Chinese populations it is DR9/DR9[8]. Additionally, HHEX polymorphisms increase the risk of type 2 diabetes mellitus in Asian and white populations, but not in Indian or African American populations[34]. These findings underscore the need for more inclusive and diverse genetic studies in diabetes research.

RPS26 is located on chromosome 12q13.2 and encodes a highly conserved ribosomal protein[10,35,36]. The RPS26 protein is situated on the cytoplasmic side of the rough ER membrane. It is part of the ribosomal complex and participates in protein translation. Recent research has suggested that ER stress can lead to impaired β-cell function in type 1 diabetes mellitus[37,38]. Furthermore, compared with individuals without diabetes, individuals with type 1 diabetes mellitus show higher levels of ER stress[39]. Additionally, during the autoimmune reaction observed in type 1 diabetes mellitus patients, there is a loss of ER homeostasis and subsequent β-cell death[40]. This suggests that *RPS26* might play a role in the development and progression of type 1 diabetes mellitus through its involvement in ER stress. However, autoimmunity is correlated with the pathogenesis of both type 1 diabetes mellitus and LADA, and T lymphocytes are crucial for this process[41]. Previous studies have shown that *RPS26* is highly expressed in T lymphocytes, and that the ablation of *RPS26* in T lymphocytes disrupts peripheral T lymphocyte homeostasis and impairs T lymphocyte development in the thymus[15]. This role of *RPS26* in T lymphocyte development and homeostasis might also be a potential mechanism influencing autoimmune diabetes. Currently, *RPS26* has been identified as a susceptibility gene for type 1 diabetes mellitus in multiple large-scale GWASs in white populations[16,17], which was subsequently confirmed by studies integrating human and mouse genotype and expression data[17]. Nevertheless, there is a lack of studies on *RPS26* in LADA and type 2 diabetes mellitus, and the exact role of *RPS26* in type 1 diabetes mellitus remains to be shown.

Furthermore, the *RPS26* rs1131017 selected in the present study has been found to be in linkage disequilibrium with rs10876864, rs11171739 and rs773125 (all $R^2 > 0.80$ in Estonian population or Northern Europeans from Utah)[20]. These variants have previously been shown in several large GWASs to be relevant to various autoimmune diseases, such as vitiligo[21], type 1 diabetes mellitus in white people[18] and rheumatoid arthritis[22], respectively. The SNP, rs1131017, is localized in the 5′ oligopyrimidine channel of *RPS26*, which exerts a translational repressive effect[42]. The G/C allele affects the distribution of ribosomes, and the G allele can produce more protein with higher translation efficiency by interrupting an oligopyrimidine channel, as previously shown[43]. This effect might underlie the dissimilar functions of the G/C allele in type 1 diabetes mellitus and LADA patients, and thus, additional investigations are needed. In alignment with our earlier reference to the impact of *RPS26* on T lymphocytes, it has been established that rs1131017 is a prominent SNP of a trans-regulatory site that affects multiple genes in $CD4^+$ and $CD8^+$ T lymphocytes[20]. In T lymphocytes, rs1131017 can influence several genes connected with T lymphocyte activation and autoimmune disease[24], yielding a potential explanation for the linkage of this variant with type 1 diabetes mellitus and LADA.

In addition, we investigated the relationship between clinical features and rs1131017 genotypes in patients with diabetes. This polymorphism was found to feature variations in age at diagnosis, β-cell function, and GADA positivity across type 1 diabetes mellitus, LADA and type 2 diabetes mellitus patients. Specifically, the presence of the C allele was negatively correlated with PCP levels in type 1 diabetes mellitus patients and FCP levels in LADA patients. Of note, previous studies have shown that the genetic profile of LADA is akin to that of late-onset type 1 diabetes mellitus (European participants, age at diagnosis >30 years)[44]. However, differences in the *RPS26* polymorphism between LADA and late-onset type 1 diabetes mellitus were observed in the current research, regardless of whether type 1 diabetes mellitus was grouped by age at diagnosis of 15, 18 or 20 years. These differences might be attributed to variations in ethnicity and corresponding age groups. Therefore, the significance of *RPS26* in the Chinese autoimmune diabetes population is noteworthy. Concurrently, the present research indicates that, divergent from type 1 diabetes mellitus patients, individuals with LADA show elevated levels of GADA positivity and a lower prevalence of GC frequencies, inspiring us to further explore the distinctive mechanisms of the GC genotype of rs1131017 in the autoimmune processes of both type 1 diabetes mellitus and LADA.

Taking into consideration the heterogeneity of diabetes, the present study was carried out using a considerably large cohort of Chinese Han participants in Hunan Province, China. In addition, we analyzed the clinical features of diabetes patients with varying genotypes using different genetic models. Nevertheless, some limitations exist in the present study. First, the study primarily utilized samples from central China, and validation from a multicenter cohort is needed for comprehensive results. Second, as diabetes is a polygenic disease influenced by a combination of genes and the environment, this study lacked the exploration of interactions with other diabetes susceptibility loci. Finally, continued investigation is necessary to understand the underlying mechanisms of the impact of *RPS26* rs1131017 genotypes and the G/C allele on the pathogenesis and clinical features of diabetes.

In summary, the present study confirms that the SNP, rs1131017, of the *RPS26* gene is a genetic susceptibility locus for type 1 diabetes mellitus and LADA in the Chinese Han population, but it has no impact on susceptibility to type 2 diabetes mellitus. Our research provides evidence for the important role of *RPS26* in the genetic susceptibility of type 1 diabetes mellitus, offering a promising target for risk assessment and precise diagnosis of diabetes.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program of China (2018YFE0114500), the National Natural Science Foundation of China (81820108007, 82200933), the Natural Science Foundation of Hunan Province (2021JC0003, 2022JJ40689, 2022JJ40718) and the Natural Science Foundation of Changsha (kq2202404). The authors thank all the doctors, nurses and participants for their devotion to this work.

DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: This study was approved by the ethics committee of The Second Xiangya Hospital of Central South University, and carried out according to the principles of the Declaration of Helsinki in 1995 (as revised in Fortaleza, Brazil, October 2013).

Informed consent: The patients/participants provided their written informed consent to participate in this study.

Approval date of registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

REFERENCES

1. Zimmet P, Alberti KG, Magliano DJ, *et al*. Diabetes mellitus statistics on prevalence and mortality: Facts and fallacies. *Nat Rev Endocrinol* 2016; 12: 616–622.
2. Redondo MJ, Gignoux CR, Dabelea D, *et al*. Type 1 diabetes in diverse ancestries and the use of genetic risk scores. *Lancet Diabetes Endocrinol* 2022; 10: 597–608.
3. Cousminer DL, Ahlqvist E, Mishra R, *et al*. First genome-wide association study of latent autoimmune diabetes in adults reveals novel insights linking immune and metabolic diabetes. *Diabetes Care* 2018; 41: 2396–2403.
4. Laakso M, Fernandes SL. Genetics of type 2 diabetes: Past, present, and future. *Nutrients* 2022; 14: 3201.

5. Kumar A, de Leiva A. Latent autoimmune diabetes in adults (LADA) in Asian and European populations. *Diabetes Metab Res Rev* 2017; 33: 3201.
6. Fuchsberger C, Flannick J, Teslovich TM, *et al*. The genetic architecture of type 2 diabetes. *Nature* 2016; 536: 41–47.
7. Zhu M, Xu K, Chen Y, *et al*. Identification of novel T1D risk loci and their association with age and islet function at diagnosis in autoantibody-positive T1D individuals: Based on a two-stage genome-wide association study. *Diabetes Care* 2019; 42: 1414–1421.
8. Luo S, Lin J, Xie Z, *et al*. HLA genetic discrepancy between latent autoimmune diabetes in adults and type 1 diabetes: LADA China study No. 6. *J Clin Endocrinol Metab* 2016; 101: 1693–1700.
9. Spracklen CN, Horikoshi M, Kim YJ, *et al*. Identification of type 2 diabetes loci in 433,540 east Asian individuals. *Nature* 2020; 582: 240–245.
10. Belyy A, Levanova N, Tabakova I, *et al*. Ribosomal protein Rps26 influences 80S ribosome assembly in Saccharomyces cerevisiae. *mSphere* 2016; 1: e00109-15.
11. Maxel T, Smidt K, Petersen CC, *et al*. The zinc transporter Zip14 (SLC39a14) affects Beta-cell function: Proteomics, gene expression, and insulin secretion studies in INS-1E cells. *Sci Rep* 2019; 9: 8589.
12. Ahmed S, Cerosaletti K, James E, *et al*. Standardizing T-cell biomarkers in type 1 diabetes: Challenges and recent advances. *Diabetes* 2019; 68: 1366–1379.
13. Xia C, Rao X, Zhong J. Role of T lymphocytes in type 2 diabetes and diabetes-associated inflammation. *J Diabetes Res* 2017; 2017: 6494795–6494796.
14. Qin W, Sun L, Dong M, *et al*. Regulatory T cells and diabetes mellitus. *Hum Gene Ther* 2021; 32: 875–881.
15. Chen C, Peng J, Ma S, *et al*. Ribosomal protein S26 serves as a checkpoint of T-cell survival and homeostasis in a p53-dependent manner. *Cell Mol Immunol* 2021; 18: 1844–1846.
16. Todd JA, Walker NM, Cooper JD, *et al*. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 2007; 39: 857–864.
17. Schadt EE, Molony C, Chudin E, *et al*. Mapping the genetic architecture of gene expression in human liver. *PLoS Biol* 2008; 6: e107.
18. Rotival M, Zeller T, Wild PS, *et al*. Integrating genome-wide genetic variations and monocyte expression data reveals trans-regulated gene modules in humans. *PLoS Genet* 2011; 7: e1002367.
19. Zou F, Chai HS, Younkin CS, *et al*. Brain expression genome-wide association study (eGWAS) identifies human disease-associated variants. *PLoS Genet* 2012; 8: e1002707.
20. Kasela S, Kisand K, Tserel L, *et al*. Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. *PLoS Genet* 2017; 13: e1006643.
21. Tang XF, Zhang Z, Hu DY, *et al*. Association analyses identify three susceptibility loci for vitiligo in the Chinese Han population. *J Invest Dermatol* 2013; 133: 403–410.
22. Okada Y, Wu D, Trynka G, *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014; 506: 376–381.
23. Plagnol V, Smyth DJ, Todd JA, *et al*. Statistical independence of the colocalized association signals for type 1 diabetes and RPS26 gene expression on chromosome 12q13. *Biostatistics* 2009; 10: 327–334.
24. Li S, Schmid KT, de Vries DH, *et al*. Identification of genetic variants that impact gene co-expression relationships using large-scale single-cell data. *Genome Biol* 2023; 24: 80.
25. Xia Y, Chen Y, Li X, *et al*. HLA class I association with autoimmune diabetes in Chinese: Distinct implications in classic type 1 diabetes and LADA. *J Clin Endocrinol Metab* 2023; 108: e404–e414.
26. Heneberg P, Kocková L, Čecháková M, *et al*. Autoimmunity-associated PTPN22 polymorphisms in latent autoimmune diabetes of the adult differ from those of type 1 diabetes patients. *Int Arch Allergy Immunol* 2018; 177: 57–68.
27. Conde Barreiro S, Rodríguez Rigual M, Bueno Lozano G, *et al*. Epidemiology of type 1 diabetes mellitus in children in Spain. *An Pediatr (Barc)* 2014; 81: 189.e1–189.e12.
28. Brenu EW, Harris M, Hamilton-Williams EE. Circulating biomarkers during progression to type 1 diabetes: A systematic review. *Front Endocrinol (Lausanne)* 2023; 14: 1117076.
29. Hu J, Ding J, Li X, *et al*. Distinct signatures of gut microbiota and metabolites in different types of diabetes: A population-based cross-sectional study. *EClinicalMedicine* 2023; 62: 102132.
30. Li X, Cheng J, Huang G, *et al*. Tapering decay of β-cell function in Chinese patients with autoimmune type 1 diabetes: A four-year prospective study. *J Diabetes* 2019; 11: 802–808.
31. Fan B, Lim CKP, Poon EWM, *et al*. Differential associations of GAD antibodies (GADA) and C-peptide with insulin initiation, glycemic responses, and severe hypoglycemia in patients diagnosed with type 2 diabetes. *Diabetes Care* 2023; 46: 1282–1291.
32. Chen Y, Xia Y, Xie Z, *et al*. The unfavorable impact of DR9/DR9 genotype on the frequency and quality of partial remission in type 1 diabetes. *J Clin Endocrinol Metab* 2022; 107: e293–e302.
33. Pei Z, Chen X, Sun C, *et al*. A novel single nucleotide polymorphism in the protein tyrosine phosphatase N22 gene (PTPN22) is associated with type 1 diabetes in a Chinese population. *Diabet Med* 2014; 31: 219–226.
34. Cai Y, Yi J, Ma Y, *et al*. Meta-analysis of the effect of HHEX gene polymorphism on the risk of type 2 diabetes. *Mutagenesis* 2011; 26: 309–314.

35. Shi X, Huang X, Zhang Y, *et al*. Identification of a novel RPS26 nonsense mutation in a Chinese diamond-Blackfan anemia patient. *BMC Med Genet* 2019; 20: 120.
36. Liu XM, Yan MQ, Ji SY, *et al*. Loss of oocyte Rps26 in mice arrests oocyte growth and causes premature ovarian failure. *Cell Death Dis* 2018; 9: 1144.
37. Andreone L, Fuertes F, Sétula C, *et al*. Compound a attenuates proinflammatory cytokine-induced endoplasmic reticulum stress in beta cells and displays beneficial therapeutic effects in a mouse model of autoimmune diabetes. *Cell Mol Life Sci* 2022; 79: 587.
38. Chen CW, Guan BJ, Alzahrani MR, *et al*. Adaptation to chronic ER stress enforces pancreatic β-cell plasticity. *Nat Commun* 2022; 13: 4621.
39. Nakatani Y, Kaneto H, Hatazaki M, *et al*. Increased stress protein ORP150 autoantibody production in type 1 diabetic patients. *Diabet Med* 2006; 23: 216–219.
40. Zhang IX, Raghavan M, Satin LS. The endoplasmic reticulum and calcium homeostasis in pancreatic Beta cells. *Endocrinology* 2020; 161: bqz028.
41. Tan T, Xiang Y, Deng C, *et al*. Variable frequencies of peripheral T-lymphocyte subsets in the diabetes spectrum from type 1 diabetes through latent autoimmune diabetes in adults (LADA) to type 2 diabetes. *Front Immunol* 2022; 13: 974864.
42. Levy S, Avni D, Hariharan N, *et al*. Oligopyrimidine tract at the 5′ end of mammalian ribosomal protein mRNAs is required for their translational control. *Proc Natl Acad Sci USA* 1991; 88: 3319–3323.
43. Li Q, Makri A, Lu Y, *et al*. Genome-wide search for exonic variants affecting translational efficiency. *Nat Commun* 2013; 4: 2260.
44. Hernández M, Nóvoa-Medina Y, Faner R, *et al*. Genetics: Is LADA just late onset type 1 diabetes? *Front Endocrinol (Lausanne)* 2022; 13: 916698.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Age at diagnosis (thresholds of 15 and 20 years) stratified by RPS26 rs1131017 genotypes in Chinese patients with diabetes.

Table S2 PCP stratified by RPS26 rs1131017 genotypes in Chinese patients with diabetes.

Table S3 IA-2A and ZnT8A status stratified by RPS26 rs1131017 genotypes in Chinese patients with diabetes.