A Polymorphism in the Zinc Transporter Gene *SLC30A8* Confers Resistance Against Posttransplantation Diabetes Mellitus in Renal Allograft Recipients

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OBJECTIVE—Posttransplantation diabetes mellitus (PTDM) is a major metabolic complication in renal transplant recipients, and insulin secretory defects play an important role in the pathogenesis of PTDM. The R325W (rs13266634) nonsynonymous polymorphism in the islet-specific zinc transporter protein gene, *SLC30A8*, has been reported to be associated with type 2 diabetes and possibly with a defect in insulin secretion. This study investigated the association between genetic variations in the *SLC30A8* gene and PTDM in renal allograft recipients.

RESEARCH DESIGN AND METHODS—A total of 624 unrelated renal allograft recipients without previously diagnosed diabetes were enrolled. Rs13266634 was genotyped in the cohort, which consisted of 174 posttransplantation diabetic patients and 450 non-posttransplantation diabetic subjects. The genotyping of the SLC30A8 polymorphism was performed using real-time PCR.

RESULTS—The prevalence of PTDM was 33.8% in patients carrying the R/R genotype, 26.8% in patients with the R/W genotype, and 19.8% in patients with the W/W genotype. There was a strong association between the number of W-alleles and PTDM risk reduction (P for trend = 0.007). Patients with at least one T-allele showed a decreased risk of PTDM compared with those with the R/R genotype (R/W, risk ratio [RR] 0.78, $P = 0.126$; W/W, RR 0.52, $P = 0.007$). The effect of the SLC30A8 genotype remained significant after adjustments for age, sex, body weight gain, and type of immunosuppressant (R/W, hazard ratio [HR] 0.77, $P = 0.114$; W/W, HR 0.58, $P = 0.026$).

CONCLUSIONS—These data provide evidence that the *SLC30A8* rs13266634 gene variation is associated with protection from the development of PTDM in renal allograft recipients. ***Diabetes*** **57: 1043–1047, 2008**

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FPG, fasting plasma glucose; HOMA, homeostasis model assessment; PTDM, posttransplantation diabetes mellitus; SNP, single nucleotide polymorphism; ZnT, zinc transporter.

Posttransplantation diabetes mellitus (PTDM) is a serious metabolic complication and is associated with cardiovascular morbidity and mortality (1–3). Patients with PTDM have an increased risk for long-term cardiovascular events of up to 3.27 times compared with those without PTDM (4). The incidence of PTDM ranges from 2 to 53% (5). A number of risk factors for PTDM have been reported, including older age, ethnicity, obesity, family history of diabetes, cadaver donor, frequent acute rejection, hepatitis C infection, corticosteroid dose, and type of immunosuppressant therapy (2,6–12). However, there are few studies on genetic risk factors for the development of PTDM (13,14).

Although many studies have reported that insulin resistance is important to the pathophysiology of PTDM, a defect in insulin secretion may also play a role in the development of PTDM (10,15). Insulin is stored in pancreatic β-cells as zinc-insulin crystals. Zinc is believed to be an important component of the insulin secretion mechanism and may modulate insulin secretion (16–18). Zinc transporter (Znt)-8 , expressed only in pancreatic β-cells, is thought to be the β-cell zinc regulator (19–21). Recently, a polymorphism in the ZnT-8 gene (*SLC30A8*) has been associated with type 2 diabetes (22–25). This single nucleotide polymorphism (SNP), rs13266634, is a nonsynonymous SNP causing an amino acid change from arginine (R) to tryptophan (W) at position 325. We investigated the association between the *SLC30A8* polymorphism and the incidence of PTDM in renal allograft recipients.

RESEARCH DESIGN AND METHODS

A total of 805 unrelated transplant recipients were recruited from 1989 to 2007. PTDM was diagnosed according to American Diabetes Association criteria (26) at the 3rd month posttransplantation. Patients who were started on antidiabetic medication (oral medication or insulin) after transplantation and continued the medication thereafter were included in the posttransplantation diabetic group. The rest of the patients were assigned to the non-posttransplantation diabetic group. According to our previous study (15), cases of persistent PTDM (patients who developed diabetes within 1 year posttransplantation and remained diabetic) and late PTDM (patients who developed diabetes after 1 year posttransplantation) were assigned to the posttransplantation diabetic group. Transient PTDM cases (patients who developed diabetes within 1 year posttransplantation but eventually recovered to normoglycemia without medication) were classified as non-PTDM.

Patients were eligible to participate in the study if they were the recipients of a kidney allograft with no previous history of organ transplantation. Those with no previous diagnosis of diabetes and a recorded fasting plasma glucose (FPG) level <5.5 mmol/l were included. Patients were excluded if they had a history of diabetes before transplantation, severe metabolic or infectious

TABLE 1
Clinical characteristics of the study population

	PTDM	Non-PTDM	*P*
n (% women)	174 (35.1)	450 (35.6)	0.907*
Age at transplantation (years)	42.10 ± 8.99	35.42 ± 9.43	0.001
Family history of diabetes (%)	100 (57.5)	256 (56.9)	0.895*
Follow-up duration (months)	113.02 ± 58.53	100.36 ± 62.26	0.018
BW at transplantation (kg)	59.01 ± 9.69	58.76 ± 9.72	0.771
BW at 3 months after transplantation (kg)	59.58 ± 9.32	58.54 ± 8.74	0.205
BW at 6 months after transplantation (kg)	62.68 ± 8.83	60.87 ± 8.99	0.023
ΔBW during first 3 months after transplantation (kg)	−0.57 ± 4.83	−0.21 ± 4.29	0.061
ΔBW during first 6 months after transplantation (kg)	3.66 ± 5.76	2.12 ± 5.22	0.002
FPG at transplantation (mmol/l)	5.31 ± 1.35	5.16 ± 1.44	0.254
FPG at 3 months after transplantation (mmol/l)	6.90 ± 3.61†	5.34 ± 0.78	<0.001
FPG at 6 months after transplantation (mmol/l)	6.81 ± 3.18†	5.32 ± 0.73	<0.001
FPG at 12 months after transplantation (mmol/l)	6.93 ± 2.85†	5.41 ± 0.85	<0.001
Duration of dialysis (months)	17.48 ± 23.96	19.47 ± 29.98	0.449
No. of patients with acute rejection (%)	51 (29.3)	111 (24.7)	0.235*
No. of patients with tacrolimus use (%)	47 (27.0)	86 (19.1)	0.031*
Cr at 3 months after transplantation (mg/dl)	1.34 ± 0.43	1.36 ± 0.39	0.590
Cr at 6 months after transplantation (mg/dl)	1.31 ± 0.42	1.32 ± 0.34	0.749
Cr at 12 months after transplantation (mg/dl)	1.28 ± 0.42	1.33 ± 0.45	0.204

Data are means ± SD or *n* (%) unless otherwise indicated. BW, body weight; ΔBW, change in body weight. *P* values were calculated from *t* test. **P* values were calculated from the χ^2 test. †Included patients with antidiabetic medications.

disease, or a recorded FPG level ≥5.5 mmol/l. A total of 624 unrelated renal allograft recipients were enrolled in this study. Medical histories were obtained during the initial visit, and family history of diabetes refers to first-degree relatives only. Height and weight measurements were taken at the time of transplantation and at 3 and 6 months after transplantation. Blood samples were collected after an overnight fast. Frequency of patient follow-up and data collected at follow-up visits are shown in the online appendix (available at http://dx.doi.org/10.2337/db07-0761). Initial FPG level was measured on the day of transplantation using an enzymatic colorimetric assay. Insulin concentration was measured using a radioimmunoassay kit (Dainabot, Tokyo, Japan). Markers of insulin sensitivity and β-cell insulin secretory function were determined by the homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) (27). HOMA-IR was calculated as (fasting plasma insulin [μU/ml] × FPG [mmol/l])/22.5 and HOMA-β as (20 × fasting plasma insulin [μU/ml])/(FPG [mmol/l] − 3.5). The study protocol was approved by the ethics committee of the Yonsei University College of Medicine. All subjects were provided with adequate information about this study and gave informed consent.

Immunosuppression. The immunosuppressive regimens consisted of calcineurin inhibitors and glucocorticoids. Immunosuppressive regimens and schedules were as reported previously (15) (online appendix).

DNA extraction and SLC30A8 genotyping. Genomic DNA was isolated from peripheral blood lymphocytes. The genotyping of the *SLC30A8* polymorphism rs13266634 was done using the TaqMan fluorogenic 5′ nuclease assay (ABI, Foster City, CA). The final reaction volume for the PCR was 5 μl, containing 10 ng genomic DNA, 2.5 μl TaqMan Universal PCR Master Mix, and 0.13 μl 40× Assay Mix (assay ID C2684958_10). Thermal cycling conditions were as follows: 50°C for 2 min to activate the uracil *N*-glycosylase and prevent carry-over contamination, 95°C for 10 min to activate the DNA polymerase, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. All PCRs were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (ABI), and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System. Forty-eight duplicate samples and negative controls were included to ensure the accuracy of genotyping, and 100% of the duplicates replicated the original genotype. The genotype success rate was 99.48% for rs13266634.

Statistical analyses. The genotype frequencies were tested for Hardy-Weinberg equilibrium using χ^2 test. All continuous variables were expressed as means ± SD. Student's *t* test was used to compare continuous variables between the PTDM and non-PTDM posttransplantation diabetic groups. Pearson's χ^2 test was used to evaluate the difference in the incidence of diabetes between genotypes. ANOVA was used to compare continuous variables among genotypes. Mantel-Haenzel χ^2 test (*P* for trend) was used to evaluate the trend of diabetes protection with an increase in number of W-alleles. Cox proportional hazards modeling was used to identify risk factors for PTDM development and calculate the adjusted hazard ratio [HR] and 95% CIs. Diabetes-free survival was calculated from the date of transplantation to the date of PTDM diagnosis or last follow-up. Survival was estimated by Kaplan-Meier method and compared using the log-rank test. Patient baseline characteristics were assessed on transplant day. A *P* value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows software (version 12.0; SPSS, Chicago, IL). Power calculations were performed using PASS software version 2005 (NCSS Statistical Software, Kaysville, UT).

RESULTS

Clinical characteristics of posttransplantation diabetic patients. The overall incidence of PTDM in this study population was 27.9%. Baseline clinical characteristics of the recipients are shown in Table 1. The mean age of patients at transplantation was 38.7 ± 9.5 years. Patients in the posttransplantation diabetic group were older than those in the non-posttransplantation diabetic group (42.10 ± 8.99 vs. 37.42 ± 9.43 years old, $P = 0.001$). Posttransplantation diabetic patients were followed up for a significantly longer period than non-posttransplantation diabetic patients. Baseline mean body weight was 58.83 ± 9.71 kg, and although initial body weight was not significantly different between the two groups, there was a significant difference in the amount of weight gain during the first 6 months after transplantation between the two groups (Table 1). The duration of dialysis, incidence of acute rejection, and serum creatinine levels were not different between the groups (Table 1). Patients who used tacrolimus as an immunosuppressive agent had a higher incidence of PTDM ($P = 0.031$). There was no significant difference in steroid dose between the groups (online appendix).

Genotype distribution. The overall R-allele frequency was 0.577 and the overall W-allele frequency was 0.423. Overall genotype distributions were R/R, 33.17%; R/W, 49.04%; and W/W, 17.79%. Genotype distributions were in agreement with Hardy-Weinberg equilibrium ($\chi^2 = 0.013$, $P = 0.910$) (Table 2).

Association between the SLC30A8 rs13266634 genotype and PTDM. PTDM developed in 174 patients (27.2%). The W/W genotype conferred a reduced risk for PTDM

TABLE 2
Genotype distribution and corresponding RR for PTDM

			Genotype effect					
			Codominant model		Recessive model		Dominant model	
Genotype	PTDM	Non-PTDM	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*
R/R	70 (40.2)*	137 (30.4)	1					
R/W	82 (47.1)*	224 (49.8)	0.78 (0.57–1.07)	0.126				
W/W	22 (12.6)*	89 (19.8)	0.52 (0.32–0.84)	0.007	0.60 (0.38–0.94)	0.038	0.70 (0.52–0.96)	0.024

Data are *n* (%) unless otherwise indicated. The codominant model represents comparisons between R/R versus R/W genotypes and R/R versus W/W genotypes. The recessive model represents the comparison between the R/R plus R/W versus W/W genotypes. The dominant model represents the comparison of the R/R versus R/W plus W/W genotypes. *$P = 0.025$ when comparing three groups using χ^2 test and $P = 0.007$ when calculated from the Mantel-Haenzel χ^2 test (*P* trend test).

compared with the R/R genotype (risk ratio [RR] 0.52 [95% CI 0.32–0.83], $P = 0.007$; Table 2). The risk of PTDM was marginally lower in carriers of the R/W genotype than in R/R carriers (0.78 [0.57–1.07], $P = 0.126$, Table 2). The W-allele was associated with a decreased incidence of PTDM in codominant (R/R, 33.8%; R/W 26.8%; W/W, 19.8%; $P = 0.025$), dominant (R/R, 33.8%; R/W plus W/W, 24.9%; $P = 0.024$), and recessive (R/R plus R/W, 29.6%; W/W, 19.8%; $P = 0.038$) models (Table 2).

There was no difference among genotypes in age at transplantation, initial body weight, amount of body weight gain, FPG level (at baseline and at 3, 6, and 12 months after transplantation), duration of dialysis, percentage of tacrolimus use as an immunosuppressant, incidence of acute rejection, or serum creatinine levels (Table 3).

There was a linear increase in the HOMA-β value from the R/R genotype to the W/W genotype. Insulin secretory function, assessed by HOMA-β, was better preserved in patients with the W/W genotype than in those with the R/R genotype (Table 3). In contrast, no significant difference in HOMA-IR value according to genotype was observed (Table 3).

Independent risk factors of PTDM were identified using multiple Cox proportional hazard regression. We included age, sex, amount of body weight gain, type of immunosuppressant, and genotype as covariates in the multiple Cox proportional hazard regression model because they were associated with PTDM in univariate association tests (Table 1). As shown in Table 4, the *SLC30A8* genotype was a significant independent risk factor of PTDM together with age at transplantation and tacrolimus use as an immunosuppressant. The effect of the genotype remained significant after adjustments for age, sex, amount of weight gain, and type of immunosuppressant used (R/W, HR 0.77, $P = 0.114$; W/W, HR 0.58, $P = 0.026$). Age, amount of weight gain, and tacrolimus use were associated with PTDM development, but sex was not (Table 4).

The Kaplan-Meier curve for the development of PTDM is shown in Fig. 1. Patients with the W/W genotype had a significantly lower risk ($P = 0.007$) of developing PTDM than those with the R/R genotype.

TABLE 3
Characteristics of patients according to rs13266634 genotype

	R/R	R/W	W/W	*P*
n (% female)	207 (35.7)	306 (37.9)	111 (27.9)	0.168*
No. of PTDM patients (%)	70 (33.8)	82 (26.8)	22 (19.8)	0.025* 0.007†
Age at transplantation (years)	39.60 ± 9.71	38.34 ± 9.06	38.16 ± 10.41	0.269
Family history of diabetes (%)	119 (57.5)	172 (56.2)	65 (58.6)	0.901*
Follow-up duration (months)	103.66 ± 62.15	101.86 ± 59.71	109.94 ± 65.03	0.495
BW at transplantation (kg)	59.09 ± 9.22	58.70 ± 10.40	58.27 ± 8.31	0.891
BW at 3 months after transplantation (kg)	59.01 ± 8.71	58.91 ± 9.28	58.27 ± 8.31	0.762
BW at 6 months after transplantation (kg)	61.28 ± 8.91	61.59 ± 9.27	60.98 ± 8.34	0.819
ΔBW during first 3 months after transplantation (kg)	−0.09 ± 4.61	0.22 ± 4.30	−0.40 ± 4.63	0.430
ΔBW during first 6 months after transplantation (kg)	2.20 ± 5.32	2.88 ± 5.43	2.31 ± 5.55	0.338
FPG at transplantation (mmol/l)	5.32 ± 1.66	5.18 ± 1.35	5.05 ± 1.06	0.316
FPG at 3 months after transplantation (mmol/l)	5.97 ± 2.02	5.73 ± 2.46	5.53 ± 1.00	0.334
FPG at 6 months after transplantation (mmol/l)	5.74 ± 1.85	5.78 ± 2.12	5.49 ± 0.87	0.527
FPG at 12 months after transplantation (mmol/l)	6.00 ± 2.16	5.85 ± 1.80	5.47 ± 0.73	0.060
HOMA-IR at 12 months after transplantation	3.21 ± 1.45	3.02 ± 1.62	3.94 ± 1.99	0.304
HOMA-β at 12 months after transplantation	66.12 ± 45.35‡	112.41 ± 60.18§	188.32 ± 124.85	0.001
Duration of dialysis (months)	22.13 ± 31.64	16.40 ± 25.78	20.16 ± 35.90	0.079
No. of patients with acute rejection (%)	57 (27.5)	77 (25.2)	28 (25.2)	0.819*
No. of patients with tacrolimus use (%)	49 (23.7)	64 (20.9)	20 (18.0)	0.488*
Cr at 3 months after transplantation (mg/dl)	1.36 ± 0.43	1.33 ± 0.38	1.42 ± 0.38	0.099
Cr at 6 months after transplantation (mg/dl)	1.31 ± 0.38	1.31 ± 0.37	1.36 ± 0.30	0.517
Cr at 12 months after transplantation (mg/dl)	1.31 ± 0.50	1.30 ± 0.38	1.36 ± 0.49	0.481

Data are means ± SD or *n* (%). BW, body weight; ΔBW, change in body weight. *P* values were calculated from *t* test. **P* values were calculated from χ^2 test. †*P* value was calculated from the Mantel-Haenzel χ^2 test (trend *P* test). ‡$P = 0.001$ (post-hoc analysis between R/R and W/W genotypes). §$P = 0.016$ (post-hoc analysis between R/W and W/W genotypes).

TABLE 4
Multivariable Cox proportional hazard regression analysis of risk factors associated with PTDM

	HR (95% CI)	*P*
Age (years)	1.05 (1.03–1.07)	<0.001
Sex (0 = men, 1 = women)	0.93 (0.67–1.27)	0.780
ΔBW at 6 months after transplantation	1.03 (1.00–1.06)	0.036
Immunosuppressant (0 = cyclosporine A, 1 = tacrolimus)	2.66 (1.85–3.82)	<0.001
Genotype (0 = R/R, 1 = R/W)	0.77 (0.56–1.06)	0.114
Genotype (0 = R/R, 1 = W/W)	0.58 (0.36–0.94)	0.026

ΔBW, change in body weight between baseline and 3 months after transplantation.

DISCUSSION

Zinc is thought to be an important metal for insulin storage and secretion (16). Zinc homeostasis is regulated by the uptake and export of zinc by specialized transporter proteins. Insulin is stored inside secretory granules as solid hexamers bound with two Zn^{2+} ions per hexamer (17,18). Zinc is likely to play a role in the stability of the insulin hexamer and to modulate glucagon secretion in α-cells via paracrine action.

The SLC39 proteins (Zips) control the intracellular uptake of zinc (28), and SLC30 proteins (ZnTs) control the cellular efflux of zinc into the extracellular matrix or intracellular vesicles (29). Among ZnTs, ZnT-8 has recently been identified and cloned as a pancreatic β-cell–specific ZnT and is located in insulin secretory granules (19–21). ZnT-8 is encoded by the gene *SLC30A8*, which is located at chromosome 8q24.11. The polymorphism rs1326634 in the *SLC30A8* gene has been associated with type 2 diabetes (22–25). In addition, recent studies have suggested that *SLC30A8* may play a role in insulin secretion in the process of zinc incorporation into insulin vesicles (19,20).

In this study, we demonstrated the genetic influence of the *SLC30A8* polymorphism in the development of PTDM in a renal transplant cohort. We calculated the relative risks of the heterozygote R/W genotype and the mutant homozygote W/W genotype compared with the wild homozygote R/R genotype because the risk allele was the major allele (R) in a previously reported French study (22). We found the risk of PTDM to be significantly lower in patients who were W/W homozygotes than in patients who were R/R wild-type homozygotes. There was also a tendency toward a reduction in the risk of PTDM in R/W heterozygote carriers compared with R/R carriers. We observed a linear relationship between the number of R-alleles and an increased incidence of PTDM. This genotype effect remained significant after adjustments for age, sex, amount of body weight gain, and type of immunosuppressant use. These results suggest that the *SLC30A8* gene is one susceptibility gene for PTDM. Age at transplantation and tacrolimus use as an immunosuppressant were revealed as important risk factors for PTDM.

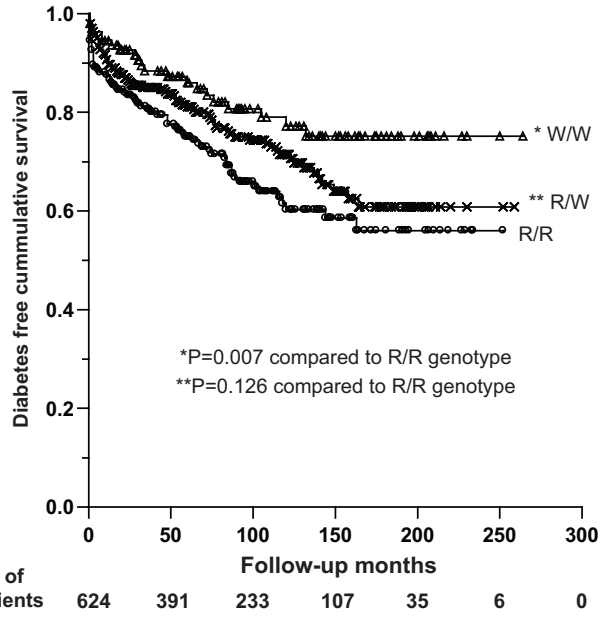

FIG. 1. Cumulative diabetes-free survival (by the Kaplan Meier method) in the development of PTDM for patients with the R/R, R/W, and W/W genotypes.

Interestingly, there was a higher mean HOMA-β value in patients carrying the W/W genotype than in those carrying the R/R genotype. These results suggest that this polymorphism may be functional and have effects on insulin secretion. There was no significant difference in HOMA-IR value according to genotype. Numakura et al. (13) reported that a vitamin D receptor (*VDR*) polymorphism was associated with PTDM in 70 renal allograft recipients. The *VDR* polymorphism is also reported to affect insulin secretion (30–32). Their results and the results of this study are consistent with our previous reports (10,15), in that a defect in insulin secretion plays a more crucial role than increased insulin resistance in the pathogenesis of PTDM.

This *SLC30A8* polymorphism site is located at the COOH-terminal region of the protein structure. The rs1326634 SNP results in a nonsynonymous mutation because the nucleotide change from C to T results in an amino acid change from arginine (R) to tryptophan (W) at position 325. This polymorphism might be a gain-of-function mutation that increases protein expression levels, thereby enhancing zinc transport into insulin secretory granules. Facilitated zinc transport could influence insulin stability and secretion. Another possibility is that the polymorphism may affect the posttranslational modification mechanism in the COOH terminus of ZnT-8. Until now, information on ZnT-interacting proteins or phosphorylation events in the intracellular COOH termini of all the ZnT subfamily members has been lacking. Interestingly, the amino acid stretch (TAASR*DSQVV) constituting R325 in the COOH terminus of ZnT-8 has a protein kinase A and protein kinase C recognition motif (R-X-S/T). The R325W polymorphism would disrupt this motif, which may possibly prevent the receptor phosphorylation otherwise regulated by serine protein kinases, leading to changes in the transporter function. There is much that still needs to be learned about the regulation of ZnT, and the exact molecular mechanisms remain to be elucidated.

A limitation of this study is that oral glucose tolerance tests were not routinely performed before transplantation.

Preexisting diabetes could lead to an overestimation in the diagnosis of PTDM. There may also be a bias toward the null because non-posttransplantation diabetic patients were followed up for a shorter duration and may have been misclassified. Another limitation is the relatively small sample size. Thus, our study had 51% power to detect an inverted reference odds ratio of 0.65 (1/1.53) at $\alpha = 0.05$ (22). Further analyses are warranted to replicate the associations in larger populations.

In conclusion, our data suggest that the *SLC30A8* rs1326634 variant is associated with a decreased risk of PTDM and may be associated with insulin secretion. The exact molecular mechanisms still need to be clarified.

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