Significance of pancreatic duodenal homeobox-1 (PDX-1) genetic polymorphism in insulin secretion in Japanese patients with type 2 diabetes

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ABSTRACT

Introduction Pancreatic and duodenal homebox factor-1 (PDX-1) is an imperative gene for insulin secretion in maturity-onset diabetes of the young 4. PDX-1 gene polymorphism was associated with lower first-phase insulin secretion in a genome-wide association study of intravenous glucose tolerance test. It was not associated with type 2 diabetes risk and insulin secretion in a genome-wide oral glucose tolerance test study. However, there have been no reports of overt type 2 diabetes and insulin resistance evaluation using a glucose clamp. We investigated PDX-1 polymorphism, insulin secretion, and insulin resistance in overt type 2 diabetes.

Research design and methods We performed a meal tolerance test (MTT) and hyperinsulinemic–euglycemic clamping on 63 Japanese subjects, 30 with type 2 diabetes and 33 non-diabetic. We analyzed the rs1124607 PDX-1 gene polymorphism and defined A/C and C/C as the high-risk group and A/A as the low-risk group.

Results HOMA-beta (homeostatic model assessment beta-cell function) was significantly lower in the high-risk group than in the low-risk group for all subjects (72.9±54.2% vs 107.0±63.5%, p<0.05). Glucose levels and glucose area under the curve (AUC) were not significantly different between both the risk groups. The insulin levels at 60 and 120 min and the insulin AUC after MTT were remarkably lower in the high-risk group than those in the low-risk group for all subjects (AUC 75.7±36.7 vs 112.7±59.5, p<0.05). High-risk subjects with type 2 diabetes had significantly lower insulin levels at 30 and 60 min and insulin AUC than low-risk subjects. Non-diabetic high-risk subjects depicted significantly lower insulin levels at 120 and 180 min. There were negligible differences in insulin resistance between the risk groups.

Conclusions These results suggest that the PDX-1 genetic polymorphism is crucial for insulin secretion in Japanese patients with type 2 diabetes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ PDX-1 gene polymorphism was associated with lower first-phase insulin secretion in a genome-wide association study of intravenous glucose tolerance tests but was not associated with type 2 diabetes risk and insulin secretion in a genome-wide oral glucose tolerance test study.

WHAT THIS STUDY ADDS

⇒ Insulin levels after meal tolerance test (MTT) were significantly lower in the high-risk group of PDX-1 polymorphism than those in the low-risk group in both type 2 diabetes and non-diabetic individuals, but there were no major differences in insulin resistance between both the risk groups.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ If we could analyze the PDX-1 genetic polymorphism, we might estimate the low insulin secretion ability of patients with type 2 diabetes.

INTRODUCTION

The key pathophysiology of type 2 diabetes mellitus (T2DM) is enhanced insulin resistance and decreased insulin secretion. 1 Pancreatic and duodenal homeobox factor-1 (PDX-1) is a gene associated with insulin secretion, mutations of which cause maturity-onset diabetes of the young (MODY) 4, 5 A rare frameshift variant of PDX1, encoding p.Gly218Alafs*12, has been reported as a risk factor for T2DM. 5 Genetic variation of PDX-1 was associated with higher fasting glucose and lower first-phase insulin secretion in a genome-wide association study (GWAS) of intravenous glucose tolerance tests (IVGTTs), but not with T2DM risk in a genome-wide oral glucose tolerance test (OGTT) study. 4 They also noted that it was not associated with OGTT-based measures of insulin secretion in another study. 5 The authors mentioned that OGTT is affected by incretins, but IVGTT remains unaffected. However, these studies were performed using IVGTT and OGTT, and
there are few studies on overt diabetes conditions due to the risk of hyperglycemia. These studies are meta-analyses of GWAS, and there is no evidence that the PDX-1 gene affects T2DM risk even in a large case–control study. Therefore, clinical studies based on detailed physiological assessments are essential. In addition, the Japanese and East Asian populations with mild obesity depict low insulin secretion and resistance. GWAS studies have been conducted in European, American and South Asian populations but not in East Asians. Hence, a PDX-1 study on East Asian and Japanese population is required. Furthermore, no study has evaluated the association between the PDX-1 gene and insulin resistance using the glucose clamp method.

Considering this, we performed a meal tolerance test (MTT) and a glucose clamp test on overt type 2 diabetic and non-diabetic healthy volunteers. We demonstrated that PDX-1 gene polymorphism is associated with insulin secretion in the MTT assay.

MATERIALS AND METHODS

Subjects

A total of 65 subjects, 30 with T2DM and 35 without diabetes (non-diabetes), participated in this study at Tottori University Hospital between 2011 and 2021. T2DM was diagnosed using the WHO criteria. We recruited subjects aged between 20 and 80 years and patients with T2DM with glycated hemoglobin (HbA1c) 6.5%–9.0%, without diabetic medication. We excluded patients with cancer, pancreatitis, viral hepatitis, liver cirrhosis, renal failure, or those on diabetic medications. All participants belonged to Japanese ethnicity and were on diet therapy alone. This study had a cross-sectional design. A previous Japanese study reported that the rs1124607 PDX-1 polymorphism indicated an AA: AC: CC ratio of 2:1:0.13. We planned to include over 30 subjects each in T2DM group and non-diabetes group so that we could obtain 10 subjects each in AA and AC group. As PDX-1 polymorphism is not an established risk factor for T2DM, in this previous study, diabetes was diagnosed by HbA1c. OGTT and IVGTT were not conducted.

We excluded two subjects due to the inconvenience of data: one had negative results for the insulinogenic index, and the other had an unreliable PDX-1 genotype (online supplemental figure 1).

This study was conducted as per the principles of the Declaration of Helsinki. It was approved by the Ethics Committee of the Faculty of Medicine of Tottori University (approval no: G161). Informed consent was obtained from all participants using a procedure approved by the ethics committee.

Meal tolerance test

We conducted an MTT assay using a test meal developed by the Japan Diabetes Society as reported previously. The participants visited our hospital after fasting overnight and consumed the test meal (460 kcal, 50% carbohydrate, 15% protein and 35% fat). Plasma glucose and serum insulin levels were measured at 0, 30, 60, 120, and 180 min after consumption of the test meal. Plasma glucose was estimated using the glucose oxidase method, and serum insulin levels were quantified using chemiluminescent immunoassays (human insulin chemiluminescent immunoassay kits; Kyowa Medix, Tokyo, Japan). We measured HbA1c using high-performance liquid chromatography. We converted HbA1c percentage values into the International Federation of Clinical Chemistry values (mmol/mol) employing the HbA1c converter from the National Institute of Diabetes and Digestive and Kidney Diseases.

Insulin secretion index was calculated as follows:

Hyperinsulinemic–euglycemic clamp

We conducted the glucose clamp test as previously reported. A hyperinsulinemic–euglycemic clamp was performed using an artificial endocrine pancreas (STG 55; Nikki-so, Shizuoka, Japan) to evaluate insulin sensitivity. We used the protocol involving primed constant infusion of insulin (100 mU/m²/min) and maintained the plasma glucose levels at 5.2 mmol/L (95 mg/dL). In previous studies, this method achieved a steady-state plasma insulin level of 1200 pmol/L (200 µU/L) in patients with T2DM. The steady-state glucose infusion rate (GIR) from 90 to 120 min was measured, and the mean GIR during this interval was defined as the glucose disposal rate (GDR), which is used as a marker for peripheral insulin sensitivity. We also calculated the M/I ratio as a measure of the quantity of glucose metabolized adjusted by the unit of plasma insulin concentration and defined the M value as the GDR and the I value as the steady-state insulin concentration.

PDX-1 gene analysis

We analyzed the PDX-1 allele rs1124607, as previously reported in a large Japanese study. The context sequence was AGGGAGGAAAGGAACTGCCACCCA[AC/G]CCCC GCAGGTTCGGCGCCTGCGGT, which was assessed using the PCR single-strand conformation polymorphism
method after DNA sequencing as previously reported. We divided the patients into low-risk (A/A) and high-risk ((A/C) or (C/C)) groups, depending on the genotype. Genomic DNA was obtained from peripheral blood leukocytes using proteinase K digestion and phenol/chloroform extraction. The PCR mixture contained genomic DNA as a template, primers, dNTPs, AmpliTaq Gold (PE Biosystems, Tokyo, Japan), and the supplemented buffer. The primer sequences were as follows: forward primer (PDX1-F): 5′-GGCTTCGGACACTACAGATC-3′, reverse G (name: PDX1-R): 5′-CCCTCCCTTACTCCCTATC-3′. PCR was performed under the following conditions: denaturation for 5 min at 95°C, followed by amplification for 35 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C in a thermal cycler. The products were separated using a 2% agarose gel to confirm their size.

DNA sequencing
A direct sequencing reaction was performed using an ABI Prism BigDye Terminator Cycle Sequencing Kit, and the sequencing samples were analyzed using an ABI PRISM 310 Genetic Analyzer (PE Biosystems). Sequencing data were evaluated using the ALF software package (Pharmacia, Tokyo, Japan) and GENETYXMAC software (Software Development, Tokyo, Japan).

**Table 1** Clinical data comparison between high-risk and low-risk subjects, classified based on PDX-1 genotype

| Parameters                  | All subjects (n=63) | High risk (n=16) | Low risk (n=47) | P value |
|-----------------------------|---------------------|------------------|----------------|---------|
| Age (years)                 | 44.6±16.0           | 44.4±13.4        | 44.0±14.0      |         |
| Sex (male/female)           | 36/27               | 7/9              | 29/18          |         |
| Type 2 diabetes/non-diabetes| 30/35               | 8/8              | 22/27          |         |
| BMI (kg/m²)                 | 23.9±4.3            | 24.0±4.3         | 23.8±4.3       | N.S.    |
| Duration of diabetes (years)| 3.2±4.2             | 3.0±3.2          | 3.3±4.6        | N.S.    |
| Fasting plasma glucose (mmol/L) | 6.01±1.51         | 6.45±1.97        | 5.83±1.31      | N.S.    |
| HbA1c (%)                   | 6.58±1.57           | 7.09±2.24        | 6.38±1.25      | N.S.    |
| HbA1c (mmol/mol)            | 47.0±16.0           | 52.0±13.0        | 45.0±13.0      | N.S.    |
| HOMA-beta (%)               | 98.1±61.9           | 72.9±54.2        | 107.0±63.5     | <0.05   |
| Insulinogenic Index         | 1.04±1.13           | 0.83±1.21        | 1.11±1.11      | N.S.    |
| Glucose AUC (0–180 min)     | 20.0±6.7            | 21.1±8.62        | 19.6±6.08      | N.S.    |
| Insulin AUC (0–180 min)     | 105.2±58.4          | 75.7±36.7        | 112.7±59.5     | <0.05   |
| HOMA-IR                     | 2.89±2.40           | 2.51±2.06        | 2.98±2.53      | N.S.    |
| Insulin sensitivity index   | 6.27±4.40           | 7.37±4.83        | 5.98±4.24      | N.S.    |
| Disposition index           | 5.63±6.07           | 5.87±8.04        | 5.63±5.40      | N.S.    |
| GDR (mg/kg/min)             | 8.21±3.32           | 8.46±2.72        | 8.13±3.55      | N.S.    |
| M/I                         | 10.9±9.9            | 12.8±7.6         | 10.4±10.6      | N.S.    |
| LDL-C                       | 3.11±0.78           | 3.25±0.67        | 3.00±0.73      | N.S.    |
| HDL-C                       | 1.51±0.44           | 1.55±0.34        | 1.50±0.50      | N.S.    |
| Triglyceride                | 1.18±0.73           | 1.21±0.86        | 1.15±0.69      | N.S.    |

Data are presented as the mean±SD values. The comparison of parameters between high-risk and low-risk groups was performed using the Mann-Whitney U test. AUC, area under the curve; BMI, body mass index; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; N.S., not significant; PDX-1, pancreatic and duodenal homeobox factor-1; T2DM, type 2 diabetes mellitus.

**Statistical analysis**
Data are expressed as mean±SD. We assessed differences in the mean values of clinical parameters between PDX-1 high-risk and low-risk subjects using the Mann-Whitney U test. We employed a multivariable linear regression model to test the differences in insulin secretion due to difference in PDX-1 genotype by adjusting for age, sex, and body mass index (BMI). PRISM9 software (GraphPad Software, San Diego, California, USA) was used for all the analyses. We conducted a power analysis to compare the HOMA-beta between PDX-1 gene high- and low-risk groups using an EZR calculator. We conducted a power analysis of the statistical test used for comparing HOMA-beta between the high- and low-risk groups. The mean HOMA-beta values in the high-risk and low-risk groups were 72.9±54.2 and 107.0±63.1, respectively. Assuming that the difference in the means of HOMA-beta in all subjects was 37.7 and the SD was 29.9, the estimated power was 86%. If the difference in means of HOMA-beta
in T2DM subjects was 34.1 and the SD was 8.9, the estimated power was >99%. Values of p<0.05 were significant.

**RESULTS**

The characteristics of all participants are presented in table 1. Fifteen subjects had a high-risk AC genotype, 1 non-DM subject had a high-risk CC genotype, and 47 had a low-risk AA genotype. The high-risk group exhibited significantly lower HOMA-beta values than the low-risk group (p<0.05). However, differences in other parameters such as BMI, fasting glucose, HbA1c, HOMA-IR and GDR were insignificant.

The characteristics of T2DM subjects are represented in table 2. Among the T2DM subjects, 8 were in the high-risk group and 22 in the low-risk group. Patients in the high-risk group had significantly lower HOMA-beta than those in the low-risk group (p<0.05), but differences in BMI, fasting glucose, HbA1c, HOMA-IR, and GDR were insignificant. Furthermore, the high-risk group exhibited significantly higher low-density lipoprotein (LDL) cholesterol than the low-risk group (p<0.05). The insulinogenic index tended to be comparatively lower in the high-risk group than that in the low-risk group, but the difference was statistically insignificant.

The non-diabetes group had 8 high-risk and 25 low-risk subjects, there were negligible differences in clinical parameter values between the two risk groups (table 3).

Figure 1 depicts the glucose and insulin responses during the MTT assay. There were inconsequential differences in glucose levels between the high-risk and low-risk groups for all subjects, the T2DM group, and the non-diabetes group (figure 1A–C). The glucose area under the curve (AUC) also indicated negligible differences between both the risk groups for all subjects, the T2DM group, and the non-diabetes group (table 1–3).

All subjects in the high-risk group exhibited remarkably lower insulin levels at 60 and 120 min after the meal than those in the low-risk group (p<0.05) (figure 1D). Furthermore, the high-risk group had lower insulin AUC after the meal than the low-risk group (p<0.05) (table 1). Patients with T2DM with the high-risk genotype had significantly lower insulin levels at 30 and 60 min than those with the low-risk genotype (p<0.05) (figure 1E). The high-risk group of T2DM subjects also exhibited significantly lower insulin AUC after the meal than the low-risk group (p<0.05) (table 2). The non-diabetes subjects with high-risk genotype had significantly lower insulin levels at 120 and 180 min after the meal than those with low-risk
genotype (p<0.05) (figure 1F). However, the insulin AUC in the non-diabetic subjects did not show a remarkable difference (table 3).

Table 3  Clinical data comparison between high-risk and low-risk non-diabetes subjects, classified based on PDX-1 genotype

| Parameters                        | All subjects (n=33) | High risk (n=8) | Low risk (n=25) | P value |
|-----------------------------------|---------------------|-----------------|-----------------|---------|
| Age (years)                       | 32.2±7.7            | 32.9±7.7        | 32.0±7.8        |         |
| Sex (male/female)                 | 20/14               | 4/4             | 16/10           |         |
| BMI (kg/m²)                       | 21.4±2.80           | 21.6±2.0        | 21.4±3.0        | N.S.    |
| Duration of diabetes (years)      | –                   | –               | –               |         |
| Fasting plasma glucose (mmol/L)   | 4.80±0.38           | 4.76±0.27       | 4.81±0.41       | N.S.    |
| HbA1c (%)                         | 5.34±0.27           | 5.35±0.25       | 5.34±0.28       | N.S.    |
| HbA1c (mmol/mol)                  | 35.0±3.0            | 35.0±3.0        | 35.0±3.0        | N.S.    |
| HOMA-beta (%)                     | 122.0±61.6          | 100.9±61.4      | 128.7±61.3      | N.S.    |
| Insulinogenic index               | 1.48±1.23           | 1.40±1.51       | 1.49±1.15       | N.S.    |
| Glucose AUC (0–180 min)           | 14.4±1.7            | 13.5±1.9        | 14.7±1.5        | N.S.    |
| Insulin AUC (0–180 min)           | 95.1±44.1           | 76.7±43.4       | 101.0±43.6      | N.S.    |
| HOMA-IR                           | 1.68±1.01           | 1.30±0.65       | 1.80±1.07       | N.S.    |
| Insulin sensitivity index         | 7.71±3.82           | 10.0±5.22       | 6.95±3.00       |         |
| Disposition index                 | 9.27±6.48           | 10.66±9.23      | 8.83±5.50       | N.S.    |
| GDR (mg/kg/min)                   | 10.3±2.88           | 10.2±2.3        | 10.4±3.0        | N.S.    |
| M/I                               | 14.0±11.5           | 15.0±5.5        | 13.7±12.8       | N.S.    |
| LDL-C                             | 2.92±0.67           | 2.81±0.41       | 2.96±0.75       | N.S.    |
| HDL-C                             | 1.78±0.39           | 1.75±0.33       | 1.79±0.41       | N.S.    |
| Triglyceride                      | 0.79±0.47           | 0.60±0.26       | 0.85±0.51       | 0.08    |

Data are presented as the mean±SD values. The comparison of parameters between high-risk and low-risk groups was performed using the Mann-Whitney U test. AUC, area under the curve; BMI, body mass index; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; N.S., not significant; PDX-1, pancreatic and duodenal homeobox factor-1; T2DM, type 2 diabetes mellitus.

Table 4 depicts the comparison of clinical parameters between T2DM and non-diabetes patients with the high-risk genotype. The disposition index of T2DM in the high-risk group was remarkably lower than the high-risk non-diabetes group (p<0.05).

We employed a multivariable linear regression model to test whether the differences in insulin secretion due to differences in PDX-1 genotype persisted after adjusting for age, sex, and BMI in all subjects. The PDX-1 genotype was shown to be a major risk factor for low insulin secretion, assessed as HOMA-beta, and insulin AUC even after adjusting for age, sex, and BMI. The standardized partial regression coefficient (95% CI) was 2.715 (−67.81 to −10.27, p<0.01) for HOMA-beta and 2.034 (−336.3 to −2.720, p<0.05) for insulin AUC (online supplemental table 1).

**DISCUSSION**

The subjects with the PDX-1 high-risk polymorphism demonstrated a lower insulin secretion ability, assessed as HOMA-beta, and lower insulin AUC during the MTT than those with the low-risk genotype in this study. The PDX-1 high-risk genotype was a significant risk factor

**Figure 1** Glucose and insulin response during meal tolerance test (MTT). MTT results. Figure shows (A) the glucose levels of all subjects after the MTT, (B) glucose levels of patients with type 2 diabetes, (C) glucose levels of non-diabetes subjects, (D) insulin levels of all subjects, (E) insulin levels of patients with type 2 diabetes, and (F) insulin levels of non-diabetes subjects. Black circles represent the data for high-risk genotype subjects, and white squares indicate low-risk genotype subjects. *p<0.05.
for low insulin secretion, assessed as HOMA-beta, and insulin AUC, even when adjusted for age, sex, and BMI. Moreover, participants with T2DM with the high-risk genotype showed lower early phase insulin secretion at 30 and 60 min and lower insulin AUC than those with the low-risk genotype. However, there were insignificant differences in insulin resistance data between the two groups. These results suggest that PDX-1 genotype is a crucial determinant of postprandial insulin secretion rather than insulin resistance. Patients with T2DM with a high-risk genotype exhibited a remarkably lower disposition index than non-diabetic individuals with a high-risk genotype. These results indicate that PDX-1 polymorphism is associated with higher fasting glucose and lower first-phase insulin secretion in a GWAS of IVGTT, but it was not associated with OGTT-based measures of insulin secretion in another study. Our study demonstrated that the PDX-1 high-risk polymorphism in T2DM patients resulted in lower early phase insulin secretion levels. A human study reported that there was a decreased PDX-1 expression in human islets of patients with T2DM compared with that in non-diabetic subjects, which was a result of DNA methylation due to hyperglycemia. These results imply that the PDX-1 polymorphism and glucose toxicity may reduce early phase insulin secretion. Another mouse study showed that the combination of PDX-1 and GLUT4 heterozygosity markedly prolonged glucose clearance and concluded that reduced PDX-1 expression impairs islet response to insulin resistance and worsens glucose homeostasis. Our study also revealed disposition index, which is the early-phase insulin secretion ability adjusted by insulin resistance, to be remarkably lower in the high-risk DM group than in the high-risk non-DM group. However, the insulogenetic index showed negligible differences. These

### Table 4 Clinical data comparison between T2DM and non-diabetes subjects with high-risk PDX-1 genotype

| Parameters | All subjects (n=16) | Type 2 (n=8) | Non-diabetes (n=8) | P value |
|------------|---------------------|-------------|--------------------|---------|
| Age (years) | 44.4±13.4           | 59.0±13.0   | 32.9±7.7           |         |
| Sex (male/female) | 7/9               | 3/5        | 4/4                |         |
| BMI (kg/m²) | 24.0±4.3            | 26.6±4.0   | 21.6±2.0           | <0.05   |
| Duration of diabetes (years) | 3.0±3.2          | 3.0±3.2    | –                  |         |
| Fasting plasma glucose (mmol/L) | 6.5±1.97       | 8.14±1.32  | 4.76±0.27          | <0.01   |
| HbA1c (%) | 7.09±2.24           | 8.68±1.85  | 5.35±0.25          | <0.01   |
| HbA1c (mmol/mol) | 52.0±13.0       | 70.0±20.0  | 35.0±3.0           | <0.01   |
| HOMA-beta (%) | 72.9±54.2      | 44.6±26.9  | 100.9±61.4         | <0.05   |
| Insulinogenic index | 0.83±1.21  | 0.25±0.25  | 1.40±1.51          | 0.06    |
| Glucose AUC (0–180 min) | 21.1±8.62    | 28.6±4.9   | 13.5±1.9           | <0.01   |
| Insulin AUC (0–180 min) | 75.7±36.7    | 74.7±31.6  | 76.7±43.4          | N.S.    |
| HOMA-IR | 2.51±2.06           | 3.71±2.30  | 1.30±0.65          | <0.01   |
| Insulin sensitivity index | 7.37±4.83    | 4.65±2.41  | 10.0±5.22          | <0.05   |
| Disposition index | 5.87±8.04   | 1.08±1.02  | 10.66±9.23         | <0.05   |
| GDR (mg/kg/min) | 8.46±2.72    | 11.0±9.11  | 10.2±2.3           | <0.01   |
| M/I | 12.8±7.6            | 10.3±8.7   | 15.0±5.5           | N.S.    |
| LDL-C | 3.25±0.67           | 3.70±0.60  | 2.81±0.41          | <0.01   |
| HDL-C | 1.55±0.34           | 1.30±0.20  | 1.75±0.33          | <0.01   |
| Triglyceride | 1.21±0.86   | 1.80±0.83  | 0.60±0.26          | <0.01   |

Data are presented as the mean±SD values. Comparison of parameters between T2DM and non-diabetes subjects was conducted using the Mann-Whitney U test. AUC, area under the curve; BMI, body mass index; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; N.S., not significant; PDX-1, pancreatic and duodenal homeobox factor-1; T2DM, type 2 diabetes mellitus.
results imply that insulin resistance and diabetic conditions might impair early phase insulin secretion. A recent article reported saturated fatty acid-induced PDX-1 mislocalization in the nucleus of islet beta-cells and beta-cell dysfunction.\(^{23}\) We found considerably higher LDL cholesterol levels in the high-risk T2DM group than those in the low-risk group. These results signify that lipotoxicity might be important for PDX1 and beta-cell function.

Our study has several limitations. The small number of participants indicates that our results need to be confirmed in a large sample size study. Glucose clamp test is a very complicated technique, and it is challenging to recruit patients with overt diabetes who are not under medication. We are continuing this study for 10 years and are currently conducting a substantial study and would like to publish the results in the future. We used the MTT assay to avoid the risk of hyperglycemia due to the use of OGTT. Although the insulinoenic index did not show significant differences, there were variations in test meal consumption and pure glucose load administration, which also affect glucose and insulin levels. Although we analyzed only PDX-1, the exon variant rs1799854 of ABCC8 showed a noteworthy association with T2DM in Japanese patients.\(^{24}\) Further studies are needed, including those on ABCC8 and other genes. We analyzed only rs1124607, while rs1619319 was analyzed in a previous study.\(^{4, 5}\) We obtained a sizeable amount of Japanese PDX-1 variant data from one study, which evaluated rs1124607 and rs43430600.\(^{9}\) As we could obtain HapMap data for rs1124607, we analyzed rs1124607. We also evaluated rs9581943, which is reported to be a risk factor for T2DM in the Chinese Han population,\(^ {24}\) but this polymorphism failed to show an association with insulin secretion (data not shown). Further studies are essential to evaluate other PDX-1 polymorphic lesions. Our study subjects were Japanese and East Asian populations with low insulin secretion ability and insulin resistance with mild obesity.\(^ {2, 20, 23}\) Further studies on other ethnic populations are required. We evaluated insulin secretion ability as HOMA-beta, which is a measure of insulin secretion based on data from Caucasians. In addition, HOMA-β insulin secretion is generally considered to be decreased when it falls below 50%, and it might be insufficient to assess the decrease in insulin secretion in the high-risk group. However, insulin AUC was also significantly lower in the high-risk group, suggesting that the PDX-1 polymorphism is vital for insulin secretion. Despite these limitations, our study contributes to the daily clinical work.

In conclusion, PDX-1 is an imperative gene associated with insulin secretion in Japanese patients with T2DM.

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Contributors T0 participated in the design of the study and performed the statistical analysis. RN, YI, SK, MA, SE, NT, KM, KS, HO, KM, SI, and EU collected the data. MK, TI, S-IT, and KY conceived the study, participated in its design and coordination, and helped to draft the manuscript. TO is the guarantor of this work and takes responsibility for the integrity and accuracy of the data and its analyses. All authors read and approved the final manuscript.

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Data availability statement Data are available on reasonable request.

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