ABSTRACT

**Introduction**: East Asians are more susceptible to early-onset diabetes than Europeans and exhibit reduced insulin secretion at earlier stages. **PAX4** plays a critical role in the development of β-cells. The dysfunction-missense variants **PAX4** R192H and R192S are common in East Asians but rare in Europeans. Therefore, we aim to investigate the diabetes-associated genes, including **PAX4** R192H/S, in East Asians with early-onset diabetes.

**Methods**: Exome variants of 80 Chinese early-onset diabetes patients (onset age <35 years) after the exclusion of type 1 diabetes (T1D) were detected by a customized gene panel covering 32 known diabetes-associated genes. Then, 229 subjects with early-onset diabetes (T1D excluded) and 1679 controls from the Chinese population were genotyped to validate the association of **PAX4** R192H/S with early-onset diabetes and related phenotypes.

**Results**: The gene panel detected 11 monogenic diabetes patients with five novel mutations among the 80 early-onset diabetes patients. Asian-specifically enriched **PAX4** R192H and R192S were associated with early-onset diabetes (R192H: OR 1.88, 95% CI 1.37–2.60, \( P = 8.41 \times 10^{-5} \); R192S: OR 1.71, 95% CI 1.17–2.51, \( P = 0.005 \)). In early-onset diabetes patients, **PAX4** R192H carriers had higher haemoglobin A1c (HbA1c) levels (\( P = 0.030 \)) and lower 2 h C-peptide levels in the oral glucose tolerance test (OGTT) (\( P = 0.040 \)); R192S carriers had lower fasting C-peptide (FCP) (\( P = 0.011 \)) and 2 h C-peptide levels (\( P = 0.033 \)) in OGTT than non-variant carriers.

**Conclusions**: The ethnic-specific enrichment of **PAX4** R192H/S predisposing East Asians to early-onset diabetes with decreased C-peptide levels may be one explanation of the discrepancy of diabetes between East Asians and Europeans.
Clinical trial registration: ClinicalTrials.gov (NCT01938365).

Keywords: C-peptide; Early-onset diabetes; East Asian; PAX4

**Key Summary Points**

**Why carry out the study?**

East Asians are more susceptible to early-onset diabetes than Europeans and exhibit reduced insulin secretion at earlier stages. Therefore, we explored the genetic architecture of early-onset diabetes in young Chinese.

**What was learned from the study?**

Asian-specifically enriched PAX4 R192H/S is associated with early-onset diabetes in Chinese population.

PAX4 R192H/S carriers have lower C-peptide levels than non-variant carriers in early-onset diabetes patients.

The ethnic-specific enrichment of PAX4 R192H/S in East Asians may partially explain the discrepancy of diabetes between the East and the West from a genetic perspective.

**DIGITAL FEATURES**

This article is published with digital features to facilitate understanding of the article. You can access the digital features on the article’s associated Figshare page. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13200650.

**INTRODUCTION**

In East Asian population, the prevalence of early-onset diabetes has increased rapidly in recent years, posing serious challenges for healthcare systems [1]. Patients with early-onset diabetes are more likely to have a family history and poor metabolic control compared with patients with late onset [2], which is suggestive of the strong involvement of genetic factors underlying the pathophysiology of early-onset diabetes. Therefore, an insight into the genetic basis of early-onset diabetes may contribute to the discovery of novel pathogenic mutations and therapeutic targets [3].

Studies have reported extensive discrepancies in diabetes between East Asians and Europeans [4]. The prevalence of early-onset diabetes in East Asians is much higher than in Europeans [5]. East Asian diabetic patients usually have a younger onset age and a lower body mass index (BMI) than those in Europe, and their insulin secretion tends to decline quickly [4, 6, 7]. Of note, PAX4 plays a critical role in the development, differentiation, expansion, and survival of islet insulin-producing β-cells [8, 9]; the missense variant R192H impairs β-cell development and survival [10, 11]. Besides, PAX4 R192H and R192S were reported to be common in East Asians but rare in Europeans [12]. Thus, it would be of interest to explore the association of variants in PAX4 with early-onset diabetes in young Chinese, attempting to explain the discrepancy of diabetes between the East and the West from a genetic perspective.

Unlike the extensive genetic studies of late-onset diabetes, limited information is available on the genetic architecture of early-onset diabetes. For assessing the exome variants that may be specific in populations, gene panel testing is simple, is more economical, and has greater scalability compared with whole-genome or exome sequencing. Therefore, in the present study, we analysed the exome variants of 80 Chinese early-onset diabetes patients by a customized gene panel after the exclusion of type 1 diabetes (T1D) [2, 13]. Then, 229 Chinese early-onset diabetes cases (T1D excluded) and 1679 controls were genotyped to validate the association of the PAX4 variants detected by the gene panel with early-onset diabetes and the related clinical features.
METHODS

Study Subjects

This research was carried out in two phases, a discovery phase and a validation phase, and at the end the clinical features were compared (Fig. 1). In the discovery stage, 80 Chinese early-onset diabetes patients (onset age < 35 years) were enrolled for gene panel testing after the exclusion of 330 T1D patients [2, 13, 14]. For the association analysis of variants with early-onset diabetes, 69 of the 80 patients and 221 age and sex-matched Chinese non-diabetic controls were enrolled. In the validation phase, an additional 160 Chinese early-onset diabetes (T1D excluded) patients genotyped PAX4 R192 variations with Sanger sequencing, and 1458 ethnicity-matched controls were enrolled to validate the association of PAX4 R192H/S with early-onset diabetes. The patients were enrolled from Ruijin Hospital and The Second Affiliated Hospital of Soochow University from October 2008 to August 2017. The controls in the discovery stage were recruited from volunteers at the Shanghai Jiao Tong University School of Medicine and were non-diabetic and not receiving any treatment for diabetes. Their whole-exome sequencing (WES) data came from a previous study [15]. The WES data of controls in validation stage came from an established database [16]. Diabetes was diagnosed if fasting plasma glucose (FPG) ≥ 7 mmol/L or 2 h glucose during oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L according to the American Diabetes Association (ADA) criteria and classified based on the SEARCH study in the discovery and validation stages [14, 17]. Patients were excluded if they fulfilled the criteria of T1D: positive of glutamic acid decarboxylase antibody (GADA) or fasting C-peptide (FCP) < 0.8 ng/mL [17]. This study was approved by the Ethics Committee of Shanghai Ruijin Hospital, and all patients provided written informed consents. The study is registered at ClinicalTrials.gov (NCT01938365). The flow-chart of the overall study design is shown in Fig. 1.

The subjects underwent a standard 75 g OGTT after an overnight fast of 10–12 h. The levels of both blood glucose and C-peptide at fasting 1 h and 2 h after taking glucose were measured using an enzymatic method (Beckman CX-7 Biochemical Autoanalyzer, Brea, CA) and a radioimmunoassay kit (Roche Diagnostics, Germany), respectively, as mentioned previously [18, 19]. Serum haemoglobin A1c (HbA1c) was measured using high-pressure liquid chromatography, and serum triglyceride and total cholesterol levels were measured using an autoanalyzer (Modular E170, Roche). Subjects also underwent comprehensive physical examination. Weight, height, waist and hip circumferences were directly measured using standardized devices, and BMI was calculated as body weight in kilograms divided by height in meters squared. Blood pressure was measured using an automated electronic device (Omron, Dalian, China).

Development of the Gene Panel

Referring to Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) and the previous study, 32 genes involved in β-cell development, insulin secretion or insulin resistance were selected to comprise the gene panel [20]. These genes included 14 maturity-onset diabetes of the young (MODY) genes, 14 neonatal diabetes mellitus (NDM) genes and 4 insulin resistance associated genes (Table S1). The mutations of these genes tend to increase the risk of early-onset diabetes [20]. All of the 566 pairs of primers for candidate genes were designed using Ion Ampliseq™ Designer of the ThermoFisher Company, and the primer design details were described at https://www.ampliseq.com/. The panel covered all exome regions and intron-exon boundaries (target region of about 149.19 kb) of the candidate genes. Genomic DNA was extracted from peripheral blood cells using a TIANamp Blood DNA Kit (Tiangen, Beijing) according to the manufacturer’s guidelines. DNA libraries were constructed, purified (Ion Torrent, Life Technologies) and sequenced on Ion Torrent PGM using an Ion PGM TM 200 Sequencing Kit, which employed Ion 314 and
1. Discovery stage

Diabetes (onset age < 35, Chinese)
Numbers Screened, N = 410

Excluded
N = 330 excluded (T1D)

Gene Panel Testing
Early-onset diabetes
N = 80

Variants were interpreted by ACMG-AMP guidelines

With pathogenic mutations

Monogenic diabetes
FLPD, N = 2; MODY, N = 9

Early-onset diabetes, N = 69

Chinese non-diabetic
Controls, N = 221

Whole exome sequencing

East-Asian specifically enriched
PAX4 R192H or R192S
was associated with early-onset T2D

2. Validation stage

Early-onset diabetes
(T1D excluded)
N = 160

Ethnicity-matched controls, N = 1,458

Sanger sequencing of PAX4 R192
Whole exome sequencing

PAX4 R192H or R192S
was associated with early-onset T2D

3. Comparisons of clinical features

Discovery stage
Early-onset diabetes, N = 69

Validation stage
Early-onset diabetes, N = 160

Genotyping of PAX4 R192

MODYs, N = 9

PAX4 R192H carriers, N = 47

PAX4 R192S carriers, N = 32

PAX4 R192H/S non-carriers, N = 152

Comparisons of clinical features
Ion 316 chips. The results were mapped to the human genome reference GRCH37 using the Torrent Mapping Alignment Program (TMAP). The Torrent suit software (v2.0) was applied for all analyses.

Analysis of Non-synonymous Variations

DNA samples were subjected to gene panel testing with the mean base coverage ≥ 20 times and the depth ≥ 80% of the target regions selected for analysis. Non-synonymous variations with minor allele frequency (MAF) < 0.05 in total population, according to the databases [1000 Genomes Project, Exome Aggregation Consortium (ExAC) Project, National Heart, Lung, and Blood Institute Exome Sequencing Project] were verified by Sanger sequencing [21]. The variations were assessed and interpreted according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG-AMP) guidelines [22]. If any variation was interpreted as “pathogenic” or “likely pathogenic”, the carrier would be diagnosed with the corresponding monogenic diabetes (MD).

Statistical Analysis

SAS version 9.2 (SAS Institute, USA) was used for data analysis. Quantitative data were described as mean ± standard deviation (SD) and tested for normal distribution using the Shapiro-Wilk test. The association of PAX4 R192H/S with early-onset diabetes and other counting data was analysed with the chi-square test. Comparisons of clinical characteristics between patients and controls were conducted using the Mann-Whitney U test. Linear regression models were constructed to examine the effects of PAX4 R192 variants on the indicated clinical characteristics with adjustment for age and sex. Mann-Whitney U test was performed to compare C-peptide levels among groups. The allele was subjected to the Hardy-Weinberg equilibrium test using the chi-square test. The results were evaluated at 95% confidence intervals (CI) with a significance level of P < 0.05.

Compliance with Ethics Guidelines

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ethics Committees of Shanghai Ruijin Hospital) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

RESULTS

Detection of Monogenic Diabetes in Early-Onset Diabetes with the Customized Gene Panel

In the discovery stage, 80 early-onset diabetes patients after the exclusion of T1D were subjected to gene panel testing; the clinical characteristics of these patients are shown in Table S2. Among the 80 early-onset diabetes patients, 11 (13.75%) had MD genetically diagnosed owing to the presence of pathogenic mutations, including 9 MODYs and 2 familial partial lipodystrophy (FPLD) with high insulin resistance (HOMA-IR were 3.38 and 12.46, respectively) (Table S3). Of the nine MODYs patients, six (66.67%) had MODY3 diagnosed, and three had MODY7, MODY1 and MODY4 diagnosed, respectively (Table S3). Aside from the known pathogenic mutations [23–27], four novel pathogenic mutations of MODY3/4/7 and one novel pathogenic mutation of FPLD3 were detected by gene panel testing (Table S3).
Association of PAX4 R192H/S Variant with Early-Onset Diabetes in Chinese Subjects

After the investigation of pathogenic mutations in 11 MD patients, missense variants in PAX4 in the remaining 69 patients and in 221 age and sex-matched non-diabetic Chinese controls were further analysed [15, 28]. Clinical features of cases and controls are shown in Table 1. Among the missense variants in PAX4, R192H and R192S were associated with early-onset diabetes [R192H: odds ratio (OR) 2.22, 95% CI 1.14–4.31, \( P = 0.016 \); R192S: OR 2.17, 95% CI 1.02–4.63, \( P = 0.041 \)]. As PAX4 R192H and R192S were common single-nucleotide polymorphisms in East Asians and the rare variants were further analysed [15, 28].

Table 1 Clinical characteristics of study participants in the discovery stage

|                      | Controls \((N = 221)\) | Early-onset diabetes \((N = 69)\) | \(P\) value |
|----------------------|------------------------|-------------------------------|-----------|
| Age                  | 21.41 ± 4.36           | 21.66 ± 5.99                  | 0.911     |
| Onset age (years)    | –                      | 20.12 ± 5.72                  | –         |
| Sex (M/F)            | 94/127                 | 30/39                         | 0.890     |
| BMI (kg/m²)          | 20.25 ± 1.58           | 22.40 ± 2.99                  | < 0.001   |
| WC (cm)              | 72.7 ± 6.4             | 79.7 ± 8.7                    | < 0.001   |
| HC (cm)              | 91.1 ± 5.1             | 92.1 ± 7.5                    | 0.094     |
| SBP (mmHg)           | 110 ± 12               | 118 ± 16                      | < 0.001   |
| DBP (mmHg)           | 68 ± 9                 | 75 ± 10                       | < 0.001   |
| FPG (mmol/L)         | 4.7 ± 0.4              | 8.1 ± 2.9                     | < 0.001   |
| 2hPG (mmol/L)        | 5.2 ± 1.0              | 16.0 ± 5.5                    | < 0.001   |
| HbA1c%               | 5.3 ± 0.3              | 8.9 ± 2.7                     | < 0.001   |
| TG (mmol/L)          | 0.79 ± 0.37            | 3.34 ± 6.17                   | < 0.001   |
| TC (mmol/L)          | 4.29 ± 3.19            | 4.71 ± 1.67                   | 0.008     |

Data are expressed as the mean ± SD. \(P\) values were calculated using Mann-Whitney \(U\) test for continuous variables and chi-square tests for categorical variables. Bolded \(P\) values reached statistical significance.

MOundy maturity-onset diabetes of the young, \(M\) male, \(F\) female, BMI body mass index, WC waist circumference, HC hip circumference, SBP systolic blood pressure, DBP diastolic blood pressure, FPG fasting plasma glucose, 2hPG 2-h post-prandial glucose, TG triglyceride, TC total cholesterol.

Association of PAX4 R192H/S Variant with Early-Onset Diabetes in Chinese Subjects

After the investigation of pathogenic mutations in 11 MD patients, missense variants in PAX4 in the remaining 69 patients and in 221 age and sex-matched non-diabetic Chinese controls were further analysed [15, 28]. Clinical features of cases and controls are shown in Table 1. Among the missense variants in PAX4, R192H and R192S were associated with early-onset diabetes [R192H: odds ratio (OR) 2.22, 95% CI 1.14–4.31, \( P = 0.016 \); R192S: OR 2.17, 95% CI 1.02–4.63, \( P = 0.041 \)]. As PAX4 R192H and R192S were common single-nucleotide polymorphisms in East Asians and the rare variants were further analysed [15, 28].

Table 2 PAX4 R192H and R192S were associated with early-onset diabetes

| Stage          | PAX4 R192H; c. 575 C > T; rs2233580 | PAX4 R192S; c. 574 G > T; rs3824004 |
|----------------|-------------------------------------|-------------------------------------|
|                | MAF Case | MAF Control | \(P\) values | OR | MAF Case | MAF Control | \(P\) values | OR |
| Discovery      | 0.116    | 0.056       | \bf{0.016}    | 2.22 (1.14–4.31) | 0.087    | 0.042       | \bf{0.041}    | 2.17 (1.02–4.63) |
| Validation     | 0.113    | 0.065       | \bf{0.001}    | 1.83 (1.25–2.67) | 0.072    | 0.047       | \bf{0.047}    | 1.58 (1.00–2.50) |
| Combination    | 0.114    | 0.064       | \bf{8.41 × 10^{-5}} | 1.88 (1.37–2.60) | 0.076    | 0.046       | \bf{0.005}    | 1.71 (1.17–2.51) |

\(P\) values were calculated using chi-square tests. Bolded \(P\) values reached statistical significance.

MAF minor allele frequency, \(OR\) odds ratio, \(CI\) confidence interval.
in Europeans in Exac databases, the two risk loci in PAX4 may be involved in the different susceptibilities of early-onset diabetes in young East Asians and Europeans (Table 2).

To confirm this finding, the frequencies of PAX4 R192H and R192S were further analysed in an additional 160 Chinese early-onset diabetes (T1D excluded) subjects and 1458 controls [16]. Consistently, both R192H and R192S variants were associated with early-onset diabetes in the validation phase (R192H: OR 1.83, 95% CI 1.25–2.67, \( P = 0.001 \); R192S: OR 1.58, 95% CI 1.00–2.50, \( P = 0.047 \)) (Table 2). The combined results of two stages showed that R192H/S was associated with an increased risk of early-onset diabetes (R192H: OR 1.88, 95% CI 1.37–2.60, \( P = 8.1 \times 10^{-5} \); R192S: OR 1.71, 95% CI 1.17–2.51, \( P = 0.005 \)) (Table 2).

**PAX4 R192H and R192S Are Associated with Lower C-Peptide Levels in Early-Onset Patients**

We then compared the metabolic characteristics of PAX4 R192H/S carriers with non-carriers in the early-onset diabetes patients (T1D and MD excluded) from both discovery and validation stages and with MODYs (Table 3). R192H/S carriers with early-onset diabetes were older at onset age, had higher trends in BMI and waist circumference and had higher diastolic blood pressure (DBP) compared with MODY patients. But no significant difference was observed in blood glucose and HbA1c levels between the two groups (Table 3).

PAX4 R192H carriers had higher HbA1c levels (\( P = 0.030 \)) and lower 2 h C-peptide levels in OGTT (\( P = 0.040 \)) than non-carriers (Table 3 and Fig. 2) in early-onset diabetes patients. More importantly, R192S carriers had lower FCP (\( P = 0.011 \)), lower 2 h C-peptide (\( P = 0.033 \)) and lower area under the curve (AUC) of C-peptide levels (\( P = 0.015 \)) than non-carriers with early-onset diabetes patients. PAX4 R192H or R192S carriers showed lower FCP (\( P = 0.032 \)), 2 h C-Peptide (\( P = 0.004 \)) and AUC of C-peptide (\( P = 0.004 \)) levels than non-carriers in diabetes patients, but presented comparable levels of C-peptide with MODYs (Fig. 2). The PAX4 192 amino acid was determined by the combination c.575C > T and c.574G > T: R/R, R/H, R/S, H/S, H/H and S/S (Table S4). The changes of PAX4 192 amino acid by c.575C > T and/or c.574G > T were associated with lower FCP levels in early-onset diabetes patients (\( \beta = -0.33, 95\% \text{ CI} = -0.60 \) to \(-0.07, P = 0.015 \) (Table S4). These results imply that PAX4 R192H/S variant carriers with early-onset diabetes have a moderate impairment of islet cell function comparable to that of MODY patients.

**DISCUSSION**

In the present study, we developed a customized gene panel to explore the genetic traits in Chinese patients with early-onset diabetes. We identified nine MODY and two FPLD patients with five novel mutations in 80 patients with early-onset diabetes after the exclusion of T1D patients. We further genotyped PAX4 R192H/S in 229 early-onset diabetes (T1D excluded) patients and 1679 non-diabetes controls and found that the East Asian specifically enriched PAX4 R192H/S was associated with an increased risk of early-onset diabetes with decreased C-peptide levels.

In the present study, we detected the presence of MODY1/3/4/7, of which MODY3 (6/9) was the most common type, but MODY2 was undetected, which is consistent with the findings of a previous study on MODY in Chinese patients [29]. Previous studies have shown that MODY3 with mutations in HNF1α and MODY2 with mutations in GCK were the two major types of MODYs among Caucasians and Koreans [30]. In addition, four novel pathogenic mutations of MODY3/4/7 were detected in our study. With genotyping diagnosis, six MODY3 patients and one MODY1 patient were recommended to receive hypoglycemic sulfonylurea treatment, as they were more sensitive to sulfonylurea than to other drugs [3]. These findings indicate that the gene panel contributes to the detection of novel mutations and effectively benefits the diagnosis and precise treatment of MD.

Previous studies focused on the relatively older T2D patients (mean age > 40 or 50 years)
in Korean, Chinese and Singaporean subjects demonstrated the association of PAX4 R192H/S with T2D [12, 31–33]. Another study on Thai patients found that the frequency of the PAX4 R192H variant was higher in MODY [34]. However, in the present study, we concentrated on young Chinese diabetic patients (mean age 23.60 ± 6.51 years) and found that PAX4 R192H and R192S variants were associated with early-onset diabetes. Therefore, to the best of our knowledge, our research is the first study to report the missense variants R192H/S of PAX4 associated with early-onset diabetes in the young Chinese population. Some previous studies also reported that PAX4 R192H was associated with a younger onset age of T2D [32–34]. However, we did not observe an younger onset age in PAX4 R192H/S carriers but found a higher OR of PAX4 R192H (OR 1.88) than those in the previous studies (OR 1.39 or 1.48) [32, 33]. This may be due to the younger and narrower age range of the subjects enrolled in our study compared with the previous ones [32, 33].

Paired-homeodomain transcription factor PAX4 is a member of the paired box (PAX) family and located at chromosome 7q32. It plays an important role in the development, differentiation, expansion and survival of islet insulin-producing β-cells [8, 9]. During the early developmental stage of the pancreas, the PAX4 gene suppresses the transcription activities of insulin, glucagon, somatostatin, islet amyloid polypeptide and ghrelin promoters mainly in the α-cells, thus repressing α-cell differentiation and permitting β-cell differentiation [10, 35]. In the pancreas of PAX4 homozygous mutant mice, insulin-producing β-cells and

|                      | PAX4 R192H carrier (N = 47) | PAX4 R192S carrier (N = 32) | PAX4 R192H/S carrier (N = 77) | PAX4 R192H/S non-carrier (N = 152) | MODYs (N = 9) |
|----------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------------|--------------|
| Age                  | 23.85 ± 6.81                | 23.47 ± 5.66                | 23.62 ± 6.40                  | 23.59 ± 6.58                      | 20.22 ± 5.78 |
| Onset age            | 22.81 ± 6.66                | 23.16 ± 5.62                | 22.87 ± 6.28                  | 22.33 ± 6.48                      | 17.78 ± 5.52C |
| Gender (M/F)         | 33/14                       | 23/9                        | 54/23                         | 86/66                             | 3/6          |
| BMI (kg/m²)          | 25.29 ± 5.16                | 24.13 ± 3.72                | 24.83 ± 4.7                   | 24.93 ± 4.47                      | 20.99 ± 2.97 |
| WC (cm)              | 91.8 ± 18.6                 | 85.7 ± 9.7                  | 89.4 ± 15.8                   | 84.2 ± 14.7                       | 73.2 ± 6.6   |
| HC (cm)              | 98.7 ± 11.8                 | 97.0 ± 7.1                  | 98.1 ± 10.2                   | 96.1 ± 8.3                        | 88.5 ± 3.9   |
| SBP (mmHg)           | 122 ± 16                    | 125 ± 14                    | 123 ± 15                      | 122 ± 15                          | 112 ± 13     |
| DBP (mmHg)           | 77 ± 11                     | 79 ± 9                      | 78 ± 10                       | 75 ± 11                           | 69 ± 3C      |
| FPG (mmol/L)         | 9.0 ± 2.9                   | 8.4 ± 3.6                   | 8.8 ± 3.2                     | 8.5 ± 3.5                         | 7.4 ± 3.1    |
| 2hPG (mmol/L)        | 15.1 ± 4.5                  | 14.9 ± 6.6                  | 15.1 ± 5.5                    | 15.8 ± 5.6                        | 12.2 ± 5.8   |
| HbA1c (%)            | 10.4 ± 2.7*                 | 9.0 ± 2.7                   | 9.9 ± 2.8                     | 9.2 ± 2.7                         | 8.1 ± 3.4    |
| TG (mmol/L)          | 2.74 ± 2.47                 | 2.15 ± 1.40                 | 2.54 ± 2.15                   | 2.30 ± 2.78                       | 1.29 ± 0.64  |
| TC (mmol/L)          | 4.71 ± 1.27                 | 4.41 ± 0.87                 | 4.61 ± 1.14                   | 4.59 ± 1.16                       | 3.69 ± 0.86  |

Data are expressed as the mean ± SD. P values were calculated using linear regression adjusted for age and sex.

MODY: maturity-onset diabetes of the young, M male, F female, BMI body mass index, WC waist circumference, HC hip circumference, SBP systolic blood pressure, DBP diastolic blood pressure, FPG fasting plasma glucose, 2hPG 2-h post-prandial glucose, TG triglyceride, TC total cholesterol

*P values < 0.05 compared with PAX4 R192H/S non-carriers; **P values < 0.05 compared with PAX4 R192H/S carriers.

△ Adis
somatostatin-producing δ-cells are absent, but the population of glucagon-producing α-cells is high [8]. Moreover, insulin protein levels increase, whereas glucagon protein levels remain constant in Pax4-overexpressing islets, and PAX4 mRNA expression is elevated in human insulinomas [36, 37]. In 2007 and 2011, pathogenic mutations in PAX4 were shown to lead to MODY9 in Thai and Japanese patients, respectively [34, 38]. Previous functional experiments have shown that PAX4 R192H can reduce the repression abilities of insulin and glucagon promoters and impair β-cell development and survival [10, 11], consistent with lower C-peptide levels in PAX4 R192H carriers, which may lead to a higher HbA1c level than in non-carriers as reported in our study. More importantly, PAX4 R192S carriers showed lower FCPr and 2hCP 2 h postprandial C-peptide, AUC area under curve, MODY maturity-onset diabetes of the young, ns no significance. Mann-Whitney U test was used for the data analysis. Data are expressed with box and whiskers (minimum to maximum). *P < 0.05; **P < 0.01; ***P < 0.001

East Asians are more susceptible to early-onset diabetes than Europeans [5] and exhibit reduced insulin secretion at earlier stages [7]. In the present study, PAX4 R192H/S was associated with early-onset diabetes in Chinese and lower C-peptide levels in early-onset diabetes patients. Also, different ethnicities have distinct frequencies of PAX4 R192H and R192S according to the database gnomAD v2.1.1, the two variants are specifically higher in East Asians (R192H, MAF = 0.109; R912S, MAF = 0.039). However, the frequencies of the variants were extremely low in Europeans (MAF < 0.000). Therefore, we presume that the East Asian-specific enrichment of PAX4 R192H/S may be
one explanation for the differences in diabetes between the East and the West.

Our study was limited with a relatively small sample size. Hence, a large-scale study of the early-onset diabetes population from East Asia is needed to validate our findings. It may also be important to develop a humanized PAX4 R192H/S variant knock-in mouse model to illustrate the causality between the two risk variants and diabetes and the pathogenic mechanism that underlies early-onset diabetes.

CONCLUSIONS

In summary, we explored the genetic architecture of early-onset diabetes in Chinese subjects by applying a customized gene panel. We further identified that the East Asian-specifically enriched variants PAX4 R192H and R192S predisposed carriers to early-onset diabetes with a reduction of insulin secretion, partially explaining the discrepancy of diabetes between the East and the West from a genetic perspective.

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Compliance with ethics guidelines All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ethics Committees of Shanghai Ruijin Hospital) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Data availability Data described in the manuscript will be made available from the corresponding authors on request, after the request has been submitted and formally reviewed and approved by the Ethics Committee of the Institute of Shanghai Ruijin Hospital, Shanghai, China.

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