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PAX4 R192H is associated with younger onset of Type 2 diabetes in East Asians in Singapore

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ABSTRACT

Aims: Young-onset T2D (YT2D) is associated with a more fulminant course and greater propensity for diabetic complications. The association of PAX4 R192H (rs2233580) variation with YT2D was inconsistent partly because of its Asian-specificity and under-representation of Asians in international consortiums. Interestingly, in our preliminary YT2D (mean = 25 years old) cohort, the prevalence of PAX4 R192H variant was remarkably higher (21.4%) than the general population. Therefore, we sought to determine whether PAX4 R192H is associated with younger onset of T2D in our East Asian (Chinese) population.
Methods: Genotyping of PAX4 R192H was carried out using Illumina OmniExpress BeadChips as part of a genome-wide association study. Data analysis was performed using SPSS Ver. 22.
Results: PAX4 R192H genotype was associated with younger onset age (CC: 47.1, CT: 46.0, TT: 42.6) after adjusting for gender, $F = 5.402$, $p = 0.005$. Independently, onset of diabetes was younger among males by 2.52 years, 95% CI [−3.45, −1.59], $p < 0.0001$. HOMA-IR and HOMA-%B were not significantly different across genotypes for a subset (n = 1045) of the cohort.
Conclusions: Minor allele (T) of PAX4 R192H is associated with younger onset diabetes among Chinese in Singapore. Determining this genotype is important for identifying at-risk individuals for earlier onset diabetes and diabetic complications.

1. Introduction

Diabetes is on the rise in Singapore, with an age-standardized prevalence of 11.3% among those aged 18–69 in 2010.[1] The number of people afflicted with diabetes is expected to reach 1 million by 2050 and a nationwide effort to fight against diabetes has been initiated by the Ministry of Health centering on early prevention, detection and management.[2] A cross-sectional study of a prospective cohort under Joint Asia Diabetes Evaluation program (JADE) highlighted that one in five adult patients had young-onset (defined as below 40 years old) Type 2 diabetes (T2D). Among the 9 countries that participated in this study, Singapore had the highest proportion (about 30%) of participants with young-onset T2D (YT2D). These young-onset diabetes patients were less likely to achieve good glycemic control i.e. maintaining HbA1c of <7% despite being more intensively treated with insulin. In addition, they had a higher propensity to develop diabetes-associated complications given a longer disease duration and exposure to suboptimal glycemic control.[3–5] Given the higher incidence and growing trend of younger onset of T2D in Singapore, it would be important to identify the determinants of YT2D to aid its prevention and detection.

As part of our research study to identify and genetically diagnose maturity-onset diabetes of the young (MODY),[6] we recruited patients clinically diagnosed with YT2D (<45 years old) and subjected them to our 16-gene candidate panel next-generation sequencing. *Paired box gene 4* (*PAX4*) was included as one of the genes in our panel as it was responsible for MODY9.[7–9] PAX4 is a transcription factor that is important for beta cell development. It is expressed early during pancreas development and is required for beta cell differentiation. Pax4-null mice did not survive beyond 3 days of birth and were found to possess

Declarations of interest

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more alpha cells while almost devoid of beta and delta cells.[10] In addition, PAX4 is implicated in regulation of beta cell survival and proliferation in the mature islets. Therefore, PAX4 is recognized as an important driver of islet cell plasticity.[11]

To date, several PAX4 variants have been identified that contribute to the development of T2D in Asian populations. PAX4 R121W (rs114202595) was found to be associated with T2D in Japanese.[12–14] The heterozygotes also exhibited reduced efficacy towards repaglinide monotherapy in Chinese as compared to the wildtype.[15] Another variant, rs6467136, was identified in a meta-analysis of genome-wide association studies in East Asians to be associated with T2D.[16] In Chinese, rs6467136 GA/AA carriers showed improved glucose levels and insulin resistance upon rosiglitazone treatment but no effect on repaglinide efficacy.[17] In addition, a variant rs10229583 located near *PAX4* gene was identified to be associated with T2D in Southern Han Chinese populations and the association was replicated in multiple Asian populations.[18] The rs10229583 risk variant was associated with an earlier age at diagnosis for T2D in cases, reduced beta cell function and increased fasting plasma glucose in healthy controls. The risk allele was subsequently found to be also associated with gestational diabetes mellitus (GDM) in Han Chinese population.[19] A novel PAX4 gene knockout (KO) model was generated in rabbits using CRISPR/Cas9 system.[20] Homozygous PAX4 KO rabbits had growth retardation, persistent hyperglycemia, reduced insulin-producing beta cells and increased glucagon-producing alpha cells. In addition, they also exhibited diabetic nephropathy, hepatopathy, myopathy and cardiomyopathy as compared to the wildtype and the heterozygous PAX4 KO rabbits. These cumulative evidence suggests the importance of PAX4 in pancreatic regulation of glucose homeostasis.

Interestingly, we observed the presence of PAX4 R192H (rs2233580) genetic variation in a size-able proportion (21.4%) of our study cohort enriched with young-onset diabetes (mean age of onset of 25 years old) patients.[6] The minor allele is reported to be 10% for East Asians, suggesting that it could be of interest. The R192H variant resides in the functionally important DNA binding domain and is predicted to be deleterious by 2 out of 4 (SIFT and Polyphen-2) bioinformatics tools used.[21–24] Upon extensive literature search, it was found that the R192H variant has been reported to be 3 times more frequent in MODY patients vs healthy controls and associates with younger onset of T2D in the Thai population.[7] Association of the minor allele with T2D in both Japanese (200 T2D, 161 controls) and Thai (254 T2D, 342 controls) populations were observed but statistically insignificant.[7,12] Kooptiwut et al. also demonstrated that the R192H variant resulted in a defective protein with reduced repression of insulin and glucagon expression in in-vitro cell based assays.[25] Due to these contradicting evidence, we classified R192H as a variant of uncertain significance with respect to MODY9 following the recommended guidelines of the American College of Medical Genetics.[26]

Subsequently, a large-scale genome-wide association analysis by GoT2D and T2D-GENES consortia reported the significant association of PAX4 R192H variant with the risk of T2D at an odds-ratio of 1.79 but not with the age of diabetes diagnosis.[27] Concurrently, the Thai group led by Plengvidhya also demonstrated the significant association of the R192H variant with T2D at an odds-ratio of 1.66 and reinforced previous findings that the resulting protein is defective for its repression of insulin and glucagon expression. In addition, they found that beta cells overexpressing the defective protein were less viable upon glucotoxic stress suggesting the role of the PAX4 in beta cell maintenance.[28]

Given the high allele frequency of the R192H minor allele in Asians (Chinese, Japanese, Thai populations), we hypothesize that it is an ethnic-specific variant that could be best studied in the East Asians with the highest allele frequency of 10% (reported in Exome Aggregation Consortium). While studies have been successful in demonstrating the significant association of the variant with T2D in Asian cohorts, they have not been able to evaluate its association with the age of T2D onset despite showing consistent associations with younger onset. This could be due to factors such as low effect size, allele frequency, sample size or a combination of the above. As such, we aim to leverage on our two large T2D cohorts to investigate the effect of the PAX4 R192H variation on the age of onset of T2D specifically in our Singapore East Asian (Chinese) population.

2. Materials and methods

2.1. Subjects

2955 bio-banked samples from Chinese T2D study participants were assembled for this study from the Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in Type 2 Diabetes (SMART2D) cohort and the Diabetic Nephropathy (DN) cohort. SMART2D is a cross-sectional study conducted between August 2011 and February 2014 including a total of 2057 adults aged 21–90 years with T2D recruited with near equal proportion from a primary care clinic and secondary care diabetes center. The DN cohort is an ongoing study conducted from 2002 at Khoo Teck Puat Hospital, a secondary-care institution in Singapore, including over 3000 adults with T2D. Self-reported age of onset of diabetes of these study participants were documented during consent-taking and data collection. These data were verified against medical records when available. These studies have been approved by our Singapore National Healthcare Group Domain-Specific Review Board (DSRB reference no. 2000/00541, 2011/00232 and 2014/01237). Informed consent was obtained from all study participants.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood samples with the QIAamp DNA Blood Mini kit (Qiagen Inc.). Genotyping was performed using the Illumina HumanOmniExpress BeadChips. Quality control (QC) procedures of samples were performed. Samples with call-rate <95.0%, extremes in heterozygosity (> or <3SD), gender discordant samples and known duplicates were excluded from analyses. Identity-by-state measures were performed by pair-wise comparison of samples to detect 1st and 2nd degree related samples and one sample, with the lower call-rate, from each relationship was excluded from further analysis. Principle component analysis (PCA) using 1000 Genomes Projects reference populations was performed to identify possible outliers from reported ethnicity for exclusion. After QC and removing samples with missing clinical data, 2886 samples (checked for Hardy-Weinberg equilibrium, $\chi^2 = 0.17$, $p = 0.68$) remained for subsequent analyses. Power calculation was performed using Quanto software (http://biostats.usc.edu). Based on the minor allele (T) frequency of 10% in East Asians reported in public databases, our sample size would be able to achieve >80% power to detect a difference in age of onset (younger) at $\alpha = 0.05$ with our sample size of 2886.

2.3. Homeostatic model assessment (HOMA) 2.0

The HOMA model was first described in 1985[29] for the estimation of insulin resistance/sensitivity and beta cell function using fasting plasma insulin and glucose concentrations. This method was derived from the basis that a feedback loop exists between the liver and beta cells and the steady state plasma glucose and insulin reflects the insulin resistance/sensitivity and beta cell function.[30] Subsequently, computer modeling was adopted in the updated HOMA 2.0 model[31] which accounted for variations in hepatic and peripheral glucose resistance, increases in insulin secretion curve in response to a plasma glucose concentration beyond 10 mmol/L, proinsulin secretion and renal glucose losses. In this study, the HOMA calculator version 2.2.3[32] was used to calculate HOMA-IR and HOMA-%B values based on the HOMA 2.0 model.

2.4. Statistical analysis

Linear regression analysis was used to determine the association of PAX4 R192H variant with age of onset of T2D while controlling for gender using IBM SPSS Statistics (Version 22).

3. Results and discussion

Baseline characteristics of the study participants are shown in Table 1. Males comprise 59.7% of the study cohort. The mean body-mass index (BMI) of 26.1 kg/m^2 falls within the overweight range and mean HbA1c (7.9%) exceeds the optimal glycemic target of 7%. Distribution of the PAX4 R192H genotypes is also shown. Participants with at least one minor T allele accounts for 27.6% of the study population. The minor allele frequency is approximately 15% for this Singapore East Asian (Chinese) cohort which is higher than the 10% reported in East Asians by the Exome Aggregation Consortium (ExAC).[33]

Before we analyzed the association of the PAX4 R192H genotype on T2D age of onset, we first identified various factors that may confound the analysis. In Singapore, age-specific prevalence of T2D in Chinese males has been reported to be higher than Chinese females for those aged 30–59, which corresponds to the age range of our study participants.[1] As shown in Fig. 1A, PAX4 R192H genotype distribution is similar in males and females, suggesting that gender is unlikely to be a confounding factor. Indeed, gender and genotype do not interact in exerting their effects on T2D onset age ($F = 0.817$, $p = 0.442$) (Fig. 1B). The relationship of PAX4 R192H genotype and gender with T2D onset age is depicted in Fig. 1C.

As body-mass index (BMI) measurements for our cohort were obtained during study participation and not prior to/at the age of diagnosis, we could not include it in our analyses as it would not accurately reflect the effect of BMI on the age of onset.

Linear regression analysis was performed using SPSS to determine association of PAX4 R192H genotype with T2D onset age while controlling for the effects of gender. Analysis results are shown in Fig. 2. Means (both unadjusted and adjusted) are given in Fig. 2A. The trend of decreasing T2D onset age with increase in the alternate T allele is shown in Fig. 2B. The effect of PAX4 R192H genotype on T2D onset age after adjusting for the effect of gender was significant, $F = 5.402$, $p = 0.005$. Gender was both independently and significantly related to onset age, $F = 28.02$, $p < 0.0001$. Pairwise comparisons (Fig. 2C) between genotypes (with reference wildtype as CC) demonstrated that there was a significant difference in T2D onset age between CC and TT genotypes ($B = -4.51$, 95% CI [−7.71, −1.31], $p = 0.006$) as well as between CC and CT genotypes ($B = -1.08$. 95% CI [−2.13, −0.02], $p = 0.045$). Males in comparison to females have significantly decreased onset age by 2.52 years (95% CI [−3.45, −1.59], $p < 0.0001$). Of note, homozygous TT minors exist in a considerable proportion of 2.1% in our Chinese population and results in an earlier onset of T2D by a non-trivial 4.5 years.

Since PAX4 is implicated in beta cell development and maintenance, we used the homeostatic model assessment (HOMA) calculator to calculate HOMA-IR and HOMA-%B to assess insulin resistance and beta cell function respectively for a subset of our cohort ($n = 1045$) where input values are available (Suppl. Table 1). We did not observe statistically significant differences in HOMA-IR and HOMA-%B across the 3 genotypes although individuals with TT genotypes were found to have numerically higher HOMA-IR and lower HOMA-%B values than their CC counterparts. Any difference in the HOMA values among the 3 genotypes is likely to be trivial and will be constrained by our study power given this analysis was only based on a subset of the cohort.

In summary, we demonstrated in our local East Asian (Chinese) population that age of onset of T2D is affected independently by PAX4 R192H variation and gender. Presence of the alternate T allele and male gender contributed to a younger onset of T2D. This result was similar to that reported in two recent studies conducted on a large cohort of Chinese and Korean populations in which the PAX4 R192H variant was found to associate with T2D and the risk allele (T) to associate with younger age of diabetes diagnosis.[34,35] The effect of the risk allele is modest, given that it resulted only in a decrease of approximately 1 year in age of onset. This may also explain why previous studies with lower sample size were not able to conclude on the significance of this variant's association with T2D onset age.[7,12,27] The main limitation of our study is the difficulty in accurately ascertaining the age of onset of diabetes, given that the subjects were not recruited to our cohorts upon the diagnosis of diabetes. Nonetheless, given the insidious onset and largely asymptomatic nature of T2D, its exact date of onset often defies accurate ascertainment. Moreover, given that the participants are not aware of their PAX4 genotype status, any mis-classification arising from error in the self-reported age of onset is likely to be random or non-differential (with respect to their PAX4 genotype). Random mis-classification is often believed to lead to loss of study power but not systematic bias. Therefore, it is our considered opinion that our results have not been biased by this limitation. Since PAX4 is a transcription factor important for pancreatic beta cell development and maintenance, individuals harboring the R192H variant carry a beta cell defect burden and are expected to have a higher risk of developing T2D. However, HOMA-IR and HOMA-%B were not found to be significantly different among the 3 genotypes. As HOMA estimates the resting/steady state of beta cell function, it is conceivable that dynamic testing (e.g.

Table 1
Clinical characteristics of study participants (overall and stratified by PAX4 R192H genotype). *$p < 0.05$ denotes statistical significance.

Clinical characteristic	% or Mean ± SD				
	Overall	PAX4 genotype Chr7(GRCh37):g.127253550			
		CC (72.4%)	CT (25.5%)	TT (2.1%)	*p*-Value
Male gender ($N = 2886$)	59.7%	59.7%	59.4%	63.9%	0.784
BMI (kg/m^2) ($N = 2602$)	26.1 ± 4.6	26.0 ± 4.6	26.2 ± 4.6	25.8 ± 4.8	0.579
HbA1c (%) ($N = 2851$)	7.9 ± 1.6	7.9 ± 1.6	7.9 ± 1.8	8.2 ± 1.7	0.310
Age of onset (years) ($N = 2886$)	46.5 ± 12.6	46.9 ± 12.6	45.8 ± 12.5	42.3 ± 13.7	0.004*
Diabetes duration (years) ($N = 2783$)	12.6 ± 9.4	12.6 ± 9.5	12.7 ± 9.1	14.6 ± 10.6	0.014*
Systolic blood pressure (mm Hg) ($N = 2727$)	138 ± 20	138 ± 20	138 ± 20	139 ± 18	0.970
Diastolic blood pressure (mm Hg) ($N = 2727$)	78 ± 11	77 ± 11	77 ± 10	78 ± 11	0.861

A

PAX4 R192H genotype	Gender	
	Male	Female
CC	72.4%	72.4%
CT	25.4%	25.7%
TT	2.3%	1.9%
	χ^2=0.487, p=0.784	

B

Independent variable	Model 1	Model 2
Genotype	0.007*	0.005*
Gender	0.051	<0.0001*
Genotype*Gender	0.442	-

Dependent variable: onset age

C

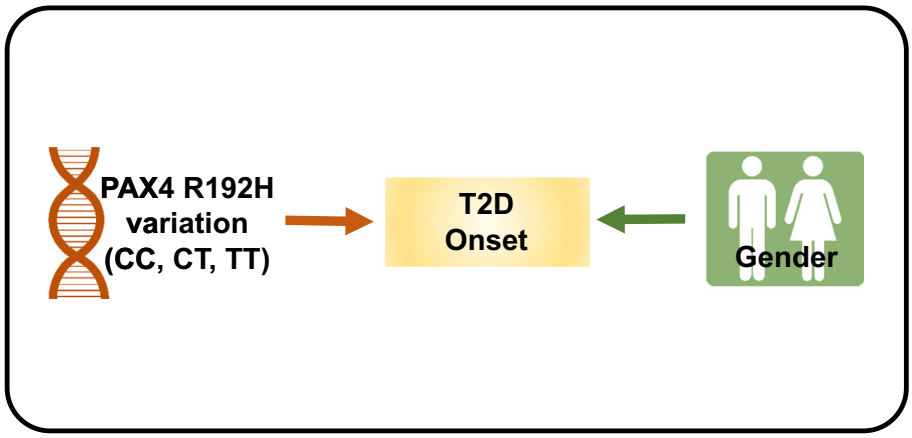

Fig. 1. Effect of PAX4 R192H genotype and gender on the age of onset of Type 2 diabetes in Singapore Chinese. (A) Distribution of genotypes is similar among males and females. (B) Association of PAX4 R192H genotype with T2D age of onset was performed considering the effect of both genotype and gender with (Model 1) and without (Model 2) their possible interaction. *$p < 0.05$ denotes statistical significance. (C) Diagram representing relationship between PAX4 R192H variation and gender on the age of onset. Arrows denote independent effects.

glucose-loading or mixed-meal challenge) may better elucidate the functional defect associated with PAX4 variant. Additionally, in a cohort heavily treated with anti-diabetic agents (including insulin replacement), the performance of HOMA model may be suboptimal.

The strengths of our study include the availability of a sufficiently large cohort of a specific ethnicity (Chinese) to examine the effect of this PAX4 R192H variant on T2D onset age, and also that the analysis was performed in consideration of gender disparity (which was absent in other studies). With cumulative and corroborative evidence gathered from other groups who have published their findings in relation to PAX4 R192H variation, we conclude that PAX4 R192H (rs2233580), an Asian-specific variant, is associated with younger onset of T2D.

4. Conclusion

PAX4 R192H, an Asian-specific variant known to affect beta cell function, is associated with younger onset age of T2D among Chinese. Given that this variant potentially confers an earlier onset of diabetes by up to 4.5 years, and therefore a longer exposure to diabetes which increases the propensity to develop diabetic vascular complications, additional studies to elucidate whether the effect of PAX4 R192H can be modified by factors like physical activity, dietary or pharmaco-intervention will also be useful for T2D prevention and progression among susceptible individuals.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2018.10.002.

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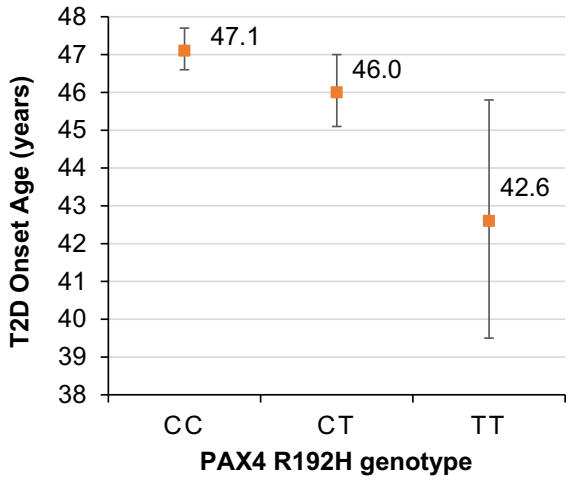
A

PAX4 genotype	Mean T2D onset age ± SD (years)	Adjusted mean T2D onset age [95% CI] (years)
CC	46.9 ± 12.6	47.1 [46.6-47.7]
CT	45.8 ± 12.5	46.0 [45.1-47.0]
TT	42.3 ± 13.7	42.6 [39.5-45.8]

B



C

Parameter		B	95% Confidence Interval		t	P-value
			Lower	Upper		
PAX4 Geno-type	TT	-4.51	-7.71	-1.31	-2.767	0.006*
	CT	-1.08	-2.13	-0.02	-2.005	0.045*
	CC					
Gender	Male	-2.52	-3.45	-1.59	-5.293	<0.0001*
	Female					

Fig. 2. Association of PAX4 R192H variation with age of onset of Type 2 diabetes. Statistical analysis of PAX4 R192H genotype with T2D age of onset was performed adjusting for the effect of gender. (A) Means and adjusted means of the three genotypes. (B) Chart showing the decreasing trend of T2D onset age (adjusted means) with increase in the number of the alternate T allele. Error bars represent the 95% confidence intervals (CI). (C) Beta coefficients, 95% CIs and *p*-values of the analysis for the effects of genotype and gender on T2D onset age. *$p < 0.05$ denotes statistical significance.

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References

1. National Health Survey 2010. Singapore: Ministry of Health. 2010.
2. Ministry of Health 2016 budget initiatives: the fight against diabetes. Singapore: Ministry of Health. 2016:2-4.
3. Zhang Y, Ning G. Diabetes: young-onset type 2 diabetes mellitus-a challenge for Asia. Nat Rev Endocrinol 2014;10:703-4.
4. Unnikrishnan R, Anjana RM, Amutha A, et al. Younger-onset versus older-onset type 2 diabetes: clinical profile and complications. J Diabetes Complications 2017;31:971-5.
5. Owen KR. Treating young adults with type 2 diabetes or monogenic diabetes. Best Pract Res Clin Endocrinol Metab 2016;30:455-67.
6. Ang SF, Lim SC, Tan C, et al. A preliminary study to evaluate the strategy of combining clinical criteria and next generation sequencing (NGS) for the identification of monogenic diabetes among multi-ethnic Asians. Diabetes Res Clin Pract 2016;119:13-22.
7. Plengvidhya N, Kooptiwut S, Songtawee N, et al. PAX4 mutations in Thais with maturity onset diabetes of the young. J Clin Endocrinol Metab 2007;92:2821-6.
8. Jo W, Endo M, Ishizu K, Nakamura A, Tajima T. A novel PAX4 mutation in a Japanese patient with maturity-onset diabetes of the young. Tohoku J Exp Med 2011;223:113-8.
9. Chapla A, Mruthyunjaya MD, Asha HS, et al. Maturity onset diabetes of the young in India - a distinctive mutation pattern identified through targeted next-generation sequencing. Clin Endocrinol (Oxf) 2015;82:533-42.
10. Sosa-Pineda B. The gene Pax4 is an essential regulator of pancreatic beta-cell development. Mol Cells 2004;18:289-94.
11. Brun T, Gauthier BR. A focus on the role of Pax4 in mature pancreatic islet beta-cell expansion and survival in health and disease. J Mol Endocrinol 2008;40:37-45.
12. Shimajiri Y, Sanke T, Furuta H, et al. A missense mutation of Pax4 gene (R121W) is associated with type 2 diabetes in Japanese. Diabetes 2001;50:2864-9.
13. Kanatsuka A, Tokuyama Y, Nozaki O, Matsui K, Egashira T. Beta-cell dysfunction in late-onset diabetic subjects carrying homozygous mutation in transcription factors NeuroD1 and Pax4. Metabolism 2002;51:1161-5.
14. Shimajiri Y, Shimabukuro M, Tomoyose T, Yogi H, Komiya I, Takasu N. PAX4 mutation (R121W) as a prodiabetic variant in Okinawans. Biochem Biophys Res Commun 2003;302:342-4.
15. Gong ZC, Huang Q, Dai XP, et al. NeuroD1 A45T and PAX4 R121W polymorphisms are associated with plasma glucose level of repaglinide monotherapy in Chinese patients with type 2 diabetes. Br J Clin Pharmacol 2012;74:501-9.
16. Cho YS, Chen CH, Hu C, et al. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 2011;44:67-72.

17. Chen M, Hu C, Zhang R, et al. Association of PAX4 genetic variants with oral antidiabetic drugs efficacy in Chinese type 2 diabetes patients. Pharmacogenomics J 2014;14:488-92.
18. Ma RC, Hu C, Tam CH, et al. Genome-wide association study in a Chinese population identifies a susceptibility locus for type 2 diabetes at 7q32 near PAX4. Diabetologia 2013;56:1291-305.
19. Xu T, Shi Y, Liu J, et al. The rs10229583 polymorphism near paired box gene 4 is associated with gestational diabetes mellitus in Chinese women. J Int Med Res 2018;46:115-21.
20. Xu Y, Wang Y, Song Y, et al. Generation and phenotype identification of PAX4 gene knockout rabbit by CRISPR/Cas9 system. G3 (Bethesda) 2018;8:2833-40.
21. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 2012;40:W452-7.
22. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248-9.
23. Tavtigian SV, Deffenbaugh AM, Yin L, et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet 2006;43:295-305.
24. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361-2.
25. Kooptiwut S, Plengvidhya N, Chukijrungroat T, et al. Defective PAX4 R192H transcriptional repressor activities associated with maturity onset diabetes of the young and early onset-age of type 2 diabetes. J Diabetes Complications 2012;26:343-7.
26. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
27. Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. Nature 2016;536:41-7.
28. Sujjitjoon J, Kooptiwut S, Chongjaroen N, et al. PAX4 R192H and P321H polymorphisms in type 2 diabetes and their functional defects. J Hum Genet 2016;61:943-9.
29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
30. Rudenski AS, Matthews DR, Levy JC, Turner RC. Understanding "insulin resistance": both glucose resistance and insulin resistance are required to model human diabetes. Metabolism 1991;40:908-17.
31. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998;21:2191-2.
32. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-95.
33. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-91.
34. Cheung CY, Tang CS, Xu A, et al. Exome-chip association analysis reveals an Asian-specific missense variant in PAX4 associated with type 2 diabetes in Chinese individuals. Diabetologia 2017;60:107-15.
35. Kwak SH, Chae J, Lee S, et al. Nonsynonymous variants in PAX4 and GLP1R are associated with type 2 diabetes in an east Asian population. Diabetes 2018;67:1892-902.