



## Association between Gene Polymorphisms of Seven Newly Identified Loci and Type 2 Diabetes and the Correlate Quantitative Traits in Chinese Dong Populations

Liya LIU<sup>1,2</sup>, \*Lizhang CHEN<sup>1</sup>, Zhanzhan LI<sup>1</sup>, Liang LI<sup>3</sup>, Jian QU<sup>4</sup>, Jing XUE<sup>1</sup>

1. Dept. of Epidemiology and Health Statistics, School of Public Health, Central South University, Changsha, China.
2. Dept. of Medical Laboratory, Hunan University of Medicine, Huaihua, China,
3. Medical Department of the First Affiliated Hospital of Xiangya, Central South University, Changsha, China
4. The Institute of Clinical Pharmacology, Central South University, Changsha, China

\*Corresponding Author: Email: chenliz@csu.edu.cn

(Received 12 Mar 2014; accepted 22 Aug 2014)

### Abstract

**Background:** There are much heterogeneity in the genetic variation of type 2 diabetes (T2D). The purpose of this study was to investigate the association of seven novel genetic loci identified in a recent genome-wide association studies (GWAS) with T2D in Chinese Dong populations.

**Methods:** A case-controlled study was performed in individuals of Chinese Dong nationality. The genotypes of PARD3B (rs849230), LOC729993 (rs149228), EPHA4 (rs16862811), HNT (rs3099797), PTPRD (rs17584499 and rs649891), TOMM7 (rs2240727) genes were determined using Multiplex PCR-SNaPshot. The independent association between each polymorphism and T2D was assessed using unconditional binary logistic regression analysis (BLR).

**Results:** A total of 136 cases of T2D and 136 control subjects were enrolled in the study. The polymorphism of rs2240727 in TOMM7 gene was associated with T2D (odds ratio (OR) = 1.65, per copy of the risk T allele,  $P = 0.004$ ). In addition, CT and TT were risk genotypes for T2D (OR (95% CIs): 2.64 (1.28–5.45) and 3.42 (1.58–7.41) respectively). After correcting for multiple testing, the above results remained significant (all  $P < 0.05$ ). After adjusting for the confounders of age, gender, and BMI, the association between T2D and rs2240727 remained significant ( $P < 0.01$ ). There were significantly statistical difference in levels of fasting plasm glucose (FPG) among genotypes of rs2240727 in controls and patients, the levels of FPG were significantly higher in CT and TT genotypes than in CC genotype in both groups (all  $P < 0.05$ ).

**Conclusions:** The rs2240727 genetic variant in TOMM7 was associated with T2D of Chinese Dong individuals, and might enhance the risk of T2D by affecting the level of FPG.

**Keywords:** Type 2 diabetes, Dong nationality, Gene polymorphism, TOMM7, SNaPshot genotyping

### Introduction

Type 2 diabetes (T2D) is a complicated and chronic metabolic disease characterized by insulin resistance and pancreatic  $\beta$ -cell dysfunction (1). Many individuals have an apparent genetic susceptibility to T2D. In recent years, many T2D susceptibility genes were identified (2-13) due to the completion of the HapMap project, an International collaborative effort to help researchers iden-

tify genes associated with human disease, as well as the wide use of genome-wide association studies (GWAS) and candidate gene association studies. However, the exact number and nature of genes involved in T2D, the underlying genetic models, and their interaction with environmental factors remain unclear. GWAS are commonly performed, and several studies searching for T2D

susceptibility genes have been performed. Recently, a GWAS and meta-analysis identified several novel T2D susceptibility loci in populations from Starr County, Texas, and Mexico City (14). Among these, six risk loci (rs2240727 in TOMM7, rs849230 in PARD3B, rs149228 in LOC729993, rs16862811 in EPHA4, rs3099797 in HNT, and rs649891 in PTPRD) were strongly associated with T2D attracted our attention. PTPRD, or protein tyrosine phosphatase receptor type D, is located on chromosome 9p23-p24.3. The protein encoded by this gene is protein tyrosine phosphatase (PTPs), which are signaling molecules that regulate various cellular processes including cell growth, differentiation, mitosis, and oncogenic transformation. PTPRD is expressed widely in tissues including skeletal muscle and the pancreas, with the highest level of expression in the brain. The rs17584499 locus of the PTPRD gene is associated with T2D in individuals from Chinese Taiwan ( $OR = 1.57$ ,  $P = 8.54 \times 10^{-10}$ ) (15). The rs649891 locus of the same gene is also associated with T2D ( $OR = 1.29$ ,  $P = 1.69 \times 10^{-4}$ ) (14). In addition, a prospective cohort study (16) performed on a Chinese Han population demonstrated that carriers of the TT genotype at the rs17584499 locus of the PTPRD gene were more likely to progress from non-diabetes to diabetes (relative risk (RR) = 8.82,  $P = 4 \times 10^{-5}$ ). Therefore, the PTPRD genetic variant appears to be associated with the progression to diabetes in the Chinese Han population, most likely due to increased insulin resistance.

However, many of the observed associations between genetic variants and T2D have not been reported in Chinese minority groups. Because T2D susceptibility genes exhibit heterogeneity among races and nationalities, we can not simply extrapolate the above findings to other ethnic populations with different living environments and genetic backgrounds. One study (17) reported that the prevalence of T2D is significantly lower in ethnic Dong individuals compared with the ethnic Han population in China; however the reason for this is unclear. Therefore, we performed this study to examine the associations between seven newly discovered diabetes-related genes, which

were identified in Mexican-American populations predominantly, and T2D in Chinese Dong populations.

## Materials and Methods

### Subjects

A case-controlled study was performed in ethnic Dong populations of Tongdao Dong Autonomous County in Hunan, China. This study included 272 subjects, with 136 T2D (43.4% males, with a mean  $\pm SD$  age of  $62.47 \pm 9.41$  years) and 136 healthy controls (39.7% males, aged  $61.23 \pm 11.20$  years) matched for ethnicity, age, and gender. T2D was diagnosed using the world health organization (WHO) criteria (18): fasting plasma glucose (FPG)  $\geq 7.0$  mmol/L, or 2-hour postprandial glucose (2hPG)  $\geq 11.1$  mmol/L. General overweight or obesity was defined as a body mass index (BMI)  $\geq 24$  or  $\geq 28$  kg/m<sup>2</sup> respectively, which was modified for Chinese Han populations (19). The inclusion criteria for controls was (i)  $\geq 40$  years of age, (ii) FPG  $< 6.1$  mmol/L and 2-hour postprandial glucose (2hPG)  $< 7.8$  mmol/L, (iii) no history of diabetes in first or second degree relatives. Patients with severe liver and kidney disease and pregnant females were excluded. Written informed consent was obtained from all participants before this study started. The study was conducted according to the principles of the Declaration of Helsinki, and was approved by the Research Ethics Committee of Central South University. Demographic and clinical data were collected from conducted interviews and physical examinations. Blood specimens were collected for genetic testing from clinically confirmed cases of T2D and the healthy controls.

All subjects underwent an oral glucose tolerance test (OGTT), 5 ml of the fasting and the 2-hour postprandial venous blood were extracted respectively, and the fasting venous blood was separated into two tubes: one tube was used for testing FPG, fasting insulin levels (FINS), total cholesterol(TC), trigalloyl glycerol(TG), high density lipoprotein cholesterol(HDL-C) and low density lipoprotein cholesterol(LDL-C), the second was a disodium ethylene diamine tetraacetic acid (EDTA) tube for genetic testing. FPG and 2hPG were measured us-

ing the plasma glucose oxidase method. The FINS was tested using the human insulin ELISA kit and the TC, TG, HDL-C, LDL-C were tested using the corresponding kits provided by the Dingguo biotechnology company in Beijing, China. Simultaneously, the homeostasis model assessment (HOMA) insulin resistance (HOMA-IR) and HOMA model B-cell function indices (HOMA-B) were calculated by the following formulae:  $HOMA-IR = FINS \times FPG / 22.5$ , and  $HOMA-B = FINS \times 20 / (FPG - 3.5)$ .

### DNA isolation

Genomic DNA was extracted from the mononuclear cells of peripheral blood using an Easy Nucleic Acid Isolation SE Blood DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA was amplified using Multiple PCR-SNaPshot (20, 21), and the amplified genomic DNA products were genotyped using GeneMapper 4.1 (Applied Biosystems Co., Ltd., USA). All the gene amplification and genotyping technologies were performed by Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co., Ltd.

### Multiplex PCR amplification of genomic DNA

As showed in Table 1, a total of 7 pairs of PCR primers were designed to amplify seven 122–250 bp fragments each containing a specific locus, and

seven extension primers adjacent to the single nucleotide polymorphism (SNP) loci were used for single base extension. PCR products were obtained from multiple PCR reactions using HotStarTaq (Qiagen), after purification using shrimp alkali enzyme (SAP; Promega) and exonuclease I (EXO I) (Epicentre). The PCR products then underwent an extension reaction using SNaPshot Multiplex kit (ABI), and the extension products were purified using Promega SAP in an ABI3130xl sampler. The SNP genotyping results were read using GeneMapper 4.1. PCR reactions were performed in 10  $\mu$ L reaction volumes containing 1 $\times$  HotStar Taq buffer, 3.0 mM  $Mg^{2+}$ , 0.3 mM dNTP, 1 U HotStar Taq Polymerase (Qiagen), 1  $\mu$ L sample DNA, and 1  $\mu$ L multiplex PCR primers. PCR cycling conditions were as follows: 95  $^{\circ}$ C for 2 min; followed by 11 cycles of 94  $^{\circ}$ C for 20 s, 65  $^{\circ}$ C ( $-0.5$   $^{\circ}$ C/cycle) for 40 s, and 72  $^{\circ}$ C for 90 s; 24 cycles of 94  $^{\circ}$ C for 20 s, 59  $^{\circ}$ C for 30 s, and 72  $^{\circ}$ C for 90 s; and 72  $^{\circ}$ C for 120 s. Samples were then cooled to 4  $^{\circ}$ C when reactions were complete. PCR products were purified using 5U SAP and 2U Exonuclease I in 15  $\mu$ L PCR product, incubated at 37  $^{\circ}$ C for 1 h, and then inactivated at 75  $^{\circ}$ C for 15 min.

**Table 1:** PCR primers used to analyze seven SNP loci

SNP locus	Primer position	Primer sequence	length	Tm
rs849230	Upstream	CAGGCAGTCAC TTCCTGTATTG	24bp	64.18
	Downstream	AAAATCTTTC TCTTTCCTTACTCGCTCTC	30bp	64.61
rs149228	Upstream	GATGATTGACATTTTGCCAGAATCA	25bp	64.27
	Downstream	GAGCCCCCTTCTGCCCCTAACT	22bp	66.00
rs16862811	Upstream	GAAACAGTCTCCCACCCTCTTGTATTG	27bp	66.10
	Downstream	TTCAGTGAGCAGGTTTCGCATTC	22bp	64.87
rs3099797	Upstream	AAATGCCCTCAGGTCAGGAACC	22bp	65.77
	Downstream	GGAAGTTTCAAGCTGTGGATGGAGA	25bp	66.83
rs17584499	Upstream	CCAGGCTTTCCTGGTCAGTAACAT	24bp	64.74
	Downstream	GCTGGGCCCAAGAAAAGACAAC	22bp	66.26
rs649891	Upstream	TTACGCGTGTGAGTATGCCTCTCT	24bp	64.43
	Downstream	TGACGGAGGTATTTGTTGTGTATAACATTT	30bp	64.49
rs2240727	Upstream	CACTGCAAAGGGAAACTGTAATCCATA	27bp	65.24
	Downstream	GGTAAAAATTAAGCCTTTTGGTACTTTCTCA	31bp	64.54

### SNaPshot multiple single-base extension reaction and genotyping

The following extending primers were used:

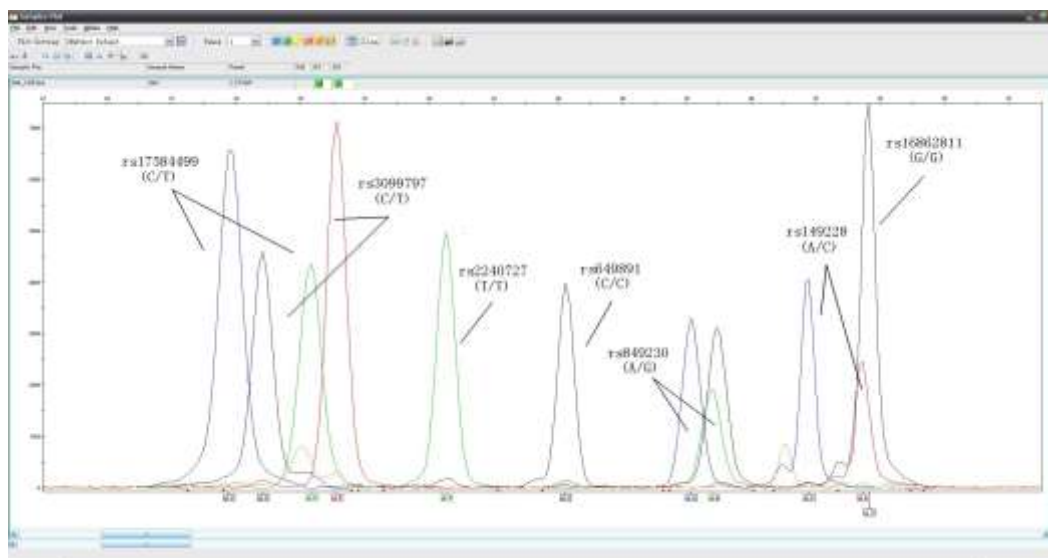
rs849230SF: TTTTTTTTTTTTTTTTTTGGTAACT-GTCTCCTCTGTTA,  $T_m$ : 60.46 °C; rs149228SR: ttTTTTTTTTTTTTTTTTTTTTTCCATGGCCAA GTGCCTCA,  $T_m$ : 66.19 °C; rs16862811SR: ttTTTTTTTTT-TTTTTTTTTTTTCGCATTCTAGTACAGGGATT-AAAAACT $m$ : 60.59 °C; rs3099797SF: CTAATCTGCAGT-ACCTGTTTAAACCA,  $T_m$ : 60.01 °C; rs17584499SR: GGACCCAGGCCTCTCTCTG,  $T_m$ : 62.3 °C; rs649891 SF: TAATATTTTGATTTAGTCTCTTATATTTAAGA-TAGTC,  $T_m$ : 57.49 °C; rs2240727SR: TTTT-TGCCTTTTGGTACTTTCTCAAACAG,  $T_m$ : 61.60 °C.

Extending reactions were then performed in a 10- $\mu$ L reaction volume consisting of 5  $\mu$ L SNaPshot Multiplex Kit (ABI), 2  $\mu$ L purified multiplex PCR products, 1  $\mu$ L extension primer mixture, and 2  $\mu$ L of ultrapure water. Reactions were then cycled at 96 °C for 1 min, followed by 28 cycles of 96 °C for 10 s, 52 °C for 5 s, and 60 °C for 30 s, and then held at 4 °C. Extension products were then

purified by the addition of 1U SAP enzyme to 10  $\mu$ L extended products, incubation at 37 °C for 1 h, and inactivation at 75 °C for 15 min.

The extended products were then genotyped using an ABI3130 XL sequencer. After purification, 0.5  $\mu$ L of the extended products were mixed with 0.5  $\mu$ L of Liz120 size standard, 9  $\mu$ L of Hi-Di, and denatured at 95°C for 5 min. The products were then added to the ABI3130XL sequencing instrument. The raw data collected from the ABI3130XL sequencing instrument were analyzed using GeneMapper 4.1. The results of SNaPshot genotyping are shown in Fig. 1.

Quality control criteria that SNPs met qualify for further analysis included: a genotyping success rate > 98.0%, a genotype concordance rate > 99%, a call minor allele frequency > 0.05 and no deviation of Hardy–Weinberg equilibrium (HWE) in cases and controls (all  $P > 0.05$ ) (22).



**Fig. 1:** The waveform and genotypes of seven polymorphic loci/Note: Fluorescently labeled extended products: G peak = blue; A peak = green; T peak = red; C peak = black

### Statistical analysis

We tested the genotype distributions of the seven SNPs described above for HWE using  $\chi^2$ -test implemented in Haploview version 4.2. (23) The differences in continuously distributed variables were assessed using the Student's  $t$ -test and one-

way analysis of variance (ANOVA), and categorical variables between the study groups were compared using chi-squared tests. Independent odds ratio (OR) and its 95% confidence interval (CI) were estimated using unconditional binary logistic regression as a measure of the association between genotype and risk of T2D. We performed power

calculations using QUANTO software version 1.2.4, and corrected for multiple testing using Bonferroni correction to control type I error. Statistical analyses were performed using the SPSS software version 17.0 (SPSS, Chicago, IL, USA);  $P$  values  $<0.05$  were considered statistically significant.

## Results

### *The basic characteristics of the study population*

A total of 272 (136 T2D patients and 136 health controls) ethnic Dong subjects were included in the final analyses. There were no differences in gender (59 and 54 males in the patient and healthy control groups, respectively) and age (mean  $\pm$  SD of  $62.47 \pm 9.41$  and  $61.23 \pm 11.20$  years, respectively) between the study groups ( $P > 0.05$ ).

The compared results of the basic characteristics between patients and controls are shown in Table 2. The levels of BMI, FPG, 2hPG, HOMA-IR and TG were significantly higher in patients than in controls (all  $P < 0.01$ ), whereas HOMA-B and HDL-C were lower in patients than in controls (all  $P < 0.05$ ). There were no significant differences between patients and controls in age, sex, FINS, TC and LDL-C.

### *Allele and genotype distribution*

All of the seven SNPs examined in the present study were in HWE, and all the minimum allele

frequencies (MAF) were  $> 1\%$ , consistent with the principles of population genetics. The response rate of each polymorphic locus was  $> 99.0\%$ . The statistical powers of the seven genetic loci ranged from 85 to 94%, which was sufficient to detect the associations between T2D and the loci.

As shown in Table 3, the frequencies of the risk T allele and the CT and TT genotypes at rs2240727 of TOMM7 were significantly higher in patients than in controls; a significant association between T2D and rs2240727 was observed (for the T allele: OR = 1.65, 95% CI 1.17–2.32,  $P = 0.004$ ; for the CT genotype: OR = 2.64, 95% CI 1.28–5.45,  $P = 0.007$ , for the TT genotype: OR = 3.42, 95% CI 1.58–7.41,  $P = 0.001$ ). After Bonferroni correcting for multiple testing, the association remained significant ( $P < 0.05$ ). We also performed unconditional binary logistic regression to assess the associations between rs2240727 and risk of T2D. The results suggested that, after adjusting for the confounders of age, gender, and BMI, the associations remained significant ( $P < 0.05$ , Table 4). However, rs849230 of PARD3B, rs149228 of LOC729993, rs16862811 of EPHA4, rs3099797 of HNT, and rs17584499 and rs649891 of PTPRD showed no significant differences between groups. Therefore, these genetic loci were not associated with the risk of T2D in this population (all  $P > 0.05$ ).

**Table 2:** Comparison of the basic characteristics of the study populations

Variable	Patients (n =136)	Controls (n =136)	Statistics	Pvalue
Age(y)	62.47 $\pm$ 9.41	61.23 $\pm$ 11.20	0.99	0.323
Sex(M/F)	59/77	54/82	0.538	0.623
BMI	24.73 $\pm$ 3.47	22.32 $\pm$ 3.00	6.13	0.000**
FPG (mmol/L)	9.59 $\pm$ 2.78	5.91 $\pm$ 0.39	15.28	0.000**
2hPG (mmol/L)	12.85 $\pm$ 4.05	6.83 $\pm$ 1.29	16.25	0.000**
FINS (uIU/ml)	14.17 $\pm$ 5.37	15.52 $\pm$ 6.05	1.95	0.052
HOMA-IR	6.02 $\pm$ 2.87	4.08 $\pm$ 1.63	6.85	0.000**
HOMA-B	53.88 $\pm$ 30.18	119.41 $\pm$ 37.15	15.97	0.000**
TC(mmol/L)	6.16 $\pm$ 1.36	6.06 $\pm$ 1.18	0.670	0.503
TG(mmol/L)	2.56 $\pm$ 1.55	1.76 $\pm$ 1.05	4.939	0.000**
HDL-C(mmol/L)	0.92 $\pm$ 0.22	0.98 $\pm$ 0.25	-2.155	0.032*
LDL-C(mmol/L)	1.50 $\pm$ 0.39	1.45 $\pm$ 0.38	1.056	0.292

Note: Values are expressed as means  $\pm$  SD. \*  $P$  value  $<0.05$ , \*\*  $P$  value  $<0.01$ ; All  $P$  values are noted for the comparison with the control group; Comparisons were performed by Student t test and Chi-square test. BMI: Body mass index, FPG: Fasting plasma glucose, 2hPG: 2-hour postprandial glucose, FINS: Fasting insulin level, HOMA-IR: HOMA model insulin resistance index, HOMA-B: HOMA model  $\beta$ -cell function index, total cholesterol(TC), trigalloyl glycerol(TG), high density lipoprotein cholesterol(HDL-C) and low density lipoprotein cholesterol(LDL-C).

Table 3: Genetic association analysis of seven polymorphic loci with type 2 diabetes

	Chr.	Position (bp)	Candidate Genes	Risk allele	Allele and genotype	Patients n (%)	Controls n (%)	OR (95% CI) <sup>a</sup>	$\chi^2$	$P_b$ value	$P_c$ value
rs2240727	7	22819037	TOMM7	T	C	99 (36.40)	132 (48.53)				
					T	173 (63.60)	140 (51.47)	1.65 (1.17-2.32)	8.194	0.004	0.028
					CC	13 (9.56)	32 (24.26)				
					CT	73 (53.68)	68 (50.00)	2.64 (1.28-5.45)	7.186	0.007	0.049
					TT	50 (36.76)	36(25.74)	3.42 (1.58-7.41)	10.125	0.001	0.007
rs849230	2	205969274	PAR3B	A	G	5 (1.84)	11 (4.04)				
					A	267 (98.16)	261(95.96)	2.25 (0.77-6.57)	2.374	0.123	
					GA	5 (3.68)	11 (8.09)				
					AA	131 (96.32)	125 (91.91)	2.31 (0.78-6.82)	2.391	0.122	
rs149228	16	13211975	LOC729993	C	A	178 (65.44)	170 (62.50)				
					C	94 (34.56)	102 (37.50)	0.88 (0.62-1.25)	0.510	0.475	
					AA	59 (43.38)	50 (36.76)				
					CA	60 (44.12)	70 (51.47)	0.73 (0.44-1.21)	1.508	0.219	
					CC	17 (12.50)	16 (11.77)	0.90 (0.41-1.96)	0.070	0.792	
rs16862811	2	222109330	EPHA4	G	T	37 (13.60)	28 (10.29)				
					G	235 (86.40)	244 (89.71)	0.73 (0.43-1.23)	1.415	0.234	
					TG	7 (5.15)	8(5.88)				
					GT	30 (22.06)	20(14.71)	1.64 (0.87-3.07)	0.837	0.360	
					GG	99 (72.79)	108 (79.41)	0.96 (0.33-2.73)	0.008	0.931	
rs3099797	11	131626596	HNT	C	T	18 (6.62)	17 (6.25)				
					C	254 (93.38)	255 (93.75)	0.94 (0.47-1.87)	0.031	0.861	
					TT	1 (0.74)	1 (0.74)				
					CT	16 (11.76)	15 (11.03)	1.08 (0.51-2.27)		1.000	
					CC	119(87.50)	120 (88.23)	1.01 (0.06-16.31)	0.000	1.000	
rs17584499	9		PIPRD	T	C	242 (88.97)	232 (85.29)				
					T	30 (11.03)	40 (14.71)	0.72 (0.43-1.19)	1.640	0.200	
					CC	107 (78.68)	98 (72.06)				
					CT	28 (20.58)	36 (26.47)	0.71 (0.41-1.25)	1.391	0.238	
					TT	1 (0.74)	2 (1.47)	0.46 (0.04-5.13)	0.005	0.946	
rs649891	9	10420602	PIPRD	C	T	60 (22.06)	69 (25.37)				
					C	212 (77.94)	203 (74.63)	1.20 (0.81-1.78)	0.823	0.364	
					TT	8 (5.88)	7 (5.15)				
					CT	44 (32.35)	55 (40.44)	0.71 (0.43-1.17)	0.415	0.519	
					CC	84 (61.77)	74 (54.41)	1.01(0.35-2.91)	0.000	0.990	

Notes: Chr chromosome, bp base-pair, OR odds ratio, CI confidence interval/<sup>a</sup> Odds ratios were calculated using the unconditional binary logistic regression to measure the ORs./  $\chi^2$  values and  $P_b$  values were calculated using the Pearson chi-square test, compared with the first genotype./  $P_c$  was adjusted  $P$  value by Bonferroni correction

**Table 4:** Multivariate Binary logistic regression analysis of diabetic risk factors

Mode	$\beta$ e	SE.	Wald- $\chi^2$	df	Sig.	OR (95% CI)
Constant	-1.752	0.409	18.393	1	0.000	0.17
Sex	0.183	0.267	0.488	1	0.485	1.21(0.71-2.03)
Age: >60 years	0.448	0.263	2.894	1	0.089	1.57(0.93-2.62)
BMI: overweight or obese	1.335	0.271	24.246	1	0.000	3.80(2.23-6.46)
rs2240727: CT	1.056	0.390	7.315	1	0.007	2.87 (1.34-6.18)
TT	1.338	0.417	10.270	1	0.001	3.81(1.68-8.64)

Note: BMI Body mass index.

#### *The association between the genotypes of rs2240727 and the biochemical indicators*

We also performed one-way analysis of variance (ANOVA) to examine the association between FPG, 2hPG, FINS, HOMA-IR, HOMA-B, TC, TG, HDL-C, LDL-C and the genotypes of rs2240727 in the controls and the patients respectively, the results were shown in Table 5 and Table 6. As shown in Table 5, there were significantly statistical difference in levels of FPG and HOMA-B among genotypes of rs2240727 in controls. The levels of FPG were significantly higher

in CT and TT genotypes than in CC genotype, whereas the levels of HOMA-B were significantly lower in CT and TT genotypes than in CC genotype (all  $P < 0.05$ ).

As shown in Table 6, there were significantly statistical difference in levels of FPG among genotypes of rs2240727 in the patients; the levels of FPG were significantly higher in CT and TT genotypes than in CC genotype, whereas there were no association between genotypes of rs2240727 and the other biochemical indicators.

**Table 5:** Association analysis between the genotypes of rs2240727 and the biochemical indicators in the controls (mean  $\pm$  SD)

Variable	rs2240727			Fvalue	Pvalue
	CC(n =32)	CT(n =68)	TT(n =36)		
FPG (mmol/L)	5.53 $\pm$ 0.28	5.78 $\pm$ 0.33	5.94 $\pm$ 0.26	16.581	0.000**
2hPG (mmol/L)	7.08 $\pm$ 1.46	6.74 $\pm$ 1.29	6.75 $\pm$ 1.10	0.719	0.490
INS (uIU/ml)	14.71 $\pm$ 5.17	13.68 $\pm$ 3.89	14.47 $\pm$ 3.79	0.824	0.441
HOMA-IR	3.62 $\pm$ 1.28	3.51 $\pm$ 1.02	3.82 $\pm$ 1.01	0.967	0.383
HOMA-B	147.49 $\pm$ 56.11	122.93 $\pm$ 40.38	120.01 $\pm$ 34.93	4.317	0.015*
TC(mmol/L)	5.98 $\pm$ 1.48	6.10 $\pm$ 1.19	6.04 $\pm$ 0.88	0.110	0.896
TG(mmol/L)	1.79 $\pm$ 0.97	1.74 $\pm$ 1.03	1.78 $\pm$ 1.17	0.023	0.977
HDL-C(mmol/L)	1.00 $\pm$ 0.28	0.95 $\pm$ 0.24	1.01 $\pm$ 0.24	1.016	0.365
LDL-C(mmol/L)	1.53 $\pm$ 0.42	1.41 $\pm$ 0.40	1.46 $\pm$ 0.29	1.183	0.310

NOTE: Comparisons were performed by the one way ANOVA. \*  $P$  value  $< 0.05$ , \*\*  $P$  value  $< 0.01$ .

**Table 6:** Association analysis between the genotypes of rs2240727 and the biochemical indicators in the patients (mean  $\pm$  SD)

Variable	rs2240727			Fvalue	Pvalue
	CC(n =13)	CT(n =73)	TT(n =50)		
FPG (mmol/L)	8.22 $\pm$ 0.90	8.64 $\pm$ 0.92	9.38 $\pm$ 2.67	3.500	0.033*
2hPG (mmol/L)	12.43 $\pm$ 3.55	13.38 $\pm$ 4.26	12.19 $\pm$ 3.82	1.353	0.262
INS (uIU/ml)	14.51 $\pm$ 5.48	14.38 $\pm$ 5.26	13.77 $\pm$ 5.58	0.216	0.806
HOMA-IR	5.23 $\pm$ 1.75	5.49 $\pm$ 1.96	5.56 $\pm$ 2.48	0.286	0.751
HOMA-B	66.73 $\pm$ 43.90	58.33 $\pm$ 26.05	54.78 $\pm$ 29.55	0.876	0.419
TC(mmol/L)	6.22 $\pm$ 1.49	6.08 $\pm$ 1.38	6.26 $\pm$ 1.33	0.261	0.771
TG(mmol/L)	2.40 $\pm$ 1.31	2.62 $\pm$ 1.60	2.50 $\pm$ 1.55	0.150	0.861
HDL-C(mmol/L)	0.93 $\pm$ 0.17	0.90 $\pm$ 0.21	0.94 $\pm$ 0.23	0.525	0.593
LDL-C(mmol/L)	1.51 $\pm$ 0.44	1.54 $\pm$ 0.43	1.45 $\pm$ 0.31	0.884	0.461

NOTE: Comparisons were performed by the one way ANOVA. \*  $P$  value  $< 0.05$ .

## Discussion

Type 2 diabetes is a complex polygenic disease. Recent increased economic development, improved living standards, and social demographic aging have caused the incidence of T2D to increase annually in both developed and developing countries. An epidemiological survey in 2003 demonstrated that there were nearly 200 million patients with diabetes worldwide, and this figure was estimated to exceed 300 million by 2025. Yang et al. (24) reported that the age-standardized prevalence of diabetes is 9.7% in adults >20 years old in 14 provinces and autonomous regions of China. The aim of this study was to assess the associations of seven novel genetic variants identified in a recent GWAS (14) in Mexican American populations, and the gene expression study (15-16) in a Chinese population.

The results of this study demonstrated that there were significant differences between the patients and the healthy controls in terms of BMI, FPG, 2hPG, HOMA-IR, HOMA-B, TG and HDL-C (all  $P < 0.05$ ). These data suggest that BMI, HOMA-IR, HOMA-B, TG and HDL-C might be environmental risk factors for T2D in Chinese Dong individuals, consistent with previous studies (25-27). Because these biochemical indicators were measured after disease onset, it was difficult to establish a causal relationship. Therefore, this should be assessed in a prospective study.

There are little studies have suggested roles for PARD3B, LOC729993, EPHA4, HNT, PTPRD, TOMM7, or intergenic regions in T2D. PARD3B is a homolog of a *C. elegans* cell polarity determinant that localizes to tight junctions in human epithelial cells. The cell polarity protein PAR3, conserved from nematodes to vertebrates, forms a complex with PAR6 and atypical protein kinase C (aPKC) at the tight junctions of mammalian epithelial cells. Previously, we cloned the human cDNA for a novel PAR3 homologue, designated PAR3beta, whose mRNA is present in a variety of tissues, but is expressed most abundantly in adult and fetal kidneys (28). PARD3B is a large gene comprised of 23 exons located on chromosome 2,

which encodes a 1205 amino acid protein from a gene 1.07 Mbp in length. Exon 20 can be alternatively spliced, resulting in three potential variants. However, the functional significance of these different isoforms remains unclear, although they may have different expression patterns (29). A previous study (14) demonstrated that the rs849230 locus was associated with T2DM (OR = 1.83, 95%CI: 1.57–2.10).

TOMM7 gene is located on chromosome 7p15.3, and is also known as a homologue of the translocate mitochondrial outer membrane 7. This gene encodes a subunit of a mitochondrial outer membrane transporter that regulates components and the stability of transportation complex. A GWAS study (14) reported that rs2240727 of the TOMM7 gene was associated with T2DM (OR = 1.37, 95% CI: 1.24–1.50).

Our study only replicated the associations between T2D and rs2240727 at TOMM7 ( $P = 0.005$ ), the frequencies of T allele, CT and TT genotypes of rs2240727 were significantly higher in patients with T2D compared with healthy controls. These associations remained significant after adjusting the confounders of age, gender, and BMI. These findings suggest that TOMM7 is the diabetes gene which is independent of ethnic background, age, gender, and BMI. Moreover the OR (1.65, 95%CI: 1.17–2.32) of rs2240727 in TOMM7 in our study was slightly higher than that identified in previous European populations (OR = 1.37, 95% CI: 1.24–1.50), which suggests some potential ethnic and regional differences between this and the current study. This suggests that rs2240727 confers a higher risk of T2D in Chinese Dong populations than in Mexican-American populations. In addition, after correcting for multiple testing, the association between T2D and rs2240727 remained significant ( $P < 0.05$ ).

This study did not find any correlation between the rs849230, rs149228, rs16862811, rs3099797, rs17584499, and rs649891 loci with T2D in Chinese Dong individuals, which differs from the GWAS study (14). This could be due to ethnic differences, and should be confirmed in further studies in different ethnic populations.



We also sought to determine whether there were any associations between rs2240727 and the biochemical indicators in controls and patients. We found that there were associations between FPG, HOMA-B and the genotypes of rs2240727 in controls, the levels of FPG were significantly higher in CT and TT genotypes than in CC genotype, whereas the levels of HOMA-B were significantly lower in CT and TT genotypes than in CC genotype (all  $P < 0.05$ ). We also found association between FPG and the genotypes of rs2240727 in patients. These findings suggested that rs2240727 of TOMM7 gene may induce the risk of T2D by affecting the level of FPG in Chinese Dong populations, but the really mechanism is not clearly. Therefore, further studies are needed to explore the pathogenesis of T2D and this gene.

## Conclusion

Our results present new evidence for the association between T2D and rs2240727 in TOMM7 gene in Chinese Dong populations, but further genetic and functional studies are needed to elucidate the biological processes underlying the association between T2D and rs2240727.

## Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

## Acknowledgements

This study was supported by the Scientific Research Project of the Department of Education in Hunan Province, China (No.:11C1007), and the Specialized Research Fund for the Doctoral Program of Higher Education of China (the class of Doctoral tutor) (No.: 20120162110071).

We thank the assistance in collection and detection of sample provided by the teachers Li-Ping Yao, Xue-Mei Huang, and Ying Zhang in the De-

partment of Medical Laboratory of Huaihua Medical College, Hunan, China. The Institute of Clinical Pharmacology of the Central South University supported this study in Changsha, China. Finally, we thank the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co., Ltd. China for genotyping support. The authors declare that they have no conflict of interests.

## References

1. Maivel H, Ghattas, Mahmoud A. Amer (2012). Possible role of microsomal epoxide hydrolase gene polymorphism as a risk factor for developing insulin resistance and type 2 diabetes mellitus. *Endocrine*, 42(3):577-583.
2. Han X, Luo Y, Ren Q, Zhang X, Wang F, Sun X, et al. (2010). Implication of genetic variants near SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, FTO, TCF2, KCNQ1, and WFS1 in Type 2 Diabetes in a Chinese population. *BMC Medical Genetics*, 11:81-89.
3. Xu M, Bi Y, Xu Y, Yu B, Huang Y, Gu L, et al.(2010). Combined Effects of 19 Common Variations on Type 2 Diabetes in Chinese: Results from Two Community-Based Studies. *PLoS one*, 11: e14022.
4. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. (2010). Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet*, 7(42): 579-589.
5. Zheng YY1, Xie X, Ma YT, Yang YN, Fu ZY, Li XM, et al. (2012). Relationship between type 2 diabetes mellitus and a novel polymorphism C698T in C5L2 in the Chinese Han population. *Endocrine*, 41(2):296-301.
6. Burgdorf KS, Grarup N, Justesen JM, Harder MN, Witte DR, Jorgensen T, et al. (2011). Studies of the Association of Arg72Pro of Tumor Suppressor Protein p53 with Type 2 Diabetes in a Combined Analysis of 55, 521 Europeans. *PLoS one*, 1: e15813.
7. Rees SD, Hydrie MZ, Shera AS, Kumar S, O'Hare JP, Barnett AH, et al.(2011). Replication of 13 genome-wide association (GWA)-validated risk variants for type 2 diabetes in Pakistani populations. *Diabetologia*, 6(54):1368-1374.

8. Lili Q, Bangshun H, Yuqin P, Yongfei X, Chan ZH, Zhipeng T, et al.(2011). Association between polymorphisms in RAPGEF1, TP53, NRF1 and type 2 diabetes in Chinese Han population. *Diabetes Res Clin Pract*, 12(91): 171 – 176.
9. Raza ST, Fatima J, Ahmed F, Abbas S, Zaidi ZH, Singh S, et al. (2013). Association of angiotensin- converting enzyme (ACE) and fatty acid binding protein 2 (FABP2) genes polymorphism with type 2 diabetes mellitus in Northern India. *J Renin Angiotensin Aldosterone Syst.* (Epub ahead of print)
10. Ali S, Chopra R, Manvati S, Singh YP, Kaul N, Behura A, et al. (2013). Replication of type 2 diabetes candidate genes variations in three geographically unrelated Indian population groups. *PLoS One*, 3:e58881.
11. Pueyo N, Ortega FJ, Mercader JM, Moreno-Navarrete JM, Sabater M, Bonàs S, et al.(2013). Common genetic variants of surfactant protein-D (SP-D) are associated with type 2 diabetes. *PLoS One*, 4:e60468.
12. Lili Cao, Wei Zhou, Yanbei Zhu, Wenwen Guo, Zhenming Cai, Xuan He, et al.(2013). Combined analysis of polymorphism variants in hMTH1, hOGG1 and MUTYH genes on the risk of type 2 diabetes in the Chinese population. *Gene*, 1(519):50-54.
13. Saxena R, Saleheen D, Been LF, Garavito ML, Braun T, Bjornes A, et al.(2013). Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. *Diabetes*, 5(62):1746-1755.
14. Below JE, Gamazon ER, Morrison JV, Konkashbaev A, Pluzhnikov A, McKeigue PM, et al.(2011). Genome-wide association and meta-analysis in populations from Starr County, Texas, and Mexico City identify type 2 diabetes susceptibility loci and enrichment for expression quantitative trait loci in top signals. *Diabetologia*, 54(8):2047–2055.
15. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, et al. (2010). A Genome-Wide Association Study Identifies Susceptibility Variants for Type 2 Diabetes in Han Chinese. *PLoS Genetics*. 2:e1000847.
16. Chang YC, Chiu YF, Liu PH, Shih KC, Lin MW, Sheu WH, et al. (2012). Replication of genome-wide association signals of type 2 diabetes in Han Chinese in a prospective cohort. *Clin Endocrinol (Oxf)*, 76(3):365-372.
17. Jun Y, Yan J, Yanwen H (2007).The analysis on the correlated indicators of diabetes screening in the Chinese Dong nationality in Guizhou province. *Guizhou medicine*, 5(12) : 1125.
18. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, et al. (2002). Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract*, 55(1):65-85.
19. Zhou B F (2002). Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults--study on optimal cut-off points of body mass index and waist circumference in Chinese adults (J). *Biomed Environ Sci*, 15(1):83-96.
20. Zhang X, Qiao H, Zhao Y, Wang X, Sun H, Liu A, et al.(2012).Association of Single Nucleotide Polymorphisms in TCF2 with Type 2 Diabetes Susceptibility in a Han Chinese Population. *PLoS One*, 12: e52938.
21. Zhang NN, Yao L, Zhuang MQ, Wang GC, Chen TT, Yang YJ, et al.(2012). Association between mannose-binding lectin 2 gene and protein kinase C-beta 1 gene polymorphisms and type 2 diabetic macrovascular complications in northern Chinese Han population. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, 12(6):709-714.
22. Xiao Yun Bao, Bin Peng, Mao Sheng Yang (2012). Replication study of novel risk variants in six genes with type 2 diabetes and related quantitative traits in the Han Chinese lean individuals. *Mol Biol Rep*, 39(3):2447-54.
23. Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*, 21(2): 263–265.
24. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, et al.(2010). Prevalence of Diabetes among Men and Women in China. *N Eng J Med*, 362(12):1090-1101.
25. Zhang J, Dong Z, Li G, Jiao S, Xie J, Zhou Y, et al. (2011). The cross-sectional investigation and the study on risk factors of type 2 diabetes of the residents in Beijing. *Chinese J Epidemiol*, 4:357-360.
26. Tan S, Avalos G, Dineen B, Burke A, Gavin J, Brennan M, et al. (2009). Traveler health: prev-

- alence of diabetes, pre diabetes and the metabolic syndrome. *Ir Med J*, 6:176-178.
27. Moraes SA, Freitas IC, Gimeno SG, Mondini L (2010). Diabetes mellitus prevalence and associated factors in adults in Ribeirão Preto, São Paulo, Brazil, 2006: OBEDIARP Project. *Cad Saude Publica*, 26(5):929-941.
28. Kohjima M, Noda Y, Takeya R, Saito N, Takeuchi K, Sumimoto H, et al.(2002). PAR3beta, a novel homologue of the cell polarity protein PAR3, localizes to tight junctions. *Biochem Biophys Res Commun*, 299(4):641-646.
29. Gao L, Macara IG, Joberty G (2002). Multiple splice variants of Par3 and of a novel related gene, Par3L, produce proteins with different binding properties. *Gene*, 29(1-2):99-107.