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Regular paper

Association between *UBE2E2* variant rs7612463 and type 2 diabetes mellitus in a Chinese Han Population

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***UBE2E2* encodes ubiquitin-conjugating enzyme E2E2, which plays an important role in the synthesis and secretion of insulin. Two previous studies indicated that SNPs in *UBE2E2* were associated with risk for type 2 diabetes mellitus (T2DM) in the Japanese and Korean populations, respectively. We examined the association of one SNP in this gene, rs7612463, with the risk of T2DM in 1957 Han participants in northeastern China, using an SNPscan™ Kit. rs7612463 genotype was significantly associated with risk for T2DM under various genetic models, including an additive model ($P=0.004$), a dominant model ($P=0.024$), and a recessive model ($P=0.008$). The AA genotype was associated with a significantly decreased risk for T2DM ($P=0.004$, OR=0.513, 95% CI=0.325–0.810) after adjustment for age, gender, and BMI. The heterozygous genotype, AC, was associated with increased risk for total cholesterol (mmol l-1; $P=0.031$) and triglycerides (mmol l-1; $P=0.039$) in control individuals. Our results show that rs7612463 is associated with T2DM, with homozygotes of the AA genotype at decreased risk for T2DM in the Chinese population. Additionally, heterozygotes may have decreased risk of T2DM due to insulin resistance.**

Key word: Type 2 diabetes mellitus; UBE2E2; rs7612463; Single nucleotide polymorphism; SNPscan

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INTRODUCTION

Currently, about 300 million people suffer from type 2 diabetes mellitus (T2DM), while the incidence continues to grow exponentially (Wild *et al*., 2004). Although the mechanisms by which T2DM develops remain partly unclear, many studies show that T2DM is a genetic disease and is not only due to the influence of environmental factors (Stumvoll *et al*., 2005).

To date, more than 75 T2DM susceptibility genes have been identified, mostly through genome-wide association studies (GWAS) (Sanghera & Blackett, 2012). The initial studies, and indeed the majority of them, have been performed in the European population. However, recent studies of the development of T2DM are also beginning to appear in the Japanese, Korean and Chinese populations (Park, 2011). The association at the *UBE2E2* locus was first identified in a Japanese GWAS of T2DM (Yamauchi *et al*., 2010).

UBE2E2 encodes ubiquitin-conjugating enzyme E2E 2. UBE2E2 is expressed in the pancreas, liver, and adipose tissue, as well as in an insulin-secreting cell line (Hicke, 1999; Strous *et al*., 1999). It is a component of the ubiquitination system (Jentsch, 1992), which influences the quality of proteins, gene transcription, and cell function (Hartley *et al*. 2009). UBE2E2 plays an important role in the synthesis and secretion of insulin.

In recent years, there have been several studies which explored the relationship between *UBE2E2* polymorphisms and T2DM. Some of these studies indicated the presence of an association between SNPs in *UBE2E2* and risk for T2DM in the Japanese and Korean populations (Park, 2011; Yamauchi *et al*., 2010). However, analysis in European and Saudi subjects could not replicate the association between the *UBE2E2* locus and risk for T2DM (Alharbi, 2014).

To improve our understanding of the role of *UBE2E2* in T2DM predisposition, it is important to understand the consequences of genetic diversity in other ethnic populations. Han Chinese constitutes the largest ethnic group in the world, but spurious associations due to its population structure pose a challenge to genetic studies. One approach to counteract this problem may be to study a greater number of populations to obtain a broader spectrum of data from East Asia. To our knowledge, the role of *UBE2E2* on T2DM in the Han population has not been previously assessed. Therefore, we conducted a case-control study to investigate the role of the leading *UBE2E2* SNP on T2DM risk in the Han population from northeastern China using SNPscan.

MATERIALS AND METHODS

Study population and clinical parameters. A total of 1957 participants were enrolled from the Second Affiliated Hospital of Harbin Medical University (Harbin, China), including 964 T2DM patients and 993 controls. These participants gave informed consent and were of Han Chinese ancestry, residing in northeast China.

Criteria for inclusion in the control group were the following: 1) a HbA1c level $<6.0\%$; 2) an FPG level <5.1 mmol/L; 3) no history of a diagnosis of diabetes; 4) no family history of T2DM; 5) not taking drugs that affect lipid and carbohydrate exchange; and 6) no systemic diseases.

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Abbreviations: T2DM, type 2 diabetes mellitus; UBE2E2, ubiquitin-conjugating enzyme E2E 2; GWAS, genome-wide association study; BMI, body mass index; SNP, single nucleotide polymorphism; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus

Table 1. Baseline characteristics of individuals in the cases and controls

Variable	Case (n=993)	Control (n=964)	P value
Gender (male:female)	567:397	610:383	0.238
Age (years)	46.09±12.54	42.96±11.69	0.000
BMI (kg m^{-2})	25.78±3.58	23.34±3.35	0.000
WHR	0.94±0.06	0.85±0.07	0.000
Systolic pressure (mm Hg)	130.17±17.52	121.32±15.06	0.000
Diastolic pressure (mm Hg)	84.63±11.12	79.28±9.63	0.000
Total cholesterol (mmol l^{-1})	4.99±1.29	4.88±1.01	0.025
Triglyceride (mmol l^{-1})	2.38±2.25	1.42±0.95	0.000
HDL-C (mmol l^{-1})	1.21±0.32	1.47±0.35	0.000
LDL-C (mmol l^{-1})	2.91±0.96	2.92±0.86	0.805
FPG (mmol l^{-1})	7.48±7.42	4.84±4.43	0.000
Fasting insulin (uU ml^{-1})	10.49±3.40	7.88±4.43	0.000
HbAlc（%）	9.29±2.37	5.12±0.47	0.000
Homa-β	12.89±7.58	130.77±150.31	0.000
Homa-IR	59.32±169.49	1.70±0.98	0.000

Abbreviations: BMI, body mass index; WHR, waist–hip ratio; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-β, HOMA of β cell function; HOMA-IR, homeostasis model assessment of insulin resistance; Data are presented as mean ± S.D.

T2DM was diagnosed according to the criteria of the World Health Organization (WHO) in 1999 (Alberti and Zimmet, 1998), defined by the following features: FPG ≥ 7.0 mmol/L and/or 2 h postprandial plasma glucose ≥ 11.1 mmol/L. Diabetes was diagnosed not more than 6 months ago, and they were not treated with insulin.

Exclusion criteria for the study group were: 1) renal and hepatic failure; 2) cardiovascular disease; 3) acute diabetic complications; 4) malignant tumors, severe injury, infections, or other endocrine diseases; or 5) other types of diabetes.

First, gender, age, height, weight, waist circumference, hip circumference, and blood pressure were recorded. Second, clinical parameters of the patients were tested, including FPG, plasma lipids, insulin concentration, and HbA1c. Finally, HOMA-β, HOMA-IR, and BMI were calculated.

Genotyping. A total of 4 ml of venous blood was collected from each person. Blood samples were collected from patients using Vacutainers and transferred to tubes lined with EDTA. SNP genotyping was performed using a custom-design 2×48-Plex SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which was based on double ligation and multiplex fluorescence PCR. In order to validate the genotyping accuracy using the SNPscan™ Kit, a 5% random sample of cases and controls were genotyped twice for all SNPs by different analysts. Specifically, we included 100 pairs of blind duplicates, and the concordance rate was more than 98%.

Statistical analyses. The Hardy–Weinberg equilibrium was evaluated using Pearson's χ^2 test separately for cases and controls. Differences between cases and controls in demographic characteristics and risk factors were evaluated using a χ^2 test (for categorical variables) or Student's *t*-test (for continuous variables). The allele frequencies between cases and controls were compared using a χ^2 test or Fisher's exact probability test, where appropriate. Statistical evaluations for testing genetic effects of association between the case–control status and each individual SNP, measured by the odds ratios (ORs) and 95% confidence intervals (CIs), were estimated using unconditional logistic regression after adjusting for age, gender and BMI. SPSS 17.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses. All presented *P*-values are two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population

Characteristics of the cases and controls are shown in Table 1. A total of 964 T2D patients and 993 non-diabetic controls were genotyped for one SNP (rs7612463) and analyzed for association with T2DM. The difference in the distributions of gender ($P = 0.238$) and LDL-C ($P = 0.805$) between cases and controls were not statistically significant. The other clinical parameters did show statistically significant associations between cases and controls.

Table 2. Association between rs7612463 and risk for type 2 diabetes

SNP	rs7612463	Cases N (%)	Controls N (%)	*p*-value	OR(95%CI)
allele	A	392（19.7%)	463（24.0%)		
	C	1594（80.3%	1465（76.0%)	0.001	1.056 (1.022–1.092)
total		1968 (100%)	1928 (100%)		
genotype	AA	36 (3.6%)	62 (6.4%)		
	AC	637 (64.2%)	563 (58.4%)		
	CC	320 (32.2%)	339 (35.2%)		
total		993 (100%)	964 (100%)	0.003	

Abbreviations: CI, confidence interval: OR, odds ratio

The genotype distributions of the investigated SNP did not deviate from Hardy–Weinberg equilibrium in either cases or controls. The allele and genotype distributions of rs7612463 in cases and the controls are shown in Table 2. We found that allele and genotype frequencies were different between T2DM patients and healthy controls ($P = 0.001$ and $P = 0.003$, respectively).

Association analysis of this SNP revealed that the homozygous AA carriers had a significantly decreased T2DM risk compared to homozygous carriers of CC after adjustment for age, sex and BMI. These results were significant under different ge-

Table 3. Effects of rs7612463 genotype on the risk of type 2 diabetes under different genetic models

SNP	genotype	Additive model		Dominant model		Recessive model	
		p-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)
rs7612463	AA	0.004	0.513 (0.325–0.810)				
	AC	0.120	0.851 (0.694–1.043)				
	CC			0.024	0.799 (0.657–0.971)	0.008	0.544 (0.346–0.853)

Abbreviations: CI, confidence interval; OR, odds ratio; BMI, body mass index; a, adjusted for age, gender, BMI

netic models (Table 3): in an additive model ($P = 0.004$, OR = 0.513, 95% CI = 0.325–0.810), a dominant model ($P = 0.024$, OR = 0.799, 95% CI = 0.657–0.971), and a recessive model ($P = 0.008$, OR = 0.544, 95% CI = 0.346–0.853). Additionally, rs7612463 was significantly associated with total cholesterol (mmol l^{-1}; $P = 0.031$) and triglycerides (mmol l^{-1}; $P = 0.039$) in the control individuals (Table 4).

DISCUSSION

UBE2E2 plays a critical role inside the beta cell and has been linked with various insulin-related diseases in addition to T2DM, including obesity and atherosclerosis (Marfella *et al.*, 2006; Chang *et al.*, 2009). However, UBE2E2 is beginning to draw interest due to its newly discovered association with lung cancer (Dmitriev *et al.*, 2012). In 2010, rs7612463 was shown to be associated with T2DM in the Japanese population (combined $P = 2.27 \times 10^{-9}$).

In this case–control study, we evaluated the association between rs7612463 at *UBE2E2* and T2DM in the Chinese Han population. We observed different allele frequencies between the case and control groups. The risk of carries of the C allele for T2DM increased by about 5.6% compared to carriers of the A allele. Genotype at rs7612463 was significantly associated with risk for T2DM under different genetic models, including additive, dominant, and recessive. Previously, it was reported that carriers of the AA genotype at rs7612463 have a significantly decreased risk for T2DM. This association was also seen in a GWAS in the Japanese population (Yamauchi *et al.*, 2010). Moreover, GWAS showed that the risk genotypes (CC+CA) had a significantly lower HOMA-β than those with the AA genotype, suggesting a role for this variant in the reduction of insulin secretion. However, the heterozygous state (AC) was associated with increased total cholesterol and triglycerides in the Chinese population. In diabetes, postprandial regulation of lipids is impaired as a result of insulin resistance and inadequate secretion of insulin, resulting in increased levels of triglycerides and cholesterol (Laakso *et al.*, 1993; Alberti & Zimmet, 2006). Insulin also promotes the synthesis of lipids, and inhibits their degradation (Saltiel & Kahn, 2001). Plasma triglycerides and high-density lipoprotein (HDL) cholesterol levels are independently associated with insulin resistance and are independent predictors of cardiovascular disease (CVD) (Manninen *et al.*, 1992; Criqui *et al.*, 1993). Indian researchers have pointed out that inappropriate regulation of the ubiquitin-proteasome system could lead to the destruc-

Table 4. The cumulative effect of rs7612463 genotype on baseline characteristics of control individuals

Variable	AA	AC	CC	*p* value
BMI (kg m^{-2})	23.51±3.30	23.37±3.48	23.13±3.15	0.456
WHR	0.85±0.07	0.85±0.07	0.84±0.07	0.581
Systolic pressure (mm Hg)	120.53±13.52	121.66±15.57	121.38±15.40	0.646
Diastolic pressure (mm Hg)	78.92±8.82	79.87±10.31	78.51±8.92	0.152
Total cholesterol (mmol l^{-1})	4.79±1.02	4.96±0.98	4.79±1.05	0.031
Triglyceride (mmol l^{-1})	1.33±0.83	1.49±1.07	1.34±0.80	0.039
HDL-C (mmol l^{-1})	11.47±0.38	1.48±0.35	1.46±0.34	0.736
LDL-C (mmol l^{-1})	2.86±0.87	2.96±0.80	2.90±0.95	0.314
FPG (mmol l^{-1})	4.83±0.28	4.84±0.28	4.84±0.31	0.876
Fasting insulin (uU ml^{-1})	8.24±4.93	7.72±4.32	7.85±4.17	0.340
HbAlc（%）	5.09±0.46	5.13±0.49	5.13±0.44	0.497
Homa-β	133.86±102.59	122.77±150.31	142.49±238.51	0.225
Homa-IR	1.77±1.07	1.67±0.98	1.69±0.92	0.416

Data are presented as mean ± S.D.

tion of important proteins for insulin signaling, such as insulin receptor substrates, which lead to insulin resistance (Balasubramanyam *et al*., 2005). Our study suggests that the mechanism by which the heterozygous allele at rs7612463 affects T2DM risk may be different between the Chinese and Japanese populations. For example, it may be that the CA genotype increases insulin resistance in the Chinese population, while it reduces insulin secretion in the Japanese population.

rs7612463 was associated with GDM in the Korean population, but no associations with HOMA-B or HOMA-IR were seen. This may be due to the pathological difference between patients with GDM and T2DM, but further studies are required (Park, 2011). Iwata and others reported that rs7612463 was not associated with T2DM in the European population. They explained that the lack of replication might have been due to an insufficient sample size included in their study (Iwata *et al*., 2012). Moreover, other studies suggest that the discordance between European and Asian populations may be attributed to genetic heterogeneity and different linkage disequilibrium (LD) structures existing among Europeans (Yamauchi *et al*., 2010).

An association does not necessarily mean that the nucleotide change at the associated SNP leads to direct effects on the phenotype under study. Therefore, it is likely that some causal variants could be ethnic-specific or could be present elsewhere in the same gene or in a nearby gene. It has been suggested that differences in the patterns of LD between this SNP and functional variants at this loci could underlie these disparate findings. Alternatively, gene-environment interactions may operate in the pathogenesis of T2DM, and differences in the levels of environmental risk factors in different populations may alter the impact of susceptibility loci on the risk for T2DM (Yamauchi *et al*., 2010).

In the current sample, drawn from the Han population in northeastern China, false-positive or false-negative associations due to population substructure are less likely to exist. According to the careful ascertainment in this study, the relatively homogeneous case and control samples belong to a single geographic location of the city of Harbin and the residents in the area must be stable. This population is relatively homogeneous: they live in the same area with a sharp-continental climate, have the same taste preferences in the ratio of fat and carbohydrates in the diet, and belong to the same ethnic group.

To our knowledge, this study is the first to demonstrate that rs7612463 is associated with T2DM in the northeastern Han Chinese. However, several inherent limitations must be noted. Because controls were collected from hospitals, some level of selection bias cannot be ruled out. However, all control individuals in our study came to hospitals for routine health examinations and were not hospitalized with specific diseases, probably making the controls more representative of the general population. Under the existing conditions, we believe the potential selection bias to be minimal.

CONCLUSIONS

In conclusion, our comprehensive analysis of rs7612463 indicates that *UBE2E2* may be associated with T2DM risk. Further large-scale association studies and functional studies will be useful to replicate these promising findings and to fully delineate the role of *UBE2E2* in the pathogenesis of T2DM.

Conflict of interests

The authors have declared that no competing interests exist.

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