Pharmacogenomics and Personalized Medicine

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ORIGINAL RESEARCH

Association of *CYP7A1* and *CYP2E1* Polymorphisms with Type 2 Diabetes in the Chinese Han Populations

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Background: Type 2 diabetes mellitus (T2DM) is caused by diverse environmental and genetic risk factors. Previous studies have reported that cytochrome P450 (*CYP*) is a promising gene for T2DM. Therefore, we aimed to determine the effects of *CYP7A1* and *CYP2E1* polymorphisms on T2DM susceptibility among the Chinese Han population.

Methods: A case-control study was conducted to assess the potential relationship of four polymorphisms (rs8192879, rs12542233, rs2070672 and rs2515641) with T2DM susceptibility in the Chinese population, involving 512 T2DM patients and 515 age- and gender-matched healthy individuals. We used the Agena MassARRAY platform to detect *CYP7A1* and *CYP2E1* polymorphisms. The relationship between genetic polymorphisms and T2DM risk was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs) in various genetic models.

Results: After adjusting for age and gender, rs12542233 in the *CYP7A1* gene was significantly associated with decreased T2DM risk (recessive: OR = 0.67, 95% CI = 0.49–0.91, $p = 0.012$; after FDR correction, $p = 0.048$). The *CYP7A1* rs12542233 was associated with a reduced risk of T2DM in people over 59 years of age ($p = 0.010$). In the population with BMI ≤ 24 kg/m^2, *CYP7A1* rs12542233 was associated with an increased risk of T2DM ($p < 0.05$). In the population with BMI > 24 kg/m^2, *CYP2E1* rs2515641 can significantly reduce the risk of T2DM ($p < 0.05$). And rs8192879, rs2070672 and rs2515641 could significantly increase the risk of diabetes retinopathy in T2DM patients ($p < 0.05$). Furthermore, the $T_{rs8192879}C_{rs12542233}$ haplotype was significantly associated with T2DM ($p = 0.019$).

Conclusion: *CYP7A1* and *CYP2E1* polymorphisms may contribute to T2DM susceptibility in the Chinese Han population, especially in stratified analysis.

Keywords: diabetes mellitus, type 2, gene polymorphisms, case-control study, *CYP7A1*, *CYP2E1*

Background

Diabetes mellitus (DM) is a complex metabolic disease characterized by hyperglycemia, which is caused by deficiencies in insulin secretion and function. According to the International Diabetes Federation (IDF), there were about 463 million people with diabetes worldwide in 2019 (diabetes prevalence was 9.3%). It is estimated that the number of diabetes patients in the world will reach 578 million (diabetes prevalence is 10.2%) by 2030, and will increase to 700 million (diabetes prevalence is 10.9%) by 2045.[1] Type 2 diabetes mellitus (T2DM) accounts for approximately 90% of patients with diabetes.[2] Diabetes complications are the main cause of death in diabetes patients, including diabetes retinopathy, renal failure, diabetes foot and cardiovascular disease. One-third of diabetes patients will have diabetes retinopathy, which is related to the risk of systemic vascular complications.[3] It is well known that genetic factors, lifestyle and environmental factors have an important impact on susceptibility to diabetes. Numerous studies have demonstrated that the significance of genetic polymorphisms in cytochromes P450 (CYP) in the pathogenesis of T2DM, such as *CYP2C9, CYP2C19, CYP2D6, CYP3A4*, and *CYP2J2*.[4–6] Cholesterol 7 α-hydroxylase (cytochrome P450 7A1, *CYP7A1*), a member of the CYP family, plays an essential role in regulating the homeostasis of cholesterol and bile acids. It has been reported that bile acids are signaling molecules, which can activate bile acid receptors to regulate bile acid synthesis and glucose metabolism.[7] Some studies have demonstrated that the bile acid pool and excretion increase in diabetic

Received: 24 March 2022
Accepted: 30 August 2022
Published: 21 September 2022

human patients and diabetic animals.[8] Gerhard et al have found that diabetic patients have significantly higher serum bile acid levels than healthy controls.[9] In addition, Prawitt et al showed that the administration of a bile acid sequestrant decreased plasma glucose and HbA1c concentrations.[10] Moreover, previous studies indicated that insulin inhibits the expression of *CYP7A1* in rat hepatocytes, the key enzyme in bile acid synthesis pathways.[11] These findings suggested that *CYP7A1* may be involved in the development of T2DM through bile acid synthesis pathways. *CYP7A1* polymorphisms have been associated with the susceptibility of coronary heart disease, gall bladder stone disease and cancers.[12–15] However, the relationship between *CYP7A1* and T2DM is unclear.

Cytochrome P450 2E1 (*CYP2E1*) is another member of the CYP superfamily and is responsible for the metabolic activation of many low-molecular weight compounds, including ethanol, benzene, vinyl chloride, and N- nitrosamines.[16] The overexpressed *CYP2E1* exhibits a high capacity of produce free radicals that probably cause liver damage and lipid peroxidation in obese T2DM patients.[17] It has been shown that there is elevated activity of *CYP2E1* in the liver of obese patients with T2DM.[18] Previous studies also have shown that T2DM could induce increased levels of *CYP2E1* protein and increase the activities of liver-related enzymes. Moreover, T2DM enhances the toxicity and/or carcinogenic effects of chemicals in the liver by inducing *CYP2E1*-dependent drug-metabolizing enzymes.[19] This evidence suggests that *CYP2E1* plays a crucial role in T2DM development. In addition, a large body of literature has demonstrated that *CYP2E1* polymorphisms are correlated with the risk of cancer and other diseases.[20,21] However, no studies have focused on the association of *CYP2E1* polymorphisms with susceptibility to T2DM.

To explore the relationship of *CYP7A1* and *CYP2E1* polymorphisms with T2DM risk in the Chinese Han populations, we designed this case-control study and focused on the correlations between four polymorphisms (*CYP7A1*: rs8192879 and rs12542233; *CYP2E1*: rs2070672 and rs2515641) and T2DM susceptibility.

Methods

Study Populations

A total of 512 diabetic patients and 515 healthy controls were enrolled from the First affiliated hospital of Xi'an Jiaotong University in China. All patients were diagnosed based on the 2022 American Diabetes Association (ADA) diagnostic criteria for diabetes. Criteria for the diagnosis of diabetes: 1) fasting blood glucose (FBG) ≥7.0 mmol/L, 2) 2-h plasma glucose (2-h PG) ≥200 mg/dL (11.1 mmol/L) during oral glucose tolerance test (OGTT), 3) A1C ≥6.5% (48 mmol/mol), 4) In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).[22] A person who accord with one of these criteria is diagnosed diabetes. Patients suffering from type 1 diabetes, gestational diabetes, inflammation, malignancy, renal dysfunction, other chronic or endocrine disease, and who have receiving any drugs like antidiabetics were excluded. The controls were age- and gender-matched healthy population and had no history of diabetes, metabolic disorders or severe diseases. The demographic and clinical characteristics of the study population, such as age, gender, body mass index (BMI), smoking status, drinking status, Complication, Antidiabetes drug, Insulin, diabetic retinopathy, Fasting blood glucose (FBG), hemoglobin A1C (HbA1C), total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Urea, Creatinine, Cystatin C, glomerular filtration rate (GFR) were required from their medical records (Table 1). The study was approved by the ethical committee of the First affiliated hospital of Xi'an Jiaotong University, and informed consent forms were signed by all individuals before the study according to the Helsinki Declaration.

Genotyping

Genomic DNA was isolated using GoldMag–Mini Purification Kit (GoldMag Co. Ltd. Xi'an, China) and stored at −80°C until analysis. The genetic variations of *CYP7A1* and *CYP2E1* were obtained through Ensembl (http://grch37.ensembl.org/Homo_sapiens/Tools/VcftoPed) database.[23] Using Haploview software, we selected tagSNPs based on Hardy-Weinberg equilibrium (HWE) > 0.01, minor allele frequency (MAF) > 0.05, Minimum genotype (Min Genotype) > 75%, and Tagger r^2 >0.8. Combined MassARRAY, HWE > 0.05, MAF > 0.05 and the call rate > 95% in our study population, four candidate SNPs (rs8192879, rs12542233, rs2070672 and rs2515641) were randomly selected in order to study their potential role in T2DM risk (Table 2). We applied the Agena MassARRAY Assay Design 3.0 software (Agena

Table 1 Demographic and Clinical Characteristics of Participants

Variables	Cases (n = 512)	Controls (n = 515)	*p*
Age (mean ± SD), years	59.23 ± 9.59	59.27 ± 10.97	0.962
>59	264 (52%)	272 (53%)	
≤59	248 (48%)	243 (47%)	
Gender			
Male	281 (55%)	283 (55%)	
Female	231 (45%)	232 (45%)	
BMI			
≤24	130 (25%)	126 (24%)	
>24	190 (37%)	123 (24%)	
Smoking status			
Yes	135 (26%)	132 (26%)	
No	231 (45%)	137 (27%)	
Drinking status			
Yes	69 (13%)	98 (19%)	
No	278 (54%)	138 (27%)	
Complication			
One	108 (21%)		
Multiple	141 (27%)		
Antidiabetes drug			
Yes	128 (25%)		
No	204 (40%)		
Insulin			
Yes	175 (34%)		
No	157 (31%)		
Diabetic retinopathy			
Yes	213 (42%)		
No	149 (29%)		
FBG (mmol/L)	9.95 ± 4.69	5.67 ± 0.78	**< 0.000**
HbA1C (%)	9.30 ± 2.47	5.88 ± 0.79	**< 0.000**
TC (mmol/L)	4.62 ± 1.32	4.94 ± 0.95	**0.004**
TG (mmol/L)	2.49 ± 2.25	1.76 ± 1.42	**< 0.000**
LDL (mmol/L)	2.77 ± 0.95	2.68 ± 0.69	0.325
HDL (mmol/L)	1.22 ± 0.64	1.20 ± 0.24	0.742
Urea (mmol/L)	6.38 ± 3.33	5.05 ± 1.27	**< 0.000**
Creatinine (μmol/L)	63.30 ± 19.91	60.26 ± 13.20	0.069
Cystatin C (mg/L)	0.97 ± 2.17	0.88 ± 0.20	0.904
GFR (mL/min)	122.78 ± 36.00	94.09 ± 15.93	**< 0.000**

Notes: *p* values were calculated by two sided Chi-square test with logistic regression analysis. Bold indicates significant difference, $p < 0.05$ indicates statistical significance.

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; HbA1C, hemoglobin A1C; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; GFR, glomerular filtration rate.

Bioscience, San Diego, California, USA) to design PCR and extension primers of each SNP (Table 3). All SNPs were genotyped by the MassARRAY iPLEX platform. Then, we performed data management and analysis using the Agena Typer 4.0 Software. HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg /haploreg.php)[24] was conducted to predict the potential functions of the candidate variants.

Data Analysis

Data analysis was performed using SPSS version 20.0 software (SPSS, Chicago, IL, USA). The differences in demographic variables and genotype distribution were assessed by the chi-square test and student's *t* test between diabetes patients and healthy controls. We used Fisher's exact test to evaluate the HWE of each SNP in controls. The association of diabetes risk

Table 2 Primary Information of *CYP7A1* and *CYP2E1* Polymorphisms

Gene	SNP	Location	dbSNP	Alleles A/B	MAF		*p HWE*	HaploReg	OR (95% CI)	*p* *	FDR Correction	
					Case	Control					k	FDR Q= *p* * (m/k)
CYP7A1	rs8192879	Chr8: 59,403,576	3'-UTR Variant	T/C	0.286	0.280	0.584	DNAse, GRASP QTL hits, Selected eQTL hits	1.03 (0.85–1.25)	0.743	3	0.990
	rs12542233	Chr8: 59,414,401	2KB Upstream Variant	T/C	0.421	0.454	0.214	Motifs changed, Selected eQTL hits	0.87 (0.73–1.04)	0.128	1	0.510
CYP2E1	rs2070672	Chr10:133,527,044	2KB Upstream Variant	G/A	0.183	0.185	0.379	Promoter and Enhancer histone marks, Motifs changed, DNAse,	0.99 (0.79–1.24)	0.909	4	0.909
	rs2515641	Chr10:133,537,858	Synonymous Variant (p.Phe421=)	C/T	0.164	0.154	0.865	Enhancer histone marks, Motifs changed, Selected eQTL hits	1.08 (0.85–1.37)	0.521	2	1.041

Notes: *p HWE: p* values of Hardy-Weinberg equilibrium were calculated using Chi-square test with logistic regression analysis, *p* *: *p* values were calculated by two sided Chi-square test with logistic regression analysis, k: sequential number sorted by *p* *, m: numbers of SNP.

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy - Weinberg equilibrium; OR, odds ratio; CI, confidence interval; FDR, false discovery rate.

Table 3 Primer Sequence of *CYP7A1* and *CYP2E1* SNPs for PCR and UEP Used in This Study

Gene	SNPs	First Primer (5'-3')	Second Primer (5'-3')	UEP_DIR	UEP SEQ (5'-3')
CYP7A1	rs8192879	ACGTTGGATGAGCCTGGACAGCTTAGTGAG	ACGTTGGATGCAATCTGCCAATTAGAATAC	R	gcttcGTGAGATCCCGTCTCC
CYP7A1	rs12542233	ACGTTGGATGGACTGGGATATCTTGCTGTG	ACGTTGGATGGCATTTATTTAGACAGGTGG	R	TGTATAAGATCAGCTTTTCTATAA
CYP2E1	rs2070672	ACGTTGGATGACTCCAAACAAATGCATGGG	ACGTTGGATGCCAACCCATAGTTAAGAACG	F	aaAGTTCCCCGTTGTCTA
CYP2E1	rs2515641	ACGTTGGATGGCCAGAACACTTCCTGAATG	ACGTTGGATGTCTCACCTGTGGAAAATGGC	F	gTCCTGAATGAAAATGGAAAGTT

Abbreviations: SNP, single nucleotide polymorphism; UEP, unextended sequencing primer; DIR, direction.

https://doi.org/10.2147/PGPM.S367806

and genetic polymorphisms was assessed using odds ratios (ORs) and 95% confidential intervals (95% CI) using logistic regression adjusted by sex and age. In addition, linkage disequilibrium (LD) and haplotype analysis were analyzed by Haploview software (version 4.0) and PLINK software.[25,26] Multifactor dimensionality reduction (MDR) analysis was used to identify the best SNP–SNP interaction model. The p values <0.05 were considered significant in our study.

Results

Characteristics of Participants

The demographic and clinical characteristics of study participants are shown in Table 1. The mean age was 59.23 ± 9.59 years and 59.27 ± 10.97 years in cases and controls, respectively. No significant differences were observed in the distributions of age, gender BMI and other characteristics of subjects. However, there were significant differences in FBG, HbA1c, TC, TG, urea, GFR and other clinical indicators between the T2DM patients and the healthy control.

Association of *CYP7A1* and *CYP2E1* Polymorphisms with Diabetes Risk

In Table 2, the primary information of *CYP7A1* and *CYP2E1* polymorphisms are presented. The results of stratified analysis by age, gender, BMI, etc. are listed in Supplemental Table 1. All genetic polymorphisms were in accordance with HWE (P> 0.05). HaploReg showed that candidate polymorphisms were associated with the regulation of promoter and/or enhancer histone, DNase, motifs changed, GRASP QTLhits and selected eQTL hits.

The genotypes frequencies of diabetes patients are shown in Table 4. *CYP7A1* rs12542233 was significantly related to T2DM risk in recessive model (p = 0.012, OR = 0.67, 95% CI = 0.49–0.91; after FDR correction, p = 0.048). In addition, in order to further explore the relationship between these four variants genotypes and T2DM susceptibility, we performed subgroup analysis stratification on age, sex, BMI, etc. (Supplemental Table 2).

Table 4 The Association of *CYP7A1* and *CYP2E1* Genotypes with Susceptibility of Diabetes

Gene	SNP	Model	Genotype	OR (95% CI)	p*	FDR Correction	
						k	FDR Q = p (m/k)
CYP7A1	rs8192879	Homozygous	TT vs CC	0.91 (0.57–1.46)	0.687	3	0.916
		Heterozygou	TC vs CC	1.14 (0.88–1.48)	0.306	1	1.222
		Dominant	TT + TC vs CC	1.10 (0.86–1.41)	0.436	2	0.873
		Recessive	TT vs TC + CC	0.86 (0.54–1.35)	0.503	2	1.006
		Additive		1.03 (0.85–1.25)	0.741	3	0.988
	rs12542233	Homozygous	TT vs CC	0.71 (0.49–1.02)	0.06	1	0.240
		Heterozygou	TC vs CC	1.10 (0.83–1.46)	0.495	3	0.660
		Dominant	TT + TC vs CC	0.98 (0.75–1.27)	0.861	4	0.861
		Recessive	TT vs TC + CC	0.67 (0.49–0.91)	**0.012**	1	**0.048**
		Additive		0.87 (0.73–1.04)	0.125	1	0.502
CYP2E1	rs2070672	Homozygous	GG vs AA	1.20 (0.58–2.47)	0.624	2	1.249
		Heterozygou	GA vs AA	0.94 (0.72–1.22)	0.631	4	0.631
		Dominant	GG + GA vs AA	0.96 (0.74–1.24)	0.742	3	0.989
		Recessive	GG vs GA+ AA	1.22 (0.60–2.51)	0.583	3	0.777
		Additive		0.99 (0.79–1.24)	0.91	4	0.910
	rs2515641	Homozygous	CC vs TT	0.85 (0.35–2.08)	0.721	4	0.721
		Heterozygou	CT vs TT	1.15 (0.87–1.51)	0.327	2	0.655
		Dominant	CC + CT vs TT	1.13 (0.86–1.47)	0.39	1	1.560
		Recessive	CC vs CT + TT	0.82 (0.34–1.99)	0.658	4	0.658
		Additive		1.08 (0.85–1.38)	0.512	2	1.023

Notes: p: p values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference, p < 0.05 indicates statistical significance, k: sequential number sorted by p *, m: numbers of SNP.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; FDR, false discovery rate.

Stratification Analysis by Age and BMI

The relationship between genotypes of four variants and T2DM susceptibility stratified by age and BMI are shown in Table 5. The *CYP7A1* rs12542233 Recessive model was associated with a reduced risk of T2DM in people over 59 years of age (p = 0.010, OR = 0.55, 95% CI = 0.35–0.87). In the population with BMI ≤ 24 kg/m^2, *CYP7A1* rs12542233 Heterozygou (p = 0.023, OR = 2.08, 95% CI = 1.11–3.90) and Dominant (p = 0.026, OR = 1.94, 95% CI = 1.08–3.49) models were associated with an increased risk of T2DM. In the population with BMI > 24 kg/m^2, *CYP2E1* rs2515641 Homozygous (p = 0.038, OR = 0.10, 95% CI = 0.01–0.89) and Recursive (p = 0.038, OR = 0.10, 95% CI = 0.01–0.88) models can significantly reduce the risk of T2DM.

The Association of *CYP7A1* and *CYP2E1* Polymorphisms with Susceptibility of Diabetic Retinopathy

We stratified the T2DM patients according to diabetes retinopathy (Table 6). The results showed that rs8192879 Heterozygou (p = 0.032, OR = 1.65, 95% CI = 1.05–2.60) and Dominant (p = 0.033, OR = 1.61, 95% CI = 1.04–2.48) models, rs2070672 Heterozygou (p = 0.028, OR = 1.75, 95% CI = 1.06–2.87), Dominant (p = 0.027, OR = 1.71, 95% CI = 1.06–2.75) and Additive (p = 0.046, OR = 1.53, 95% CI = 1.01–2.32) models, rs2515641 Dominant (p = 0.045, OR = 1.64, 95% CI = 1.01–2.67) and Additive (p = 0.042, OR = 1.61, 95% CI = 1.02–2.55) models could significantly increase the risk of diabetes retinopathy in T2DM patients.

Haplotype Analysis

Then, we conducted LD and haplotype analyses on the polymorphisms of *CYP7A1* and *CYP2E1*. These analyses revealed one block in *CYP7A1*, including rs8192879 and rs12542233 (Figure 1). Haplotype frequencies and their association with T2DM risk

Table 5 Stratification Analyses of the Association of *CYP7A1* and *CYP2E1* Polymorphisms with Susceptibility of Diabetes

Gene	*SNP*	Model	Genotype	OR (95% CI)	*p*
Age				> 59	
CYP7A1	rs12542233	Homozygous	TT vs CC	0.61 (0.37–1.02)	0.061
		Heterozygou	TC vs CC	1.21 (0.82–1.78)	0.336
		Dominant	TT + TC vs CC	1.01 (0.70–1.45)	0.969
		Recessive	TT vs TC + CC	0.55 (0.35–0.87)	**0.010**
		Additive		0.84 (0.66–1.07)	0.161
BMI (kg/m2) ≤ 24					
CYP7A1	rs12542233	Homozygous	TT vs CC	1.75 (0.88–3.47)	0.111
		Heterozygou	TC vs CC	2.08 (1.11–3.90)	**0.023**
		Dominant	TT + TC vs CC	1.94 (1.08–3.49)	**0.026**
		Recessive	TT vs TC + CC	1.08 (0.63–1.87)	0.774
		Additive		1.30 (0.92–1.82)	0.136
> 24					
CYP2E1	rs2515641	Homozygous	CC vs TT	0.10 (0.01–0.89)	**0.038**
		Heterozygou	CT vs TT	1.01 (0.61–1.69)	0.968
		Dominant	CC + CT vs TT	0.87 (0.53–1.43)	0.587
		Recessive	CC vs CT + TT	0.10 (0.01–0.88)	**0.038**
		Additive		0.76 (0.49–1.18)	0.227

Notes: *p*: *p* values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference, $p < 0.05$ indicates statistical significance.

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 6 The Association of *CYP7A1* and *CYP2E1* Polymorphisms with Susceptibility of Diabetic Retinopathy

Gene	*SNP*	Model	Genotype	OR (95% CI)	*p*
Diabetic retinopathy					
CYP7A1	rs8192879	Homozygous	TT vs CC	1.39 (0.58–3.36)	0.461
		Heterozygou	TC vs CC	1.65 (1.05–2.60)	**0.032**
		Dominant	TT + TC vs CC	1.61 (1.04–2.48)	**0.033**
		Recessive	TT vs TC + CC	1.12 (0.48–2.65)	0.792
		Additive		1.39 (0.98–198)	0.068
CYP2E1	rs2070672	Homozygous	GG vs AA	1.42 (0.41–4.97)	0.582
		Heterozygou	GA vs AA	1.75 (1.06–2.87)	**0.028**
		Dominant	GG + GA vs AA	1.71 (1.06–2.75)	**0.027**
		Recessive	GG vs GA + AA	1.22 (0.35–4.23)	0.752
		Additive		1.53 (1.01–2.32)	**0.046**
	rs2515641	Homozygous	CC vs TT	2.60 (0.28–24.19)	0.402
		Heterozygou	CT vs TT	1.61 (0.99–2.64)	0.057
		Dominant	CC + CT vs TT	1.64 (1.01–2.67)	**0.045**
		Recessive	CC vs CT + TT	2.27 (0.25–21.11)	0.470
		Additive		1.61 (1.02–2.55)	**0.042**

Notes: *p*: *p* values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference, $p < 0.05$ indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

are listed in Table 7. We found that $C_{rs8192879}T_{rs12542233}$ was markedly related to lower risk of diabetes ($p = 0.019$, OR = 0.75, 95% CI = 0.59–0.95). The results of hierarchical analysis are listed in Supplemental Table 3.

MDR Analysis

MDR was used to analyze the interactions of these four SNPs. The results of the MDR model analysis of the SNP-SNP interactions are demonstrated in Table 8. The results showed that rs12542233 was the best single-locus model to predict trait anxiety (cross-validation consistency, 9/10; testing accuracy, 0.5010; $p = 0.011$). The best multi-loci model was the three-locus model, a combination of rs12542233, rs2070672 and rs2515641, with cross-validation consistency (7/10), the testing accuracy (0.4902) and $p = 0.001$.

Association of *CYP7A1* and *CYP2E1* Polymorphisms with Clinical Characteristics Among T2DM Patients

Among the T2DM patients, the different genotypes of *CYP7A1* rs819287 were significantly correlated with the levels of total cholesterol (TC, $p = 0.036$) and low-density lipoprotein (LDL, $p = 0.031$). And, TC level also was related to *CYP2E1* rs2070672 genotypes ($p = 0.040$, Table 9).

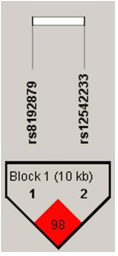

Figure 1 Haplotype block map for the SNPs of *CYP7A1*. Block includes rs8192879 and rs12542233. The LD between two SNPs is standardized by D′.

Table 7 Haplotype Frequencies and the Association with the Risk of Diabetes

Gene	Chr	Haplotypes	OR (95% CI)	*p*
CYP7A1	8	rs8192879|rs12542233 - TT	1.02 (0.84–1.24)	0.838
	8	rs8192879|rs12542233 - TC	0.75 (0.59–0.95)	**0.019**
	8	rs8192879|rs12542233 - CC	0.88 (0.74–1.05)	0.149

Notes: *p: p* values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference, $p < 0.05$ indicates statistical significance.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 8 SNP–SNP Interaction Models of the *CYP7A1 and CYP2E1* Genes for T2DM Predisposition

Model	Training Bal. Acc.	Testing Bal. Acc.	CVC	OR (95% CI)	*p*
rs12542233	0.5322	0.5010	9/10	1.50 (1.10–2.07)	**0.011**
rs12542233, rs2515641	0.5438	0.4912	5/10	1.38 (1.08–1.77)	**0.011**
rs12542233, rs2070672, rs2515641	0.5548	0.4902	7/10	1.50 (1.18–1.92)	**0.001**
rs8192879, rs12542233, rs2070672, rs2515641	0.5632	0.4893	10/10	1.70 (1.31–2.20)	**<0.000**

Notes: *p: p* values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference, $p < 0.05$ indicates statistical significance.
Abbreviations: MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross–validation consistency; OR, odds ratio; CI, confidence interval.

Discussion

In this study, we assessed the influence of four SNPs in *CYP7A1* and *CYP2E1* on susceptibility to T2DM. Our results showed that *CYP7A1* rs12542233 was significantly associated with T2DM risk in the Chinese Han populations. Stratified analysis revealed that rs12542233 was associated with the reduced risk of T2DM patients without diabetic retinopathy. *CYP7A1* rs12542233 had strong relationship with diabetes risk in the subgroups of age >59 years and BMI ≤ 24 kg/m^2. Additionally, haplotype analysis showed $C_{rs8192879}T_{rs12542233}$ was associated with lower risk of T2DM.

In recent years, studies generally believed that genetic variation is one of the genetic factors of cancer and other diseases. And SNPs are important genetic variation. Several studies have found that genetic polymorphisms are associated with the risk of diabetes in Iranian population. Genome-wide association studies indicated that *HHET* rs1111875G/A and rs5015480C/T variants significantly increased the risk of T2DM.[27] Hamidreza Galavi et al found the *SREBF-2* gene rs2267439C/T polymorphism increased T2DM susceptibility.[28] *IGF2BP2* rs11705701 and rs1470579 gene polymorphisms may be associated with T2DM.[29] Case-control study showed that functional *miR-143/145* variants may affect the risk of T2DM.[30] In addition, the *SIRT1* functional variants rs12778366 significantly increased the risk of T2DM, and the rs3758391 was associated with a reduced risk of T2DM.[31] *SLC30A8* gene rs2466293 and rs2466294 variants are associated with increased risk of T2DM, and different genetic models of rs13266634 are associated with decreased risk of T2DM.[32] In T2DM patients, the mean levels of HbA1c were significantly different between CC and TT genotype carriers of the rs28514894 polymorphism.[33] HOTAIR rs920778 C/T, rs12826786 C/T, rs4759314 A/G polymorphisms were positively correlated with T2DM, while rs1899663 G/T was negatively correlated with T2DM susceptibility.[34] In our study, the levels of TC and LDL were significantly different in rs8192879TT, TC and CC genotypes in T2DM patients. Genome-wide association studies revealed that *CYP7A1* rs2081687 is an SNP associated with lipids in African Americans.[35] However, there are no studies on the role of rs8192879 and rs12542233 in T2DM development. In our study, we found *CYP7A1* rs125422333 significantly decreased T2DM risk in recessive model. More studies are required to confirm this result in a larger and well-designed study. The prevalence of diabetes was varied in factors, such as age, sex, and BMI. Sex and age are major risk variables in epidemiology of multiple diseases. The incidence, prevalence or mortality of non-insulin-dependent T2DM increases steeply with age.[36,37] Diabetes is particularly prevalent in adults who are thin at birth but have a high BMI later in life.[38,39] LeRoith et al showed that T2DM is becoming more and more common in people, especially in individuals over 65 years old.[40] Our results showed that rs12542233 and rs2515641 could protect individuals from T2DM among the elderly Chinese population (age >59 years).

Table 9 Clinical Characteristics of Diabetes Patients Based on *CYP7A1* and *CYP2E1* Polymorphisms

Characteristics		rs8192879				rs12542233				rs2070672				rs2515641		
	TT	TC *	CC	*p*	TT	TC *	CC	*p*	GG	GA	AA	*p*	TT	CT	CC	*p*
FBG (mmol/L)	9.65±4.75	10.26±5.93	9.75±3.43	0.630	9.53±5.46	10.27±5.10	9.64±3.42	0.448	10.08±4.54	9.74±5.25	9.04±1.62	0.700	8.96±1.95	9.47±4.76	10.14±4.69	0.489
HbA1C (%)	8.96±1.96	9.33±3.02	9.32±2.02	0.805	8.82±2.10	9.53±2.84	9.16±1.92	0.172	9.33±2.21	9.27±3.13	9.00±1.25	0.909	8.40±0.73	9.22±3.08	9.35±2.22	0.701
TC (mmol/L)	4.77±1.20	4.39±1.18	4.78±1.42	**0.036**	4.59±1.11	4.50±1.20	4.81±1.57	0.183	4.71±1.38	4.33±1.15	5.08±1.02	**0.040**	4.67±0.44	4.44±1.14	4.68±1.39	0.358
TG (mmol/L)	2.19±1.42	2.35±1.93	2.64±2.56	0.475	1.98±1.34	2.40±1.96	2.90±2.94	0.053	2.62±2.57	2.19±1.38	2.41±0.86	0.321	2.63±1.15	2.23±1.28	2.59±2.54	0.447
LDL (mmol/L)	3.00±0.98	2.61±0.76	2.88±1.06	**0.031**	2.84±0.88	2.75±0.89	2.78±1.08	0.844	2.81±0.96	2.62±0.91	3.15±0.93	0.106	2.70±0.47	2.72±0.92	2.79±0.97	0.814
HDL (mmol/L)	1.13±0.34	1.20±0.53	1.25±0.74	0.643	1.18±0.30	1.25±0.67	1.21±0.71	0.766	1.24±0.68	1.19±0.56	1.14±0.20	0.747	1.12±0.26	1.19±0.56	1.24±0.67	0.770
Urea (mmol/L)	6.30±1.81	6.20±1.99	6.52±4.20	0.727	6.70±3.68	6.54±3.95	5.94±1.58	0.279	6.41±3.65	6.40±2.58	5.48±1.90	0.664	6.50±2.15	6.24±2.29	6.43±3.67	0.904
Creatinine (μmol/L)	68.32±30.27	64.29±18.80	61.80±18.87	0.263	62.39±24.73	62.97±18.69	64.30±19.19	0.821	63.12±20.62	64.26±18.99	59.00±12.21	0.695	67.62±13.09	63.11±19.25	63.29±00.31	0.907
Cystatin C (mg/L)	0.83±0.18	0.85±0.28	1.08±3.00	0.665	0.80±0.17	1.08±2.95	0.87±0.66	0.641	1.00±2.59	0.83±0.27	1.33±1.85	0.714	2.39±3.00	0.83±0.27	0.99±2.54	0.357
GFR (mL/min)	120.02 ±36.47	122.63 ±34.59	123.32 ±37.19	0.918	127.72 ±36.01	123.18 ±38.14	119.50 ±32.24	0.428	122.55 ±36.75	123.02 ±35.22	125.44 ±28.70	0.968	102.97 ±29.45	125.89 ±34.57	121.93 ±36.59	0.450

Notes: TC *: genotype – TC, *p*: *p* values were calculated by two sided Chi-square test with logistic regression analysis, Bold indicates significant difference. $p < 0.05$ indicates statistical significance.
Abbreviations: FBG, fasting blood glucose; HbA1C, hemoglobin A1C; TC, total cholesterol; TC, total cholesterol; TG, triglycerides; LDL, low density lipoprotein; HDL, high density lipoprotein; GFR, glomerular filtration rate.

Rs12542233 and rs2515641 also had strong relationships with diabetes risk in the individuals with different BMI. Meanwhile, the exact mechanism of the genetic variants in development of T2DM needs to be further studied.

Inevitably, this study has several limitations. First, the sample size is limited, so future large-scale studies are needed to verify our findings. Secondly, data on the potential function of these SNPs were predicted in silico only; thus, further functional assay is necessary to explore the underlying functions and mechanisms of these polymorphisms. Third, our study did not conduct cell or animal experiments, so we can supplement this part of the study in the future to make the relationship between *CYP7A1* and *CYP2E1* and diabetes more definite.

Conclusion

In summary, our study firstly evaluated the association of *CYP7A1* and *CYP2E1* polymorphisms with T2DM risk in a Chinese population. We have found that *CYP7A1* and *CYP2E1* gene polymorphisms were significantly associated with the risk of T2DM, especially in stratified analysis. It provides evidence that *CYP7A1* and *CYP2E1* may be associated with diabetes susceptibility, and suggests a vital role for *CYP7A1* and *CYP2E1* in the progression of diabetes.

Abbreviations

T2DM, type 2 diabetes mellitus; *CYP*, cytochrome P450; ORs, odds ratios; CIs, confidence intervals; DM, diabetes mellitus; *CYP7A1*, cytochrome P450 7A1; *CYP2E1*, cytochrome P450 2E1; BMI, body mass index; CHB, the Han Chinese in Beijing; CHS, Southern Han Chinese; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; LD, linkage disequilibrium; MDR, multifactor dimensionality reduction; LDL, low-density lipoprotein.

Data Sharing Statement

All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

Ethics Approval and Informed Consent

The study was approved by the ethical committee of the First affiliated hospital of Xi'an Jiaotong University, and informed consent forms were signed by all individuals before the study according to the Helsinki Declaration.

Consent for Publication

All authors agree to publish.

Acknowledgment

We sincerely thank all participators in this study. Specially, we thank First affiliated hospital of Xi'an Jiaotong University for providing samples.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

This study was supported by Shaanxi key research and development plan (2017SF-080).

Disclosure

The authors report no conflicts of interest in this work.

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