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/m², CYP7A1 rs12542233 was associated with an increased risk of T2DM (p < 0.05). In the population with BMI > 24 kg/m², 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<sub>rs8192879C<sub>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
human patients and diabetic animals. Gerhard et al have found that diabetic patients have significantly higher serum bile acid levels than healthy controls. In addition, Prawitt et al showed that the administration of a bile acid sequestrant decreased plasma glucose and HbA1c concentrations. Moreover, previous studies indicated that insulin inhibits the expression of CYP7A1 in rat hepatocytes, the key enzyme in bile acid synthesis pathways. 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. 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. The overexpressed CYP2E1 exhibits a high capacity of produce free radicals that probably cause liver damage and lipid peroxidation in obese T2DM patients. It has been shown that there is elevated activity of CYP2E1 in the liver of obese patients with T2DM. 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. 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. 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). 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. 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 r² > 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
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) was conducted to predict the potential functions of the candidate variants.

**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.

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 k | FDR Q= p * (m/k) |
|------|--------------|----------------------|----------------------|-------------|-----|-------|-----------------------------------------|-------------|-------------|------------------|-----------------|
|      |              |                      |                      |             |     |       | Case | Control |                  |              |             |                  |                 |
| 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, selected eQTL hits | 0.99 (0.79–1.24) | 0.909        | 4                | 0.909           |
|      | rs2515641    | Chr10:133,537,858    | Synonymous Variant   | 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| ACGTTGGATGAGGCCCTGGACAGCTTAGTGAG | ACGTTGGATGCAATCTGCCAATTAGAATAC | R       | gcttcGTGAGATCCCCGTCTCC |
| CYP7A1 | rs12542233| ACGTTGGATGGACTGGGATATCTTGCTGTG | ACGTTGGATGGCATTTATTTAGACAGGTTG | R       | TGTATAAGATCAGCTTTTCTATAA |
| CYP2E1 | rs2070672| ACGTTGGATGACTCCAAACAAATGCATGGG | ACGTTGGATGCCAACCCATAGTTAAGACG | F       | aaAGTTCCCCGTGTCTA |
| CYP2E1 | rs2515641| ACGTTGGATGGCCAGAAACATTCCTGAAATG | ACGTTGGATGTCTCAACCTGTGAAAATGGC | F       | gTCCTGAATGAAAATGGAAAGTT |

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

| Gene       | SNP     | Model       | Genotype       | OR (95% CI) | $p^*$   | FDR Correction |
|------------|---------|-------------|----------------|-------------|---------|----------------|
|            |         |             |                |             |         | k              |
| CYP7A1     | rs8192879| Homozygous  | TT vs CC       | 0.91 (0.57–1.46) | 0.687   | 3              |
|            |         | Heterozygous| TT vs CC       | 1.14 (0.88–1.48) | 0.306   | 1              |
|            |         | Dominant    | TT + TC vs CC  | 1.10 (0.86–1.41) | 0.436   | 2              |
|            |         | Recessive   | TT vs TC + CC  | 0.86 (0.54–1.35) | 0.503   | 2              |
|            |         | Additive    | TT vs TC + CC  | 1.03 (0.85–1.25) | 0.741   | 3              |
| CYP7A1     | rs12542233| Homozygous  | TT vs CC       | 0.71 (0.49–1.02) | 0.06    | 1              |
|            |         | Heterozygous| TT vs CC       | 1.10 (0.83–1.46) | 0.495   | 3              |
|            |         | Dominant    | TT + TC vs CC  | 0.98 (0.75–1.27) | 0.861   | 4              |
|            |         | Recessive   | TT vs TC + CC  | 0.67 (0.49–0.91) | 0.012   | 1              |
|            |         | Additive    | TT vs TC + CC  | 0.87 (0.73–1.04) | 0.125   | 1              |
| CYP2E1     | rs2070672| Homozygous  | GGA vs AA      | 1.20 (0.58–2.47) | 0.624   | 2              |
|            |         | Heterozygous| GGA vs AA      | 0.94 (0.72–1.22) | 0.631   | 4              |
|            |         | Dominant    | GGA + GA vs AA | 0.96 (0.74–1.24) | 0.742   | 3              |
|            |         | Recessive   | GGA vs GA+ AA  | 1.22 (0.60–2.51) | 0.583   | 3              |
|            |         | Additive    | GGA vs GA+ AA  | 0.99 (0.79–1.24) | 0.91    | 4              |
| CYP2E1     | rs2515641| Homozygous  | CC vs TT       | 0.85 (0.35–2.08) | 0.721   | 4              |
|            |         | Heterozygous| CT vs TT       | 1.15 (0.87–1.51) | 0.327   | 2              |
|            |         | Dominant    | CC + CT vs TT  | 1.13 (0.86–1.47) | 0.39    | 1              |
|            |         | Recessive   | CC vs CT + TT  | 0.82 (0.34–1.99) | 0.658   | 4              |
|            |         | Additive    | CC vs CT + TT  | 1.08 (0.85–1.38) | 0.512   | 2              |

**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 Heterozygous ($p = 0.023$, 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 Heterozygous ($p = 0.032$, 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 Heterozygous ($p = 0.028$, 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**  |             |             |              |                 |      |
| CYP7A1   | rs12542233  | Homozygous  | TT vs CC     | 0.61 (0.37–1.02)| 0.061|
|          |             | Heterozygous| 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)| 1.61 |
|          |             |             |              |                 |      |
| BMI (kg/m$^2$) ≤ 24 |       |             |              |                 |      |
| CYP7A1   | rs12542233  | Homozygous  | TT vs CC     | 1.75 (0.88–3.47)| 0.111|
|          |             | Heterozygous| 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|
|          |             |             |              |                 |      |
| BMI > 24 |       |             |              |                 |      |
| CYP2E1   | rs2515641   | Homozygous  | CC vs TT     | 0.10 (0.01–0.89)| 0.038|
|          |             | Heterozygous| 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 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 rs8192879 T vs 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).

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**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 |
|        |        | Heterozygous | 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 |
|        |        | Homozygous | GG vs AA | 1.42 (0.41–4.97) | 0.582 |
|        |        | Heterozygous | 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 |
|        |        | Homozygous | CC vs TT | 2.60 (0.28–24.19) | 0.402 |
|        |        | Heterozygous | 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.

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Figure 1 Haqlotype block map for the SNPs of CYP7A1. Block includes rs8192879 and rs12542233. The LD between two SNPs is standardized by D’.
In this study, we assessed the influence of four SNPs in \textit{CYP7A1} and \textit{CYP2E1} on susceptibility to T2DM. Our results showed that \textit{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. \textit{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\textit{rs8192879}T\textit{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 \textit{HHET} rs1111875G/A and rs5015480C/T variants significantly increased the risk of T2DM. Hamidreza Galavi et al found the \textit{SREBF-2} gene rs2267439C/T polymorphism increased T2DM susceptibility. \textit{IGF2BP2} rs11705701 and rs1470579 gene polymorphisms may be associated with T2DM. A case-control study showed that functional \textit{miR-143/145} variants may affect the risk of T2DM. In T2DM patients, the mean levels of HbA1c were significantly different between CC and TT genotype carriers of the rs28514894 polymorphism. HOTAIR rs920778 C/T, rs12826786 C/T, rs4759314 A/G polymorphisms were positively correlated with T2DM, while rs1889663 G/T was negatively correlated with T2DM susceptibility. 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 \textit{CYP7A1} rs2081687 is an SNP associated with lipids in African Americans. However, there are no studies on the role of rs8192879 and rs12542233 in T2DM development. In our study, we found \textit{CYP7A1} rs12542233 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. Diabetes is particularly prevalent in adults who are thin at birth but have a high BMI later in life. LeRoith et al showed that T2DM is becoming more and more common in people, especially in individuals over 65 years old. Our results showed that rs12542233 and rs2515641 could protect individuals from T2DM among the elderly Chinese population (age >59 years).

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

| Gene     | Chr | Haplotype       | 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**: \( p \) 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 \textit{CYP7A1} and \textit{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             | 0.4693            | 10/10| 1.70 (1.31–2.20) | <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**: 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 \textit{CYP7A1} and \textit{CYP2E1} on susceptibility to T2DM. Our results showed that \textit{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. \textit{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\textit{rs8192879}T\textit{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 \textit{HHET} rs1111875G/A and rs5015480C/T variants significantly increased the risk of T2DM. Hamidreza Galavi et al found the \textit{SREBF-2} gene rs2267439C/T polymorphism increased T2DM susceptibility. \textit{IGF2BP2} rs11705701 and rs1470579 gene polymorphisms may be associated with T2DM. Case-control study showed that functional \textit{miR-143/145} variants may affect the risk of T2DM. In addition, the \textit{SIRT1} functional variants rs12778366 significantly increased the risk of T2DM, and the rs3758391 was associated with a reduced risk of T2DM. \textit{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. In T2DM patients, the mean levels of HbA1c were significantly different between CC and TT genotype carriers of the rs28514894 polymorphism. 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. 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 \textit{CYP7A1} rs2081687 is an SNP associated with lipids in African Americans. However, there are no studies on the role of rs8192879 and rs12542233 in T2DM development. In our study, we found \textit{CYP7A1} rs12542233 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. Diabetes is particularly prevalent in adults who are thin at birth but have a high BMI later in life. LeRoith et al showed that T2DM is becoming more and more common in people, especially in individuals over 65 years old. 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         | TT       | TC *       | CC        | p         | TT       | TC *       | CC        | p         |
| FBG (mmol/L)    | 9.65±4.75 | 10.26±5.93 | 9.75±3.43 | 0.620     | 9.53±5.46 | 10.27±5.10 | 9.64±3.42 | 0.448     | 10.08±6.54 | 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 | 4.46±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±1.98 | 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 | 0.707     |
| Urea (mmol/L)   | 6.30±3.18 | 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 | 0.644     | 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±0.31 | 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     | 122.63     | 123.32     | 0.918     | 127.72     | 123.18     | 119.50     | 0.428     | 122.55     | 123.02     | 125.44     | 0.968     | 102.97   | 125.89     | 121.93     | 0.450     |

Notes: TC *: genotype – TC, 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; 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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