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Association of *FHL5* and *LPA* genetic polymorphisms with diabetes mellitus risk: a case-control study

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ABSTRACT

Background: China is one of the countries with the fastest growing prevalence of diabetes mellitus (DM) in the world. This study intended to investigate the association of single nucleotide polymorphisms (SNPs) of *FHL5* and *LPA* with DM risk in the Chinese population.

Methods: This case-control study involved 1,420 Chinese individuals (710 DM patients and 710 controls). Four candidate loci (rs2252816/rs9373985 in *FHL5* and rs3124784/rs7765781 in *LPA*) were successfully screened. The association of SNPs with DM risk was assessed by logistic regression analysis. Differences in clinical characteristics among subjects with different genotypes were analyzed by one-way analysis of variance.

Results: Overall analysis indicated that rs3124784 was associated with an increased risk of DM. Stratification analysis showed that rs3124784 significantly increased DM risk in different subgroups (male, non-smoking, non-drinking, and BMI $>$ 24), while rs7765781 increased DM risk only in participants with BMI $\leq$ 24. Rs2252816 was associated with the course of DM. We also found that rs2252816 GG genotype and rs9373985 GG genotype were linked to the increased cystatin c in DM patients.

Conclusion: The genetic polymorphisms of *LPA* may be associated with DM risk in the Chinese population, which will provide useful information for the prevention and diagnosis of DM.

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Introduction

Diabetes mellitus (DM), characterized by chronic hyperglycemia and disturbances in carbohydrate, protein and fat metabolism, is mainly caused by defective or dysfunctional insulin secretion. DM can be classified as type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus (GDM), and other types of diabetes from other causes [1], of which T2D is the most common type of DM, accounting for more than 90% of all types [2]. With economic development, diet changes and the aging of population, DM has become a major public health problem worldwide [3]. China is one of the countries with the fastest growing prevalence of DM in the world and the country with the largest number of diabetic patients. In 2021, the number of diabetic patients in China reached 140 million [4]. As we all know, the acute and chronic complications caused by DM, including multiple organ damage such as blood vessels, eyes, kidneys, and feet, seriously endanger people's health and quality of life and are the main causes of disability and death in DM patients. The study on the burden of DM has shown that in 2016, the number of DM deaths in China exceeded 140,000, and the mortality rate increased from 6.3/100,000 in 1990 to 10.3/100,000 in 2016 [5]. Genetic factors (mainly the polymorphisms of susceptibility genes) and risk factors (age [6], obesity, smoking, drinking, etc.) play a key role in the occurrence and development of DM and its complications [7,8]. In recent years, a number of genetic polymorphisms related to DM have been found, especially some hotspot genes, such as *TCF7L2* [9,10], *SLC30A8* [11,12], *KCNJ11* [13,14] and so on.

Four-and-a-half LIM domain 5 (*FHL5*), also known as the *ACT* gene, is a member of the four-and-a-half LIM

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domain (*FHL*) protein family, located on chromosome 6q16.1, containing eight exons [15]. The study has found that *FHL5* is expressed in tumor cell lines from leukemia, melanoma and squamous cell carcinoma [16]. Several studies based on genome-wide association studies (GWAS) and meta-analysis have found that polymorphisms of this gene may be associated with the susceptibility to migraine [15,17]. Meanwhile, the study has revealed that *FHL5* is correlated with the susceptibility to Alzheimer's disease (AD) [18]. Some recent studies have shown that there is a certain correlation between AD and T2D. Huang et al. have shown an increased risk of neurocognitive impairment in patients with T2D and AD, and the enrichment analysis of AD and T2D showed that these two diseases are both closely related to synaptic vesicle function and MAPK signaling pathway [19]. Lynn et al. have provide a detailed review of the relationship between DM and AD. Firstly, insulin resistance is present in AD and is also a major component of the pathogenesis of T2D, which can lead to impaired brain glucose metabolism, neurodegeneration and cognitive impairment. Secondly, dysregulation of the insulin receptor and the components of insulin signaling pathway has been reported in these two diseases. In addition, inflammation, oxidative stress, mitochondrial dysfunction, amyloid deposition, and advanced glycation end products can co-occur in these two diseases, AD has been labeled as "type 3 diabetes" [20]. Huang et al. have demonstrated that immune-related biological functions and pathways are closely related to AD, T2D and microvascular complications [21]. Taken together, based on the above research results, we speculated that *FHL5* was closely related to the occurrence of DM. Genetic polymorphisms can affect the function of the certain gene [22]. Therefore, *FHL5* polymorphisms might be involved in DM.

Lipoprotein(a) gene (*LPA*) is located on chromosome 6q26 and contains 40 exons. The protein encoded by the *LPA* gene is a serine protease that constitutes an important part of Lp(a). The study has suggested that Lp(a) may be a biomarker for T2D [23]. The study has shown that increased Lp(a) levels are associated with reduced risk of T2D in a Chinese population with very high cardiovascular risk [24]. Gudbjartsson et al. have reported that Lp(a) concentrations of lower than 10% can increase T2D risk [25]. A study on Lp(a) and DM has pointed out that Lp(a) is negatively correlated with the prevalence of DM and the risk of new-onset DM [26]. Kollerits et al. have shown that high Lp(a) concentrations and low-molecular weight apolipoprotein(a) isoforms are risk predictors of death (infection) in diabetic hemodialysis patients [27]. The study showed that a large number of *LPA* kringle IV type 2 repeats are associated with an increased risk of T2D in a Chinese population with very high cardiovascular risk [28]. In addition, the study by Singh et al. have demonstrated that two SNPs (rs10455872 and rs3798220) of *LPA* could affect plasma Lp(a) levels [29]. Hence, we speculated that *LPA* genetic polymorphisms might be associated with DM. In other words, the genetic polymorphisms of *FHL5* and *LPA* may be involved in the occurrence of DM, but this relevancy has not been studied in the Chinese population.

To best our knowledge, rs2252816, rs9373985, and rs3124784 had not been reported before. While rs7765781 has only been reported to be associated with premature myocardial infarction [30] and premature coronary artery disease [31]. However, an association study by Jiang et al. has found that alleles of the *FHL5* polymorphism are associated with reduced expression levels of the *FHL5* gene and reduced pre-mRNA alternative splicing levels of the *FHL5* gene in a variety of human tissues [17]. Meanwhile, the study has found that SNPs do not lead to amino acid changes in proteins, but may be in linkage disequilibrium with other genetic variants that could potentially affect gene expression or function [32]. And there is evidence that SNPs affect gene expression [33]. Another study has shown that *LPA* mRNA levels are higher in carriers of *LPA* SNPs than in noncarriers [34]. Thus, the polymorphisms studied in our study may affect the development of DM through the above functional effects.

Therefore, this study selected four candidate SNPs in *FHL5* and *LPA* in 1,420 Chinese individuals, and we overall assessed SNPs that affect the susceptibility to DM. Meanwhile, the association between these SNPs and DM was further stratified by age, gender, smoking, drinking, the course of DM, retinal degeneration and Body Mass Index (BMI). Our study will enrich the genetic data related to DM in the Chinese population, and the discovered susceptibility loci can be identified as genetic markers for DM risk prediction, so as to better offer theoretical reference for the early prevention and diagnosis of DM.

Materials and methods

Study participants

This was a case-control study officially approved by the Ethics Committee of People's Hospital of Wanning. All study subjects were enrolled from People's Hospital of Wanning, Hainan Province, and they gave

written informed consent. According to the inclusion and exclusion criteria, a total of 710 unrelated DM patients were assigned to the DM group, and 710 individuals without DM were assigned to the control group. DM patients were newly diagnosed and confirmed by experienced specialists according to the World Health Organization (WHO) 1999 criteria [35]. The inclusion criteria for the DM group were [1]: age $\geq$ 18 years old [2]; fasting plasma glucose (FPG) $\geq$ 7.0 mmol/L, or 2-h plasma glucose (2h PG) $\geq$ 11.1 mmol/L during the oral glucose tolerance test (OGTT), or Hemoglobin A1C (HbA1C) $\geq$ 6.5%, or previous diagnosis of DM [3]; no family history of DM [4]; Chinese Han [5]; no family history of DM; and [6] good understanding and communication. The inclusion criteria for the control group were [1]: age $\geq$ 18 years old [2]; FPG $<$ 6.1 mmol/L and OGTT 2h PG $<$ 7.8 mmol/L [3]; no family history of DM [4]; Chinese Han; and [5] good understanding and communication. The exclusion criteria for both groups were [1]: severe consumptive diseases, such as viral infection, hyperthyroidism, malignant tumors, etc. [2]; those who were unwilling to sign the informed consent form and fill in the questionnaire; and [3] the examination items required for the study were incomplete. Besides, a baseline survey of general demographic data (gender, age, height, weight, smoking/drinking status etc.) and disease history (the course and retinal degeneration in DM patients) was conducted by a professional physician for all participants.

Blood sample collection and DNA extraction

Peripheral blood samples were collected from all participants into blood collection tubes containing anticoagulant EDTA and stored in a refrigerator at −20 °C. After that, genomic DNA were extracted using a DNA extraction kit according to the manufactory's instruction (GoldMag Co. Ltd. Xi'an, China). Eventually, the extracted DNA was stored in an ultra-low temperature freezer (–80 °C) for future research.

Selection of SNPs and SNP genotyping

Rs2252816 G/A and rs9373985 G/C on the *FHL5* gene, as well as rs3124784 A/G and rs7765781 C/G on the *LPA* gene were selected from the 1000 Genomes Project with minor allele frequency (MAF) $>$ 0.05, Hardy-Weinberg equilibrium (HWE) $>$ 0.05, and Tagger $r^2 < 0.8$. The primers were designed by MassARRAY Assay Design software. All SNPs in this study were genotyped using the MassARRAY system (Agena, San Diego, CA, USA). SNP genotypes were generated using iPLEX chemistry. Additionally, MALDI-TOF was applied to obtain profiles of different mass peaks for multiple reactions, and finally genotyping was successfully completed.

Statistical analysis

Demographic characteristics of research respondents including age (*t*-test) and gender, smoking, and drinking (χ^2 test) were tested by SPSS 21.0 (SPSS, Chicago, IL, USA). After that, the genotype frequencies of all SNPs were tested by chi-square to determine whether they satisfied HWE. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression models to evaluate the association between all candidate SNPs and DM risk. OR $<$ 1 indicated that SNP is a protective factor for DM; OR $=$ 1 indicated that this factor has no effect on DM; and OR $>$ 1 indicated that SNP is a risk factor for DM. A variety of genetic models were evaluated using Plink 1.9 with wild-type alleles as reference. All tests were two-sided, and $p < 0.05$ was considered statistically significant. Afterwards, the results were analyzed by false positive reporting probability (FPRP) to determine whether they deserved attention. The interaction of four SNPs with the risk of DM was assessed by the multifactorial dimensionality reduction (MDR) method. Differences in clinical characteristics among subjects with different genotypes were analyzed by one-way analysis of variance (ANOVA).

Results

Sample overview

Basic demographic and epidemiological information about the case and control groups is presented in Table 1. There were 1,420 unrelated participants in our study, including 710 DM patients [483 males (68.0%) and 227 females (32.0%)] with an average age of 57.68 ± 12.46 years and 710 healthy individuals [481 males (67.7%) and 229 females (32.3%)] with an average age of 57.66 ± 10.42 years. No statistical differences were observed in mean age ($p = 0.974$), gender distribution ($p = 0.909$), smoking ($p = 0.524$), drinking ($p = 0.958$), and BMI ($p = 0.248$) between the two groups. However, there was a significant difference in the level of FPG, Total cholesterol (TC), Triglyceride (TG), Urea (Ure), Aspartate aminotransferase (AST), AST/Alanine aminotransferase (ALT), Uric acid (UA) between groups.

Association between candidate SNPs and DM risk

In this study, the genotype distribution of four SNPs conformed to HWE ($p > 0.05$), and MAF was greater than 5% in the study population. The specific information about all candidate SNPs is shown in Table 2. The functions of SNP were studied through the HaploReg database. The results showed that rs2252816 could affect enhancer histone marks. Rs9373985 could affect motifs changed and selected eQTL hits. Rs3124784 and rs7765781 could affect motifs changed (Table 2). In addition, we found significant differences in the allele frequency ($p = 0.047$) of rs3124784 between DM patients and healthy controls (Figure 1). The association between four candidate SNPs and the risk of DM is shown in Figure 2. The results showed that rs3124784 was significantly associated with DM risk, and no correlation was found between the remaining three candidate SNPs and DM risk ($p > 0.05$). Precisely, rs3124784 significantly increased DM risk under the codominant (AG vs. GG: OR = 1.32, 95% CI 1.04–1.68, $p = 0.024$), dominant (AG-AA vs. GG: OR = 1.30, 95% CI 1.03–1.64, $p = 0.028$) and log-additive (OR = 1.24, 95% CI 1.00–1.53, $p = 0.047$) models.

Association between candidate SNPs and DM risk stratified by clinical characteristics

After stratification by age and gender, the association between four candidate SNPs and DM risk is presented in Table 3. Rs7765781 could notably increase the risk of DM in subjects aged $\leq$ 60 years under a variety of genetic models (allele: OR = 1.29, 95% CI 1.06–1.57, $p = 0.010$; heterozygote: OR = 1.49, 95% CI 1.09–2.02, $p = 0.011$; homozygote: OR = 1.60, 95% CI 1.06–2.42, $p = 0.025$; dominant: OR = 1.51, 95% CI 1.13–2.03, $p = 0.005$; log-additive: OR = 1.30, 95% CI 1.07–1.59, $p = 0.010$). Rs7765781 also was a protective factor for DM patients aged >60 years under the following two genetic models (codominant: OR = 0.65, 95% CI 0.44–0.96, $p = 0.031$; dominant: OR = 0.69, 95% CI 0.48–0.98,

Table 1. Characteristics of patients with diabetes mellitus and healthy individuals.

Characteristics		Cases $n = 710$	Control $n = 710$	p
Age	Mean ± SD	57.68 ± 12.46	57.66 ± 10.42	0.974
	>60 years	288 (40.6 %)	293 (41.3 %)	
	≤60 years	422 (59.4 %)	417 (58.7 %)	
Gender	Male	483 (68.0 %)	481 (67.7 %)	0.909
	Female	227 (32.0 %)	229 (32.3 %)	
Smoking	Yes	346 (48.7 %)	334 (47.0 %)	0.524
	No	364 (51.3 %)	376 (53.0 %)	
Drinking	Yes	330 (46.5 %)	329 (46.3 %)	0.958
	No	380 (53.5 %)	381 (53.7 %)	
Course of diabets mellitus disease	>8 vs ≤8 years	305 (43.0 %)		
		396 (55.8 %)		
Retinal degeneration	Yes	251 (35.4 %)		
	No	319 (44.9 %)		
BMI	>24 kg/m^2	410 (57.7 %)	272 (55.9 %)	0.248
	≤ 24 kg/m^2	300 (42.3%)	215 (44.1 %)	
FPG	Mean ± SD	8.07 ± 3.81	5.97 ± 1.42	**<0.001**
TC	Mean ± SD	4.03 ± 1.39	4.80 ± 0.94	**<0.001**
TG	Mean ± SD	2.36 ± 2.27	1.84 ± 1.48	**<0.001**
Ure	Mean ± SD	55.02 ± 120.86	5.12 ± 1.22	**<0.001**
AST	Mean ± SD	21.81 ± 15.11	25.65 ± 9.36	**<0.001**
AST/ALT	Mean ± SD	1.02 ± 0.41	1.11 ± 0.44	**0.001**
UA	Mean ± SD	264.02 ± 150.19	329.84 ± 80.67	**<0.001**
Cys-c	Mean ± SD	1.03 ± 1.81		

BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; Ure: urea; AST: aspartate aminotransferase; ALT: alanine aminotransferase; UA: uric acid; Cys-c: cystatin c.
$p < 0.05$ and bold text indicate statistical significance.

Table 2. the Basic information and HWE about the selected SNPs.

Gene	SNP ID	Chrs	Position	Function	Alleles (A/B)	MAF Cases	MAF Control	HWE (p value)	HaploReg v4.2
FHL5	rs2252816	6	96610698	nonsynonymous	G/A	0.463	0.452	1.000	Enhancer histone marks
FHL5	rs9373985	6	96615646	nonsynonymous	G/C	0.340	0.330	0.611	Motifs changed, Selected eQTL hits
LPA	rs3124784	6	160531806	nonsynonymous	A/G	0.160	0.134	0.872	Motifs changed
LPA	rs7765781	6	160586464	nonsynonymous	C/G	0.448	0.427	1.000	Motifs changed

HWE: Hardy–Weinberg equilibrium; SNP: single nucleotide polymorphisms; Chrs: chromosome number; Alleles (A/B): minor/major allele; MAF: minor allele frequency.
$p > 0.05$ indicates that the genotypes were in Hardy–Weinberg Equilibrium.

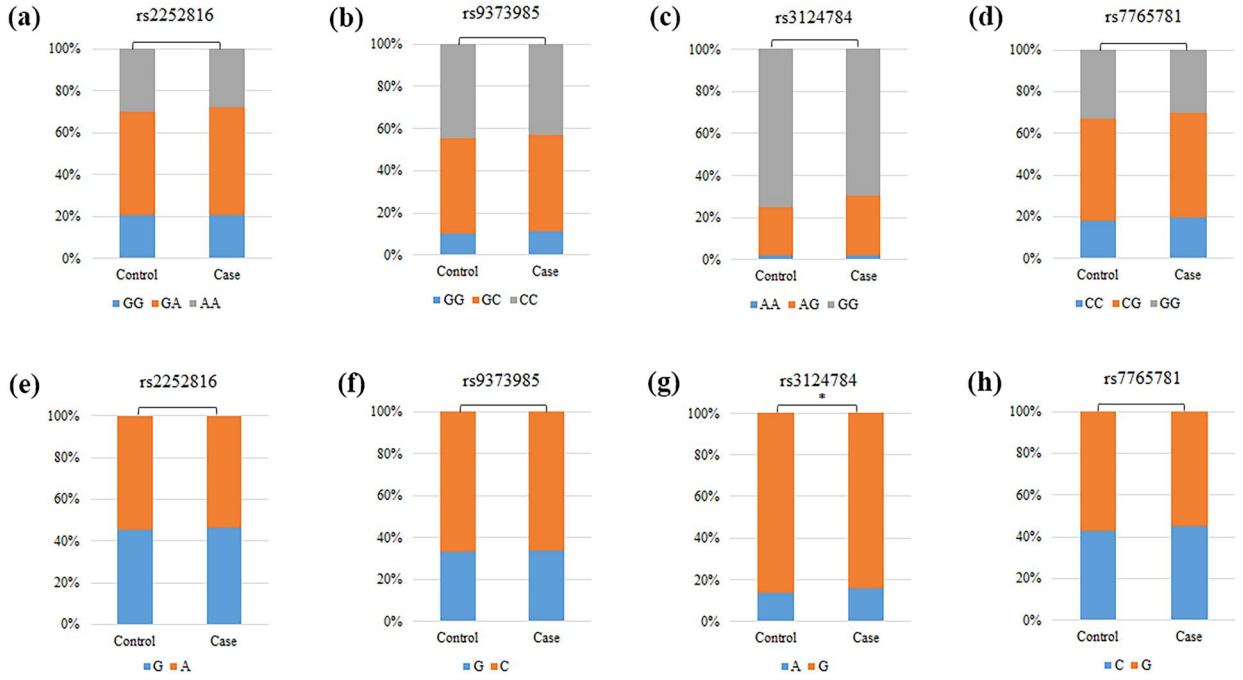

Figure 1. Comparisons of the genotype frequency and allele frequency of SNPs of *FHL5/LPA* genes in DM case and healthy control. (a) Comparisons of the genotype frequency of rs2252816 of *FHL5* gene between the two groups. (b) Comparisons of the genotype frequency of rs9373985 of *FHL5* gene between the two groups. (c) Comparisons of the genotype frequency of rs3124784 of *LPA* gene between the two groups. (d) Comparisons of the genotype frequency of rs7765781 of *LPA* gene between the two groups. (e) Comparisons of the allele frequency of rs2252816 of *FHL5* gene between the two groups. (f) Comparisons of the allele frequency of rs9373985 of *FHL5* gene between the two groups. (g) Comparisons of the allele frequency of rs3124784 of *LPA* gene between the two groups. (h) Comparisons of the allele frequency of rs7765781 of *LPA* gene between the two groups. '*' indicate statistical significance at the 0.05 level.

$p = 0.041$). In addition, the rs3124784 polymorphism could notably increase DM risk in male participants (allele: OR = 1.33, 95% CI 1.03–1.71, $p = 0.028$; codominant: OR = 1.41, 95% CI 1.06–1.89, $p = 0.020$; dominant: OR = 1.42, 95% CI 1.06–1.89, $p = 0.017$; log-additive: OR = 1.37, 95% CI 1.05–1.79, $p = 0.019$).

The association between four candidate SNPs and DM risk stratified by smoking and drinking is presented in Table 4. The results indicated that rs3124784 increased the risk of DM in both non-smoking (codominant: OR = 1.46, 95% CI 1.04–2.04, $p = 0.027$; dominant: OR = 1.40, 95% CI 1.01–1.94, $p = 0.042$) and non-drinking participants (codominant: OR = 1.42, 95% CI 1.02–1.97, $p = 0.038$; dominant: OR = 1.38, 95% CI 1.00–1.90, $p = 0.049$).

The information about the course of DM and retinal degeneration is shown in Table 5. The results indicated that rs2252816 was linked to the course of DM under a variety of genetic models (heterozygote: OR = 0.67, 95% CI 0.46–0.97, $p = 0.034$; homozygote: OR = 0.61, 95% CI 0.38–0.96, $p = 0.035$; dominant: OR = 0.65, 95% CI 0.46–0.93, $p = 0.017$; log-additive: OR = 0.77, 95% CI 0.61–0.97, $p = 0.026$).

As presented in Table 6, the association between four candidate SNPs and DM risk stratified by BMI indicated that rs3124784 increased the risk of DM in participants with BMI >24 (codominant: OR = 1.57, 95% CI 1.09–2.27, $p = 0.015$; dominant: OR = 1.57, 95% CI 1.10–2.25, $p = 0.014$; log-additive: OR = 1.50, 95% CI 1.07–2.11, $p = 0.018$). Meanwhile, rs7765781 increased the risk of DM in participants with BMI $\leq$ 24 under the allele (OR = 1.31, 95% CI 1.02–1.69, $p = 0.034$), codominant (OR = 1.92, 95% CI 1.10–3.33, $p = 0.021$), recessive (OR = 1.64, 95% CI 1.01–2.68, $p = 0.047$), and log-additive (OR = 1.36, 95% CI 1.04–1.78, $p = 0.023$) models.

FPRP analysis

Detailed results of the FPRP analysis can be discovered in Table S1. The result showed that the association between rs2252816 and the course of DM under the homozygous gene model was not noteworthy at the prior probability level of 0.25 and FPRP value of 0.2. Meanwhile, the association between rs7765781 and DM risk in participants with BMI $\leq$ 24 was not noteworthy. The FPRP values of other results were all less than 0.2 at a prior probability level of 0.25, suggesting that these positive results were noteworthy.

SNP ID	Model	Genotype	Case	Control	*p*	OR (95 % CI)
rs2252816	Allele	G	657 (46.3 %)	642 (45.2 %)	0.549	1.05 (0.90-1.21)
		A	761 (53.6 %)	778 (54.8 %)		1
	Codominant	GA	352 (49.6%)	365 (51.5 %)	0.372	1.12 (0.88-1.42)
		GG	145 (20.4 %)	146 (20.6 %)	0.609	1.08 (0.80-1.46)
		AA	213 (30 %)	198 (27.9 %)		1
	Dominant	GA-GG	497 (70 %)	511 (72.1 %)	0.387	1.11 (0.88-1.39)
		AA-GA	565 (79.6 %)	563 (79.4 %)		1
	Recessive	GG	145 (20.4 %)	146 (20.6 %)	0.950	1.01 (0.78-1.31)
		AA-GG	358 (50.4 %)	344 (48.5 %)		1
	Log-additive	–	–	–	0.550	1.05 (0.90-1.21)
rs9373985	Allele	G	483 (34.0 %)	469 (33.0 %)	0.578	1.05 (0.89-1.22)
		C	937 (66.0 %)	951 (67.0 %)		1
	Codominant	GC	321 (45.2 %)	325 (45.8 %)	0.700	1.04 (0.84-1.30)
		GG	74 (10.4 %)	79 (11.1 %)	0.621	1.09 (0.77-1.56)
		CC	315 (44.4 %)	306 (43.1 %)		1
	Dominant	GC-GG	395 (55.6 %)	404 (56.9 %)	0.625	1.05 (0.85-1.30)
		CC-GC	636 (89.6 %)	631 (88.9 %)		1
	Recessive	GG	74 (10.4 %)	79 (11.1 %)	0.694	1.07 (0.76-1.50)
		CC-GG	389 (54.8 %)	385 (54.2 %)		1
	Log-additive	–	–	–	0.582	1.05 (0.89-1.22)
rs3124784	Allele	A	227 (16 %)	190 (13.4 %)	**0.050***	1.23 (1.00-1.52)
		G	1191 (83.9 %)	1228 (86.5 %)		1
	Codominant	AG	164 (23.1 %)	201 (28.4 %)	**0.024***	1.32 (1.04-1.68)
		AA	13 (1.8 %)	13 (1.8 %)	0.857	1.07 (0.49-2.34)
		GG	532 (75 %)	495 (69.8 %)		1
	Dominant	AG-AA	177 (25 %)	214 (30.2 %)	**0.028***	1.30 (1.03-1.64)
		GG-AG	696 (98.2 %)	696 (98.2 %)		1
	Recessive	AA	13 (1.8 %)	13 (1.8 %)	1.000	1.00 (0.46-2.17)
		GG-AA	545 (76.9 %)	508 (71.7 %)		1
	Log-additive	–	–	–	**0.047***	1.24 (1.00-1.53)
rs7765781	Allele	C	634 (44.6 %)	605 (42.6 %)	0.258	1.09 (0.94-1.26)
		G	782 (55.1 %)	813 (57.3 %)		1
	Codominant	CG	347 (48.9 %)	352 (49.7 %)	0.433	1.10 (0.87-1.39)
		CC	129 (18.2 %)	141 (19.9 %)	0.272	1.18 (0.88-1.60)
		GG	233 (32.9 %)	215 (30.4 %)		1
	Dominant	CG-CC	476 (67.1 %)	493 (69.6 %)	0.312	1.12 (0.90-1.40)
		GG-CG	580 (81.8 %)	567 (80.1 %)		1
	Recessive	CC	129 (18.2 %)	141 (19.9 %)	0.410	1.12 (0.86-1.46)
		GG-CC	362 (51.1 %)	356 (50.3 %)		1
	Log-additive	–	–	–	0.257	1.09 (0.94-1.27)

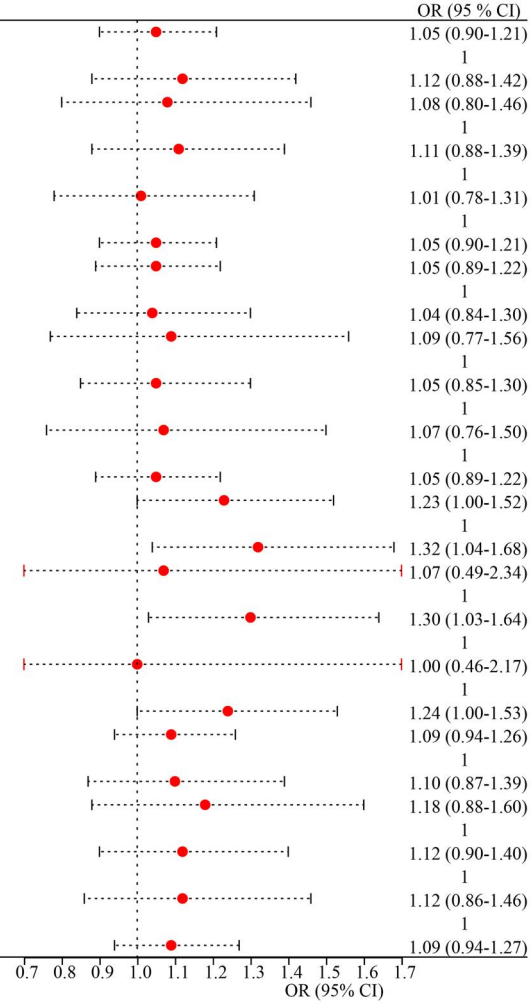

Figure 2. Analysis of the association between susceptibility of DM and SNPs. DM, diabetes mellitus; SNP, single nucleotide polymorphisms; or, odds ratio; CI, confidence interval; *p* values represent adjusted for age, gender, smoking, and drinking; $p < 0.05$, bold text and '*' indicate statistical significance.

MDR analysis

The MDR analysis was carried out to analyze and evaluate SNP-SNP interactions (Figure 3). The red lines showed that there was a synergistic effect of rs7765781 and rs9373985 on DM risk. The blue lines showed that there was a redundant effect of rs3124784 and rs2252816 on DM risk. Details of SNP-SNP interactions are presented in Table 7. The results showed that the best prediction model was the four-site model: rs2252816, rs9373985, rs3124784 and rs7765781 (the largest CVC: 10/10, testing balanced accuracy: 0.501, $p < 0.0001$).

Differences in clinical characteristics among different genotypes

The associations among the four candidate SNPs and the clinical characteristics of DM patients are shown in Table 8. The results indicated that the rs2252816 GG genotype was linked to the reduced Ure in DM patients and the increased cystatin c (Cys-c) in DM patients. The rs9373985 GG genotype was associated with the increased Cys-c.

Discussion

This study is the first to analyze the association between the polymorphisms of *FHL5* (rs2252816 G/A, rs9373985 G/C) and *LPA* (rs3124784 A/G, rs7765781 C/G) and DM risk in the Chinese population. We found that *LPA* polymorphisms (rs3124784 A/G, rs7765781 C/G) were associated with DM risk and played as risk factors in DM.

DM is one of the risk factors for coronary artery disease (CAD) [36]. Previous studies have shown that the *LPA* gene polymorphisms are associated with CAD [37], and other SNPs of *LPA* might be correlated with

Table 3. the SNPs associated with susceptibility of diabetes mellitus in the subgroup tests (age and gender).

			Age				Gender			
			≤ 60 (*N* = 839)		> 60 (*N* = 581)		Male (*N* = 964)		Female (*N* = 456)	
SNP ID	Model	Genotype	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*
rs2252816	Allele	G	1.00 (0.82–1.21)	0.975	1.12 (0.89–1.41)	0.334	1.07 (0.90–1.29)	0.435	0.99 (0.76–1.28)	0.936
		A	1		1		1		1	
	Codominant	GA	0.97 (0.71–1.33)	0.853	1.41 (0.96–2.07)	0.076	1.14 (0.85–1.54)	0.379	1.08 (0.70–1.66)	0.733
		GG	1.01 (0.68–1.48)	0.979	1.24 (0.76–2.03)	0.384	1.15 (0.79–1.66)	0.463	0.99 (0.58–1.70)	0.976
		AA	1		1		1		1	
	Dominant	GA-GG	0.98 (0.73–1.32)		1.37 (0.95–1.97)	0.092	1.14 (0.86–1.52)	0.349	1.05 (0.70–1.58)	0.806
		AA-GA	1		1		1		1	
	Recessive	GG	1.02 (0.74–1.42)		0.99 (0.65–1.52)	0.981	1.05 (0.77–1.44)	0.747	0.95 (0.59–1.52)	0.821
		AA-GG	1		1		1		1	
	Log-additive	–	1.00 (0.83–1.21)	0.997	1.15 (0.90–1.46)	0.268	1.08 (0.90–1.29)	0.427	1.00 (0.77–1.31)	0.975
rs9373985	Allele	G	1.04 (0.85–1.27)	0.698	1.05 (0.82–1.34)	0.696	1.07 (0.88–1.29)	0.512	1.00 (0.76–1.32)	0.983
		C	1		1		1		1	
	Codominant	GC	0.93 (0.69–1.24)	0.603	1.24 (0.88–1.76)	0.218	1.04 (0.79–1.36)	0.802	0.97 (0.65–1.44)	0.881
		GG	1.20 (0.76–1.89)	0.423	0.91 (0.51–1.64)	0.759	1.13 (0.73–1.73)	0.581	0.96 (0.50–1.85)	0.894
		CC	1		1		1		1	
	Dominant	GC-GG	0.98 (0.74–1.28)		1.18 (0.85–1.64)	0.332	1.05 (0.81–1.36)	0.695	0.97 (0.66–1.41)	0.864
		CC-GC	1		1		1		1	
	Recessive	GG	1.25 (0.82–1.92)		0.82 (0.47–1.44)	0.485	1.11 (0.74–1.66)	0.619	0.97 (0.52–1.82)	0.926
		CC-GG	1		1		1		1	
	Log-additive	–	1.04 (0.85–1.27)	0.715	1.06 (0.82–1.36)	0.669	1.05 (0.87–1.28)	0.598	0.97 (0.73–1.30)	0.863
rs3124784	Allele	A	1.23 (0.93–1.63)	0.141	1.23 (0.90–1.69)	0.186	1.33 (1.03–1.71)	**0.028***	1.05 (0.73–1.52)	0.787
		G	1		1		1		1	
	Codominant	AG	1.36 (0.98–1.87)	0.063	1.29 (0.89–1.87)	0.181	1.41 (1.06–1.89)	**0.020***	1.17 (0.75–1.83)	0.479
		AA	0.93 (0.31–2.80)	0.894	1.41 (0.46–4.33)	0.546	1.50 (0.51–4.41)	0.459	0.86 (0.26–2.83)	0.801
		GG	1		1		1		1	
	Dominant	AG-AA	1.32 (0.97–1.81)		1.30 (0.90–1.86)	0.158	1.42 (1.06–1.89)	**0.017***	1.14 (0.74–1.74)	0.552
		GG-AG	1		1		1		1	
	Recessive	AA	0.86 (0.29–2.59)		1.31 (0.43–4.01)	0.632	1.37 (0.47–4.00)	0.568	0.82 (0.25–2.70)	0.750
		GG-AA	1		1		1		1	
	Log-additive	–	1.25 (0.94–1.67)	0.125	1.26 (0.91–1.74)	0.162	1.37 (1.05–1.79)	**0.019***	1.08 (0.75–1.56)	0.677
rs7765781	Allele	C	1.29 (1.06–1.57)	**0.010***	0.86 (0.68–1.09)	0.209	1.11 (0.93–1.33)	0.250	1.04 (0.80–1.36)	0.744
		G	1		1		1		1	
	Codominant	CG	1.49 (1.09–2.02)	**0.011***	0.65 (0.44–0.96)	**0.031***	1.25 (0.94–1.67)	0.125	0.82 (0.53–1.27)	0.375
		CC	1.60 (1.06–2.42)	**0.025***	0.76 (0.48–1.21)	0.243	1.19 (0.82–1.72)	0.357	1.14 (0.66–1.97)	0.646
		GG	1		1		1		1	
	Dominant	CG-CC	1.51 (1.13–2.03)	**0.005***	0.69 (0.48–0.98)	**0.041***	1.24 (0.94–1.62)	0.129	0.90 (0.60–1.36)	0.616
		GG-CG	1		1		1		1	
	Recessive	CC	1.26 (0.87–1.82)	0.218	0.99 (0.66–1.46)	0.946	1.04 (0.75–1.44)	0.824	1.28 (0.80–2.07)	0.303
		GG-CC	1		1		1		1	
	Log-additive	–	1.30 (1.07–1.59)	**0.010***	0.85 (0.68–1.08)	0.180	1.11 (0.93–1.33)	0.256	1.04 (0.79–1.36)	0.798

SNP: Single nucleotide polymorphisms; OR: odds ratio; CI: Confidence interval.
$p < 0.05$, bold text and '*' indicate statistical significance.

the risk of cardiovascular disease (CVD) and CAD [38,39]. So we speculated that *LPA* polymorphisms may play a vital role in DM. In the overall analysis, this study found that *LPA* rs3124784 was associated with an increased risk of DM in the Chinese population. Although no association of *LPA* polymorphisms with DM risk has been reported, the FPRP analysis in this study further validated that our results were still significant. Therefore, we speculated that *LPA* rs3124784 was a risk factor for DM in the Chinese population. DM demonstrates high genetic heterogeneity in populations [40], and further validation of our results in different populations is needed.

Former researches have indicated that a variety of risk factors, such as age [6], gender, smoking, drinking, etc [7,8], contribute to the development of DM. However, a study has suggested that BMI and smoking are not independent risk factors for DM [6]. Consequently, our study stratified participants by the above factors, and the results indicated that rs3124784 of *LPA* significantly increased DM risk in different subgroups (male, non-smoking, non-drinking, BMI > 24), and gender differences were observed in DM risk. Consistent with our results, Zhang et al. have found that genetic polymorphisms can influence T2D risk in male participants [41]. Contrary to our findings, Cui et al. have indicated that genetic polymorphisms are associated with T2D susceptibility in females [42]. Tarnowski et al. have shown that genetic polymorphisms can influence GDM risk in female participants [43]. A study has indicated that there are significant differences in blood glucose levels among male [44]. Globally, more and more males are being diagnosed as DM. CAD mortality due to DM is higher in males

Table 4. the SNPs associated with susceptibility of diabetes mellitus in the subgroup tests (smoking and drinking).

			Smoking				Drinking			
			Yes ($N = 680$)		No ($N = 740$)		Yes ($N = 659$)		No ($N = 761$)	
SNP ID	Model	Genotype	OR (95 % CI)	p	OR (95 % CI)	p	OR (95 % CI)	p	OR (95 % CI)	p
rs2252816	Allele	G	0.99 (0.80–1.22)	0.904	1.10 (0.90–1.35)	0.351	1.05 (0.85–1.30)	0.658	1.04 (0.85–1.28)	0.684
		A	1		1		1		1	
	Codominant	GA	0.91 (0.63–1.29)	0.586	1.34 (0.95–1.88)	0.092	0.99 (0.69–1.41)	0.939	1.25 (0.90–1.74)	0.184
		GG	0.99 (0.64–1.52)	0.956	1.21 (0.79–1.85)	0.392	1.12 (0.72–1.74)	0.602	1.04 (0.69–1.58)	0.839
		AA	1		1		1		1	
	Dominant	GA-GG	0.93 (0.66–1.30)	0.673	1.30 (0.94–1.79)	0.109	1.03 (0.73–1.44)	0.884	1.19 (0.87–1.63)	0.278
		AA-GA	1		1		1		1	
	Recessive	GG	1.05 (0.73–1.52)	0.790	1.00 (0.69–1.45)	0.999	1.13 (0.78–1.65)	0.511	0.91 (0.63–1.29)	0.584
		AA-GG	1		1		1		1	
	Log-additive	–	0.99 (0.80–1.22)	0.908	1.12 (0.91–1.39)	0.290	1.05 (0.85–1.31)	0.635	1.04 (0.85–1.28)	0.693
rs9373985	Allele	G	0.91 (0.73–1.14)	0.416	1.19 (0.95–1.48)	0.123	1.05 (0.84–1.32)	0.671	1.04 (0.84–1.29)	0.717
		C	1		1		1		1	
	Codominant	GC	0.75 (0.54–1.05)	0.091	1.32 (0.97–1.79)	0.076	0.99 (0.71–1.37)	0.950	1.10 (0.81–1.48)	0.550
		GG	0.90 (0.55–1.46)	0.664	1.28 (0.75–2.18)	0.372	1.17 (0.71–1.93)	0.541	1.02 (0.61–1.68)	0.949
		CC	1		1		1		1	
	Dominant	GC-GG	0.78 (0.58–1.07)	0.124	1.31 (0.98–1.76)	0.070	1.03 (0.75–1.40)	0.875	1.08 (0.81–1.44)	0.592
		CC-GC	1		1		1		1	
	Recessive	GG	1.03 (0.66–1.63)	0.886	1.10 (0.66–1.84)	0.705	1.18 (0.73–1.89)	0.504	0.97 (0.60–1.57)	0.900
		CC-GG	1		1		1		1	
	Log-additive	–	0.89 (0.71–1.11)	0.297	1.20 (0.96–1.51)	0.114	1.05 (0.84–1.32)	0.663	1.04 (0.84–1.30)	0.724
rs3124784	Allele	A	1.20 (0.89–1.62)	0.241	1.26 (0.95–1.68)	0.110	1.18 (0.87–1.61)	0.284	1.28 (0.96–1.70)	0.093
		G	1		1		1		1	
	Codominant	AG	1.21 (0.85–1.72)	0.287	1.46 (1.04–2.04)	**0.027***	1.22 (0.86–1.73)	0.269	1.42 (1.02–1.97)	**0.038***
		AA	1.72 (0.52–5.66)	0.376	0.81 (0.28–2.38)	0.702	1.26 (0.38–4.21)	0.702	0.97 (0.35–2.72)	0.954
		GG	1		1		1		1	
	Dominant	AG-AA	1.24 (0.88–1.75)	0.225	1.40 (1.01–1.94)	**0.042***	1.22 (0.87–1.72)	0.253	1.38 (1.00–1.90)	**0.049***
		GG-AG	1		1		1		1	
	Recessive	AA	1.63 (0.50–5.37)	0.420	0.73 (0.25–2.15)	0.574	1.20 (0.36–3.98)	0.766	0.89 (0.32–2.48)	0.820
		GG-AA	1		1		1		1	
	Log-additive	–	1.23 (0.90–1.69)	0.188	1.29 (0.96–1.73)	0.093	1.20 (0.87–1.64)	0.263	1.28 (0.96–1.71)	0.088
rs7765781	Allele	C	1.20 (0.97–1.49)	0.096	1.00 (0.81–1.23)	0.981	1.09 (0.88–1.36)	0.433	1.09 (0.89–1.33)	0.415
		G	1		1		1		1	
	Codominant	CG	1.19 (0.84–1.68)	0.325	1.01 (0.72–1.41)	0.955	1.32 (0.93–1.87)	0.122	0.95 (0.68–1.31)	0.734
		CC	1.45 (0.93–2.27)	0.100	0.96 (0.63–1.47)	0.860	1.13 (0.72–1.77)	0.587	1.24 (0.82–1.88)	0.305
		GG	1		1		1		1	
	Dominant	CG-CC	1.26 (0.91–1.74)	0.171	1.00 (0.73–1.36)	0.982	1.26 (0.91–1.76)	0.165	1.02 (0.75–1.39)	0.898
		GG-CG	1		1		1		1	
	Recessive	CC	1.31 (0.88–1.94)	0.181	0.96 (0.66–1.38)	0.816	0.96 (0.65–1.41)	0.818	1.29 (0.89–1.85)	0.177
		GG-CC	1		1		1		1	
	Log-additive	–	1.20 (0.97–1.50)	0.097	0.98 (0.80–1.21)	0.884	1.09 (0.88–1.36)	0.427	1.09 (0.89–1.34)	0.400

SNP: Single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval.
$p < 0.05$, bold text and '*' indicate statistical significance.

than female [45]. The above results suggest that the susceptibility to DM may vary by gender. Contrary to our findings, Zhang et al. have found that genetic polymorphisms influence T2D risk in smoking and drinking participants [41]. Tian et al. have shown that genetic polymorphisms associated with the risk of T2D may be closely related to smoking and drinking [46]. Taken together, the association between rs3124784 and the risk of DM may be affected by smoking and drinking. Research has showed that genetic polymorphism affect BMI [44]. Consistent with our results, Matharoo et al. have revealed that genetic polymorphisms influence T2D risk in participants with high BMI and central obesity [47]. Zhang et al. have found that genetic polymorphisms influence T2D risk in participants with BMI $>$ 24 [41]. Tian et al. have shown that the association of genetic polymorphisms with the risk of T2D might be affected by BMI [46]. In conclusion, the association between rs3124784 and DM risk may be influenced by BMI. We also found that rs2252816 was associated with the course of DM. Consistent with our results, Zhang et al. have found that genetic polymorphisms are linked to the course of T2D [41]. Previous studies of chronic diseases in China have found that the risk of death increases by 13% for each 5-year increase in the course of DM [48]. Therefore, the association between genetic polymorphisms and DM may be affected by the course of the disease.

In addition, we found that the rs2252816 GG genotype and rs9373985 GG genotype were associated with an increased Cys-c in DM patients. It has been found that certain clinical blood parameters may be indicators of biological phenotypes in patients with

Table 5. the SNPs associated with susceptibility of diabetes mellitus in the subgroup tests (course of diabetes mellitus and retinal degeneration).

			Course of diabetes mellitus		Retinal degeneration	
SNP ID	Model	Genotype	>8 vs ≤ 8 years ($N = 701$) OR (95 % CI)	p	Retinal degeneration vs no retinal degeneration ($N = 570$) OR (95 % CI)	p
rs2252816	Allele	G	0.82 (0.66–1.01)	0.062	1.05 (0.83–1.33)	0.683
		A	1		1	
	Codominant	GA	0.67 (0.46–0.97)	**0.034***	1.23 (0.82–1.84)	0.314
		GG	0.61 (0.38–0.96)	**0.035***	1.10 (0.67–1.81)	0.702
		AA	1		1	
	Dominant	GA-GG	0.65 (0.46–0.93)	**0.017***	1.19 (0.81–1.74)	0.369
		AA-GA	1		1	
	Recessive	GG	0.79 (0.53–1.17)	0.238	0.96 (0.63–1.47)	0.868
		AA-GG	1		1	
	Log-additive	–	0.77 (0.61–0.97)	**0.026***	1.06 (0.83–1.36)	0.629
rs9373985	Allele	G	0.86 (0.69–1.08)	0.190	1.07 (0.84–1.37)	0.592
		C	1		1	
	Codominant	GC	0.81 (0.58–1.13)	0.212	1.36 (0.94–1.97)	0.098
		GG	0.72 (0.42–1.24)	0.240	0.99 (0.56–1.76)	0.977
		CC	1		1	
	Dominant	GC-GG	0.79 (0.57–1.09)	0.149	1.28 (0.90–1.81)	0.168
		CC-GC	1		1	
	Recessive	GG	0.81 (0.49–1.34)	0.411	0.85 (0.49–1.46)	0.552
		CC-GG	1		1	
	Log-additive	–	0.84 (0.66–1.06)	0.143	1.10 (0.85–1.42)	0.462
rs3124784	Allele	A	1.07 (0.80–1.42)	0.664	0.85 (0.61–1.18)	0.328
		G	1		1	
	Codominant	AG	1.06 (0.74–1.50)	0.755	0.74 (0.50–1.10)	0.135
		AA	0.93 (0.29–2.95)	0.901	1.79 (0.45–7.06)	0.407
		GG	1		1	
	Dominant	AG-AA	1.05 (0.74–1.48)	0.785	0.78 (0.53–1.14)	0.201
		GG-AG	1		1	
	Recessive	AA	0.91 (0.29–2.89)	0.880	1.94 (0.49–7.63)	0.343
		GG-AA	1		1	
	Log-additive	–	1.03 (0.76–1.41)	0.837	0.85 (0.60–1.20)	0.348
rs7765781	Allele	C	1.11 (0.90–1.37)	0.348	1.00 (0.79–1.26)	0.994
		G	1		1	
	Codominant	CG	1.29 (0.89–1.86)	0.176	1.15 (0.77–1.71)	0.496
		CC	1.19 (0.75–1.87)	0.460	0.99 (0.61–1.61)	0.962
		GG	1		1	
	Dominant	CG-CC	1.26 (0.89–1.78)	0.193	1.10 (0.75–1.60)	0.627
		GG-CG	1		1	
	Recessive	CC	1.02 (0.68–1.51)	0.938	0.91 (0.59–1.39)	0.653
		GG-CC	1		1	
	Log-additive	–	1.11 (0.89–1.39)	0.370	1.01 (0.79–1.28)	0.955

SNP: Single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval.
$p < 0.05$, bold text and '*' indicate statistical significance.

metabolic diseases [49]. Consistent with our results, the study indicated that the increased levels of Cys-c lead to adverse outcomes in women with high-risk pregnancies and GDM [50]. Therefore, rs2252816 G/A and rs9373985 G/C are risk factors for DM patients.

Taken together, we first studied the correlation of between the polymorphisms of *FHL5* and *LPA* with DM risk. The results presented that *LPA* is a risk gene for DM. This study increased the understanding of this gene and provided a basis for subsequent studies. Meanwhile, association studies of this gene with DM will help to develop new therapeutic targets for DM. Furthermore, in terms of individualized health management, early screening of these susceptible loci related to DM may help to understand the pathogenesis and progress of DM, and provide reference for the early detection, prevention and personalized treatment of DM.

However, our study has certain limitation due to the molecular mechanism of the polymorphisms of *FHL5* and *LPA* in DM in the Chinese population has not been studied. In the future, we will center on this issue. Despite the above shortcoming, our study provides new insights into the association between the gene polymorphisms of *FHL5* (rs2252816 G/A, rs9373985 G/C) and *LPA* (rs3124784 A/G, rs7765781 C/G) and DM risk.

Conclusion

In summary, this study first investigated DM risk in the Chinese population based on polymorphisms of *FHL5* (rs2252816 G/A, rs9373985 G/C) and *LPA* (rs3124784

Table 6. the SNPs associated with susceptibility of diabetes mellitus in the subgroup tests (BMI).

			BMI			
			≤ 24 ($N = 515$)		>24 ($N = 682$)	
SNP ID	Model	Genotype	OR (95 % CI)	*p*	OR (95 % CI)	*p*
rs2252816	Allele	G	1.11 (0.86–1.42)	0.427	1.00 (0.80–1.24)	0.969
		A	1		1	
	Codominant	GA	1.22 (0.81–1.84)	0.347	1.11 (0.77–1.61)	0.572
		GG	1.27 (0.76–2.13)	0.367	1.01 (0.65–1.58)	0.966
		AA	1		1	
	Dominant	GA-GG	1.23 (0.84–1.82)	0.292	1.08 (0.76–1.53)	0.664
		AA-GA	1		1	
	Recessive	GG	1.12 (0.72–1.76)	0.615	0.94 (0.65–1.38)	0.765
		AA-GG	1		1	
	Log-additive	–	1.14 (0.88–1.47)	0.328	1.01 (0.81–1.26)	0.918
rs9373985	Allele	G	1.17 (0.90–1.52)	0.249	0.88 (0.70–1.11)	0.287
		C	1		1	
	Codominant	GC	1.11 (0.76–1.61)	0.598	0.91 (0.65–1.27)	0.563
		GG	1.59 (0.85–2.97)	0.146	0.77 (0.46–1.30)	0.333
		CC	1		1	
	Dominant	GC-GG	1.18 (0.83–1.69)	0.357	0.88 (0.64–1.21)	0.420
		CC-GC	1		1	
	Recessive	GG	1.51 (0.83–2.74)	0.174	0.81 (0.50–1.33)	0.411
		CC-GG	1		1	
	Log-additive	–	1.20 (0.92–1.58)	0.184	0.89 (0.70–1.12)	0.322
rs3124784	Allele	A	1.03 (0.73–1.46)	0.849	1.36 (0.99–1.87)	0.054
		G	1		1	
	Codominant	AG	1.08 (0.72–1.62)	0.703	1.57 (1.09–2.27)	**0.015***
		AA	1.18 (0.32–4.33)	0.805	1.48 (0.36–6.15)	0.590
		GG	1		1	
	Dominant	AG-AA	1.09 (0.73–1.61)	0.675	1.57 (1.10–2.25)	**0.014***
		GG-AG	1		1	
	Recessive	AA	1.15 (0.32–4.21)	0.831	1.32 (0.32–5.43)	0.703
		GG-AA	1		1	
	Log-additive	–	1.08 (0.76–1.55)	0.662	1.50 (1.07–2.11)	**0.018***
rs7765781	Allele	C	1.31 (1.02–1.69)	**0.034***	0.99 (0.79–1.23)	0.898
		G	1		1	
	Codominant	CG	1.27 (0.85–1.91)	0.243	1.02 (0.72–1.46)	0.903
		CC	1.92 (1.10–3.33)	**0.021***	0.91 (0.58–1.43)	0.686
		GG	1		1	
	Dominant	CG-CC	1.40 (0.95–2.07)	0.087	0.99 (0.71–1.38)	0.947
		GG-CG	1		1	
	Recessive	CC	1.64 (1.01–2.68)	**0.047***	0.90 (0.61–1.34)	0.601
		GG-CC	1		1	
	Log-additive	–	1.36 (1.04–1.78)	**0.023***	0.96 (0.77–1.20)	0.737

SNP: Single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval.
$p < 0.05$, bold text and '*' indicate statistical significance.

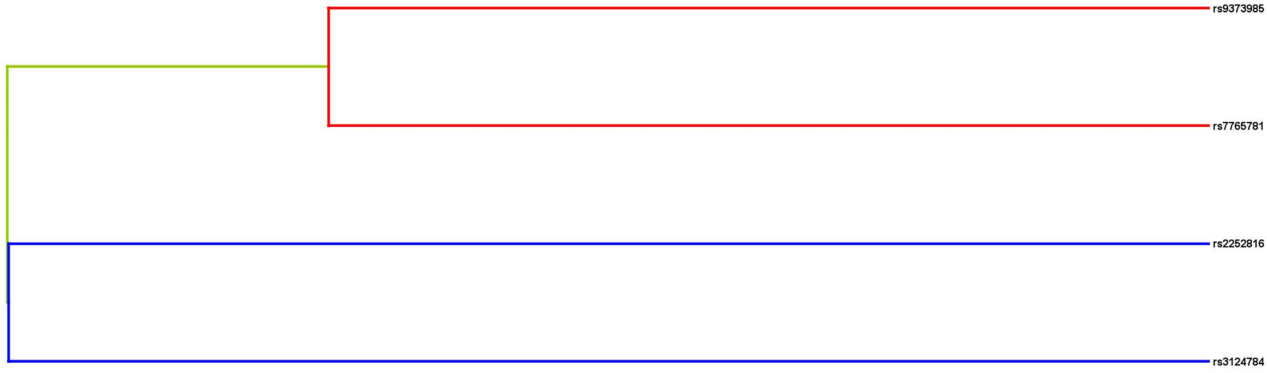

Figure 3. Dendrogram of SNP-SNP interactions. The colors in the tree diagram represent synergy or redundancy.

A/G, rs7765781 C/G). The results showed that there was a certain association between *LPA* gene polymorphisms (rs3124784 A/G) and DM risk. Our study further enriched the genetic data on DM susceptibility in the Chinese population, and provided a preliminary molecular basis for DM risk.

Table 7. SNP–SNP interaction models analyzed by the MDR method.

Model	Training Bal. Acc	Testing Bal. Acc	OR (95 % CI)	*p* value	CVC
rs3124784	0.527	0.506	1.30 (1.03–1.64)	**0.0282**	9/10
rs2252816, rs3124784	0.534	0.489	1.28 (1.04–1.58)	**0.0208**	6/10
rs9373985, rs3124784, rs7765781	0.549	0.489	1.45 (1.18–1.79)	**0.0005**	6/10
rs2252816, rs9373985, rs3124784, rs7765781	0.561	0.501	1.60 (1.30–1.97)	***p* < 0.0001**	10/10

MDR: multifactor dimensionality reduction; Bal. Acc.: balanced accuracy; CVC: cross-validation consistency; OR: odds ratio; 95 % CI: 95 % confidence interval.
p values were calculated using χ^2 tests; $p < 0.05$ and bold text indicate statistical significance.

Table 8. Clinical characteristics of patients ($N = 710$) based on the genotypes of selected SNPs.

	rs2252816				rs9373985			
Characteristics	GG	GA	AA	*p*	GG	GC	CC	*p*
FPG	7.84 ± 4.15	8.06 ± 3.73	8.27 ± 3.70	0.594	8.57 ± 4.87	7.82 ± 3.55	8.20 ± 3.76	0.207
TC	4.03 ± 1.36	4.02 ± 1.38	4.06 ± 1.43	0.941	4.04 ± 1.56	4.05 ± 1.36	4.01 ± 1.37	0.917
TG	2.35 ± 2.28	2.44 ± 2.33	2.22 ± 1.16	0.554	2.62 ± 2.70	2.33 ± 2.21	2.32 ± 2.22	0.553
LDL-C	2.53 ± 0.79	2.57 ± 0.95	2.64 ± 1.00	0.565	2.44 ± 0.80	2.61 ± 0.97	2.58 ± 0.93	0.360
HDL-C	1.17 ± 1.18	1.09 ± 0.60	1.08 ± 0.30	0.405	1.27 ± 1.56	1.10 ± 0.63	1.06 ± 0.30	0.065
Ure	53.50 ± 120.64	44.84 ± 106.56	75.39 ± 142.58	**0.018***	55.63 ± 120.52	46.90 ± 108.26	63.50 ± 132.86	0.234
Cr	66.76 ± 31.73	67.71 ± 44.62	68.84 ± 55.68	0.916	67.92 ± 34.39	68.52 ± 47.34	67.17 ± 46.57	0.935
GFR	105.19 ± 29.79	106.28 ± 31.06	106.20 ± 25.35	0.940	103.88 ± 32.66	105.33 ± 28.54	107.40 ± 29.10	0.599
AST	22.31 ± 15.79	21.93 ± 16.83	21.24 ± 10.91	0.827	23.08 ± 18.54	21.78 ± 15.26	21.53 ± 13.97	0.762
ALT	27.30 ± 30.04	25.43 ± 26.75	24.24 ± 19.65	0.616	28.57 ± 36.62	24.90 ± 19.83	25.21 ± 27.40	0.577
AST/ALT	0.97 ± 0.32	1.02 ± 0.41	1.07 ± 0.46	0.119	0.97 ± 0.29	1.00 ± 0.40	1.06 ± 0.44	0.147
TBA	5.36 ± 6.20	5.68 ± 5.31	5.60 ± 5.33	0.874	6.23 ± 7.98	5.59 ± 5.35	5.40 ± 4.77	0.566
UA	271.22 ± 150.94	271.92 ± 146.02	243.66 ± 155.57	0.134	272.03 ± 145.09	271.53 ± 150.38	254.29 ± 151.32	0.398
GHbA1c	8.38 ± 2.22	8.39 ± 2.30	8.43 ± 2.06	0.978	8.56 ± 2.52	8.38 ± 2.17	8.37 ± 2.19	0.789
Cys-c	1.38 ± 3.87	0.95 ± 0.44	0.94 ± 0.47	**0.035***	1.78 ± 5.22	0.94 ± 0.45	0.94 ± 0.42	**0.001***

FPG: fasting plasma glucose; TC: total cholesterol; TG: triacylglycerol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Ure: Urea; Cr: creatinine; GFR: glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TBA: total bile acid; UA: uric acid; GHbA1c: glycosylated hemoglobin A1c; Cys-c: cystatin c.
$p < 0.05$, bold text and '*' represent statistical significance.

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Ethical approval and consent to participation

This study fully followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of People's Hospital of Wanning. Informed consent from all participants was obtained.

Author contributions

Xuezhong Xu wrote the paper; Xuezhong Xu, Fangyun Liang, Jinmei Chen analyzed and interpreted the data; Yipeng Ding conceived and designed the experiments; Feihong Chen, Lingyi Kong performed the experiments. All authors reviewed the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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