

Genetic Variants in the *SAMM50* Gene Create Susceptibility to Nonalcoholic Fatty Liver Disease in a Chinese Han Population

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Background: Genome-wide association studies have shown that *rs738491*, *rs2143571*, and *rs3761472* in the sorting and assembly machinery component 50 homolog (*SAMM50*) gene are significantly associated with susceptibility to nonalcoholic fatty liver disease (NAFLD).

Objectives: The present study evaluated the association between the three genetic variants in the *SAMM50* gene and susceptibility to NAFLD in a Chinese Han population.

Patients and Methods: Genotypes for 3 single nucleotide polymorphisms (SNPs), viz *rs738491*, *rs2143571*, and *rs3761472*, in the *SAMM50* gene were determined using an improved multiplex ligation detection reaction technique in 340 B-type ultrasonography-diagnosed NAFLD patients and 452 healthy controls. Meanwhile, serum lipid profiles and liver enzymes were estimated using standard clinical laboratory methods. The SNP-SNP interactions were analyzed by performing multifactor dimensionality reduction (MDR) and generalized multifactor dimensionality reduction (GMDR).

Results: The genotype and allele frequencies of the *SAMM50* polymorphisms between the NAFLD group and the control group were significantly different (all P s < 0.05). In the multivariate analysis adjusted for gender, age, and body mass index, the carriers of the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele had significantly increased susceptibility to NAFLD (OR, 1.507; 95% CI, 1.035 to 2.195; P = 0.032; OR, 1.761; 95% CI, 1.232 to 2.517; P = 0.002; OR, 1.483; 95% CI, 1.039 to 2.115; P = 0.030, respectively). Moreover, the *rs738491* T allele carriers had significantly higher levels of alanine aminotransferase (ALT) (P = 0.017) than did the noncarriers. However, differences in the levels of serum triglyceride (TG) and aspartate aminotransferase (AST) were not statistically significant (P = 0.123; P = 0.107). The *Rs2143571* A allele and the *rs3761472* G allele were both deeply associated with increased levels of serum TG, ALT, and AST (all P s < 0.05). Furthermore, the MDR and GMDR showed that a synergistic relationship might exist between *rs738491*, *rs2143571*, and *rs3761472* in the *SAMM50* gene and the pathophysiology and genetics of NAFLD.

Conclusions: We first demonstrated that the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele in the *SAMM50* gene created susceptibility to NAFLD in a Chinese Han population. The combination of the three SNPs in the *SAMM50* gene may have synergism to predict the predisposition to NAFLD.

Keywords: Nonalcoholic Fatty Liver Disease; Polymorphism; Single Nucleotide; *SAMM50*

1. Background

Nonalcoholic fatty liver disease (NAFLD) is the main cause of chronic liver disease and a major public health problem worldwide (1). Simple fatty liver is generally considered a benign pathological process. However, 20% of simple fatty liver patients display lobular inflammation and hepatocyte injury, a condition designated as "nonalcoholic steatohepatitis" (NASH), which may even progress toward liver cirrhosis and hepatocellular carcinoma (2, 3). The detailed pathogenesis of NAFLD remains unclear. Generally, it is believed that the genetic factor is important in the development of NAFLD (4-6), and specific ethnic groups or environmental conditions have been proven to influence the results. Identifying genetic associations with NAFLD in dif-

ferent ethnic groups will lead to the development of new noninvasive biomarkers for the early diagnosis of NAFLD and may allow early preventive and therapeutic strategies for people at high risk in different ethnic groups.

Recently, 3 single nucleotide polymorphisms (SNPs), viz *rs738491*, *rs2143571*, and *rs3761472*, in the sorting and assembly machinery component 50 homolog (*SAMM50*) gene were shown to be deeply associated with NAFLD after adjusting for age, gender, and body mass index (BMI) in the Japanese population (7). In Indian subjects, a subsequent study demonstrated a significant link between *rs2143571* and NAFLD (8).

Now, NAFLD has been confirmed as the fastest growing etiology for hepatocellular carcinoma in China.

2. Objectives

Because the association between the 3 SNPs and NAFLD susceptibility in a Chinese Han population had not been investigated previously, the present study sought to explore the association between the genetic variants in *rs738491*, *rs2143571*, and *rs3761472* in the *SAMM50* gene and susceptibility to NAFLD in a Chinese Han population. The findings should, in turn, provide new insights into the pathophysiology and genetics of NAFLD.

3. Patients and Methods

3.1. Patients and controls

From September 2012 to October 2014, 340 unrelated Chinese NAFLD patients diagnosed by B-type ultrasonography (female, 189; male, 151; mean age, 41.54 ± 8.19 years) (9) and 452 healthy control subjects matched for sex and age (female, 228; male, 224; mean age, 40.18 ± 11.03 years) were enrolled in this study. All the subjects were recruited during the same study period from the Department of Gastroenterology and the Medical Center of Qingdao Municipal Hospital. The diagnosis of NAFLD was made according to the guidelines for the management of NAFLD of the Chinese Medical Association in 2010 (10). The exclusion criteria comprised alcohol abuse (≥ 210 g/week for males and ≥ 140 g/week for females), chronic viral hepatitis (hepatitis B and C), drug-induced steatosis, autoimmune liver disease, Wilson's disease, and alpha-1-antitrypsin deficiency (5). The healthy controls were chosen from among persons without evidence of abnormal lipid profiles, abnormal liver enzymes, or any of the features of the metabolic syndrome (5).

The study was conducted according to the guidelines of the World Medical Association Declaration of Helsinki (11). All the subjects provided written informed consent in accordance with the study protocol approved by the Ethics Committee of Qingdao Municipal Hospital.

3.2. Demographic and Clinical Assessments

Demographic parameters such as BMI were determined in all the subjects. Venous blood samples from each participant were obtained and collected into sterile tubes containing ethylenediaminetetraacetic acid (EDTA). Serological assays, including serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), fasting plasma glucose (FPG), uric acid (UA), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were carried out in accordance with the standard clinical laboratory methods in the Central Laboratory of Qingdao Municipal Hospital.

3.3. Genetic Analysis

Genomic DNA was isolated from noncoagulated blood samples using the Genomic DNA Purification Kit

(BioTeke, Biotechnology, Beijing, China) following the user's protocol. The genotyping of the 3 SNPs, viz *rs738491*, *rs2143571*, and *rs3761472*, in the *SAMM50* gene was conducted using an improved multiplex ligation detection reaction technique (Genesky Biotechnologies Inc. Shanghai, China). Primers for *rs738491*, *rs2143571*, and *rs3761472* were 5'-CTTCATCCCCCTCCCATCATC-3' (forward), 5'-ACAGGGGATGCAAGCCAAGAA-3' (reverse), 5'-GGAGTCCTGCTGCCACTTCTC-3' (forward), 5'-GGAAACCAGCAGTACCCACAGC-3' (reverse), 5'-CAATGGGAACCCAAAGTAAATAACA-3' (forward), and 5'-GCCTGT AGCATAACCATACTGCCTTCA-3' (reverse). The polymerase chain reaction (PCR) was performed as follows: denaturation at 95°C for 2 minutes, 11 cycles \times (denaturation at 94°C for 20 seconds, annealing at 65°C - 0.5°C/cycle for 40 seconds, and elongation at 72°C for 1 minute 30 seconds), 24 cycles \times (denaturation at 94°C for 20 seconds, annealing at 59°C for 30 seconds, and elongation at 72°C for 1 minute 30 seconds) at 72°C for 10 minutes, and keeping at 4°C. The ligation products were loaded into the ABI 3730XL, and the raw data were analyzed using GeneMapper 4.1 software (Applied Biosystems).

3.4. Statistical Analysis

The statistical analyses were performed using statistical package for the social sciences (SPSS), version 17.0 (SPSS Inc. Chicago, IL, USA). All the data are presented as Mean \pm Standard Deviation (SD) for the continuous variables and as percentages for the categorical variables. The Hardy-Weinberg equilibrium was assessed using the χ^2 test. Genotypes and allele frequencies were calculated using the χ^2 test, and the distributions between the NAFLD patients and the healthy controls were analyzed using the Pearson χ^2 test or the Fisher exact test. The strength of the association between the genetic variants and NAFLD was evaluated by logistic regression analysis and estimated by the odds ratio (OR) with a 95% confidence interval (CI). Differences in the baseline characteristics between the 2 groups were examined using the Student t-test, paired samples t-test, or the χ^2 test. The SNP-SNP interactions were analyzed by open-source Java software multifactor dimensionality reduction (MDR), version 3.0.2 (12), and generalized multifactor dimensionality reduction (GMDR), version 0.9 (13). A P value < 0.05 was considered statistically significant.

4. Results

4.1. Demographic and Clinical Characteristics of the Nonalcoholic Fatty Liver Disease Patients and Healthy Controls

Table 1 shows the demographic and clinical characteristics of the NAFLD patients and the healthy controls. The 2 groups were matched for sex and age (both $P_s > 0.05$). The NAFLD patients had higher BMI, waist and hip circumferences, serum levels of ALT, AST, GGT, FPG, TG, TC,

and LDL, and lower HDL than did the healthy controls (all $P_s < 0.05$).

4.2. Genotypes and Allele Frequencies of *rs738491*, *rs2143571*, and *rs3761472*

The genotypes of each SNP were in the Hardy-Weinberg equilibrium in the two groups ($P_{\text{NAFLD}} = 0.112, 0.077, 0.071$; $P_{\text{control}} = 0.879, 0.709, 0.767$). As is described in Table 2, there were significant differences in the genotype and allele frequencies of the *SAMM50* polymorphisms between the NAFLD group and the control group (all $P_s < 0.05$). Moreover, Table 3 demonstrates strong links between the 3 genetic variants in the *SAMM50* gene and NAFLD. The carriers of the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele had significantly increased susceptibility to NAFLD (OR, 1.712; 95% CI, 1.223 to 2.398; $P = 0.002$; OR, 1.940; 95% CI, 1.410 to 2.671; $P < 0.001$; OR, 1.758; 95% CI, 1.288 to 2.399; $P < 0.001$, respectively). After adjusting for confounders (i.e. gender, age, and BMI), we observed that the significant associations still existed (OR, 1.507; 95% CI, 1.035 to 2.195; $P = 0.032$; OR, 1.761; 95% CI, 1.232 to 2.517; $P = 0.002$; OR, 1.483; 95% CI, 1.039 to 2.115; $P = 0.030$, respectively).

4.3. Association Between the 3 Genetic Variants in the *SAMM50* Gene and Clinical Features

As is shown in Table 4, compared with the noncarriers,

the carriers of the *rs738491* T allele had significantly high levels of liver enzymes (serum ALT; $P = 0.017$) and lipid profiles (TC and LDL; both $P_s < 0.05$). Nevertheless, differences in the levels of serum AST, TG, and HDL between the carriers and the noncarriers of the *rs738491* T allele were not statistically significant ($P = 0.107$; $P = 0.123$; $P = 0.099$). Similar strong links, except for the AST and TG levels of serum, were observed in the other 2 variants. The *rs2143571* A allele and *rs3761472* G allele were also both deeply associated with increased levels of serum AST and TG ($P = 0.045$; $P = 0.002$; $P = 0.008$; $P = 0.040$).

4.4. Analysis of SNP-SNP Interactions

As is shown in Figures 1 and 2, the dendrogram and the Fruchterman-Reingold described the interactions between these SNPs. The results of the GMDR model analysis of the SNP-SNP interactions are demonstrated in Table 5. The results showed that *rs2143571* was the best single-locus model to predict NAFLD (testing accuracy [TA], 54.94%; $P = 0.0547$; cross-validation consistency [CVC], 8/10). The best two-locus model was the combination of *rs2143571* and *rs3761472* (TA, 62.21%; $P = 0.001$; CVC, 10/10). The three-locus model included *rs738491*, *rs2143571*, and *rs3761472* (TA, 60.79%; $P = 0.001$; CVC, 10/10). Therefore, the best model was the two-locus model, a combination of *rs2143571* and *rs3761472*, with the highest TA and perfect CVC (OR, 2.9430; 95%CI, 1.9291 to 4.4899; $P < 0.0001$).

Table 1. Demographic and Clinical Characteristics of Nonalcoholic Fatty Liver Disease Patients and Healthy Controls ^a

	NAFLD Patients	Healthy Controls	χ^2 or t	P Value
Female, %	151 (44.4)	224 (49.6)	$\chi^2 = 2.061$	0.151
Age, y	41.54 ± 8.19	40.18 ± 11.03	t = 1.909	0.057
BMI, kg/m ²	26.17 ± 2.60	23.18 ± 3.07	t = 14.796	< 0.001
ALT, U/L	42.25 ± 25.36	29.73 ± 15.54	t = 8.046	< 0.001
AST, U/L	25.86 ± 12.65	22.82 ± 8.81	t = 3.784	< 0.001
GGT, U/L	29.73 ± 25.48	18.35 ± 22.90	t = 6.500	< 0.001
FPG, mmol/L	5.51 ± 1.61	4.89 ± 0.92	t = 6.418	< 0.001
UA, mmol/L	360.54 ± 95.20	306.28 ± 82.12	t = 8.400	< 0.001
TG, mmol/L	2.40 ± 2.32	1.60 ± 1.97	t = 5.175	< 0.001
TC, mmol/L	5.00 ± 1.05	4.58 ± 1.01	t = 5.574	< 0.001
HDL, mmol/L	1.29 ± 0.46	1.47 ± 0.31	t = -6.479	< 0.001
LDL, mmol/L	3.21 ± 1.04	2.72 ± 0.83	t = 7.116	< 0.001

^a Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ -glutamyltransferase; FPG, fasting plasma glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; TG, triglyceride; TC, total cholesterol; UA, uric acid.

Table 2. Distribution of Genotypes and Allele Frequencies of Genetic Variants in *SAMM50* in Subjects

Genotypes and Allele	Nonalcoholic Fatty Liver Disease Patients ^a	Controls ^a	χ^2	P Value
rs738491				
Genotypes			25.467	< 0.001
CC	66 (19.41)	132 (29.20)		
CT	151 (44.41)	226 (50.00)		
TT	123 (36.18)	94 (20.80)		
Alleles			24.604	< 0.001
C	283 (41.62)	490 (54.20)		
T	397 (48.38)	414 (45.80)		
rs2143571				
Genotypes			33.300	< 0.001
GG	76 (22.35)	162 (35.84)		
GA	152 (44.71)	214 (47.35)		
AA	112 (32.94)	76 (16.81)		
Alleles			34.172	< 0.001
G	304 (44.71)	538 (59.51)		
A	376 (55.29)	366 (40.49)		
rs3761472				
Genotype			26.380	< 0.001
AA	85 (25.00)	167 (36.95)		
GA	153 (45.00)	213 (47.12)		
GG	102 (30.00)	72 (15.93)		
Alleles			26.527	< 0.001
A	323 (47.50)	547 (60.51)		
G	357 (52.50)	357 (39.49)		

^a Values are presented as No. (%).

Table 3. Odds Ratios for Nonalcoholic Fatty Liver Disease According to Genotypes of *SAMM50* Single Nucleotide Polymorphisms in Study Group

	Unadjusted OR, 95% CI	P Value	Adjusted OR, 95%CI ^a	P Value
rs738491				
CC	1		1	
CT + TT	1.712 (1.223 - 2.398)	0.002	1.507 (1.035 - 2.195)	0.032
rs2143571				
GG	1		1	
GA + AA	1.940 (1.410 ~ 2.671)	< 0.001	1.761 (1.232 - 2.517)	0.002
rs3761472				
AA	1		1	
AG + GG	1.758 (1.288 - 2.399)	< 0.001	1.483 (1.039 - 2.115)	0.030

^a Multiple logistic regression model was adjusted for age, gender, and body mass index.

Table 4. Association Between SAMM50 Polymorphisms and Clinical Features in Study Subjects^a

	rs738491				rs2143571				rs3761472			
	Noncarriers	Carriers	χ^2 or t	P Value	Noncarriers	Carriers	χ^2 or t	P Value	Noncarriers	Carriers	χ^2 or t	P Value
n	198	594			238	554			252	540		
Female, %	86 (43.4)	289 (48.7)	$\chi^2 = 1.622$	0.203	114 (47.9)	261 (47.1)	$\chi^2 = 0.041$	0.839	128 (50.8)	247 (45.7)	$\chi^2 = 1.760$	0.185
Age, y	41.51 ± 10.08	40.51 ± 9.88	T = 1.223	0.222	40.81 ± 10.37	40.74 ± 9.75	t = 0.090	0.929	41.61 ± 10.78	40.37 ± 9.50	t = 1.563	0.119
BM, kg/m²	23.91 ± 2.94	24.65 ± 3.31	t = -2.938	0.004	24.08 ± 3.24	24.63 ± 3.23	t = -2.192	0.029	23.93 ± 3.26	24.71 ± 3.20	t = -3.210	0.001
ALT, U/L	32.20 ± 18.82	36.08 ± 21.94	t = -2.407	0.017	31.29 ± 18.63	36.75 ± 22.10	t = -3.572	< 0.001	30.95 ± 18.27	37.05 ± 22.26	t = -4.069	< 0.001
AST, U/L	23.06 ± 9.15	24.48 ± 11.19	t = -1.613	0.107	23.09 ± 8.49	24.57 ± 11.54	t = -2.012	0.045	22.63 ± 8.50	24.82 ± 11.56	t = -2.680	0.008
GGT, U/L	20.62 ± 20.84	24.11 ± 25.78	t = -1.725	0.085	19.37 ± 15.15	24.89 ± 27.63	t = -3.605	< 0.001	18.55 ± 15.79	25.42 ± 27.62	t = -4.435	< 0.001
FPG, mmol/L	4.95 ± 0.95	5.22 ± 1.39	t = -3.075	0.002	5.07 ± 1.11	5.19 ± 1.37	t = -1.399	0.162	5.06 ± 1.20	5.20 ± 1.34	t = -1.501	0.134
UA, mmol/L	320.87 ± 95.35	332.36 ± 90.65	t = -1.521	0.129	332.03 ± 100.30	328.41 ± 88.13	t = 0.482	0.63	325.45 ± 90.31	331.38 ± 92.67	t = -0.845	0.398
TG, mmol/L	1.76 ± 1.84	2.00 ± 2.25	t = -1.546	0.123	1.62 ± 1.66	2.08 ± 2.32	t = -3.145	0.002	1.71 ± 2.08	2.05 ± 2.19	t = -2.055	< 0.001
TC, mmol/L	4.41 ± 0.89	4.88 ± 1.08	t = -6.117	< 0.001	4.57 ± 0.98	4.84 ± 1.07	t = -3.306	0.001	4.74 ± 0.94	4.77 ± 1.10	t = -0.436	0.663
HDL, mmol/L	1.44 ± 0.52	1.38 ± 0.34	t = 1.651	0.099	1.43 ± 0.51	1.38 ± 0.33	t = 1.456	0.146	1.41 ± 0.33	1.39 ± 0.42	t = 0.587	0.557
LDL, mmol/L	2.69 ± 0.89	3.01 ± 0.96	t = -4.129	< 0.001	2.74 ± 0.83	3.01 ± 0.99	t = -4.042	< 0.001	2.61 ± 0.79	3.08 ± 0.99	t = -6.691	< 0.001

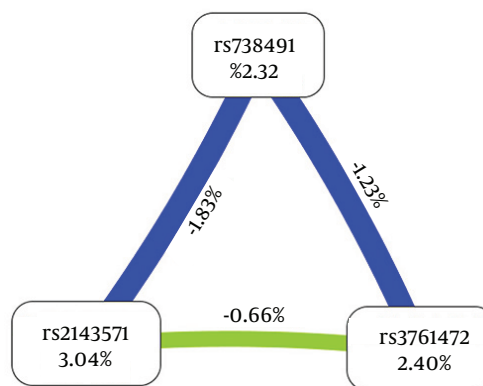
^a Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; GGT, γ -glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TC, total cholesterol; UA, uric acid.

Figure 1. SNP-SNP Interaction Dendrogram



The shorter the line connecting the 2 SNPs, the stronger the interaction. Green and blue represent redundancy or correlation.

Figure 2. Fruchterman-Reingold, Describing Interactions Between the 3 Genetic Variants



Green and blue with a negative percent entropy indicate redundancy or correlation.

Table 5. Best Models to Predict Nonalcoholic Fatty Liver Disease by Generalized Multifactor Dimensionality Reduction (GMDR)^a

Factor	Model	Training Accuracy ^b	Testing Accuracy ^b	Sign Test (P)	Cross-Validation Consistency
1	rs2143571	58.32	54.94	8 (0.0547)	8/10
2	rs2143571, rs3761472 ^a	62.67	62.21	10 (0.0010)	10/10
3	rs738491, rs2143571, rs3761472	64.37	60.79	10 (0.0010)	10/10

^a Whole dataset statistics: Training Balanced Accuracy, 0.6264; Training Accuracy, 0.6264; Training Sensitivity, 0.5294; Training Specificity, 0.7235; Training Odds Ratio, 2.9430 (1.9291, 4.4899); Training χ^2 (P), 25.7846 (P < 0.0001); Training Precision, 0.6569; Training Kappa, 0.2529; Training F-Measure, 0.5863.

^b The values' unit is (%).

5. Discussion

This study aimed to identify NAFLD susceptibility of the genetic variants in *rs738491*, *rs2143571*, and *rs3761472* in the *SAMM50* gene in a Chinese Han population. The GMDR analysis was employed to determine the SNP-SNP interactions.

Previous studies have indicated that the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele in the *SAMM50* gene may contribute to the progression from simple fatty liver to NASH, not only significantly associated with predisposition to NAFLD (7, 8), but also with decreased levels of serum TG and increased levels of ferritin and hyaluronic acid, which are high in NASH. However, our data demonstrated a controversial association inasmuch as the *rs2143571* A allele and the *rs3761472* G allele were both deeply associated with increased serum TG levels, whereas the *rs738491* T allele did not show a significant P value. Furthermore, the ALT and AST levels of serum were significantly different between the carriers and the noncarriers of the *rs2143571* A allele and the *rs3761472* G allele, while the *rs738491* T allele was only associated with increased levels of serum ALT. It is worthy of note that a similar finding has been confirmed in Indian subjects (8). This suggested that the genetic variants in *rs2143571* and *rs3761472*, but not in *rs738491*, were associated with hepatic fat accumulation, as well as liver injury (13). Furthermore, adjusting for gender, age, and BMI, we demonstrated that the carriers of the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele increased the predisposition to NAFLD compared with the noncarriers. However, the current results should be confirmed by future studies including larger and multiple ethnic populations.

The *Sam50*, encoded by the *SAMM50* gene, is a part of the sorting and assembly machinery (SAM) required for the assembly pathway of the mitochondrial outer membrane β -barrel proteins (14). It is crucial for maintaining mitochondrial shape, morphology of mitochondrial cristae, and assembly of respiratory chain complexes (14, 15). Ott et al. confirmed that a moderate reduction in the *Sam50* could result in changes in mitochondrial shape and the morphology of cristae and that depletion of the *Sam50* could influence mitochondrial respiratory complexes (14). Various studies have established that mitochondrial dysfunction (loss of mitochondrial cristae and paracrystalline inclusions) plays an important role

in insulin resistance (16, 17). Hepatic insulin resistance is known as the major pathophysiological contributor to the development and progression of NAFLD (18). In addition, mitochondrial abnormalities have been observed in the liver biopsy specimens of patients with NASH (19). These findings and our data indicate that the *rs738491* T allele, *rs2143571* A allele, and *rs3761472* G allele in the *SAMM50* gene may be involved in mitochondrial dysfunction and subsequent insulin resistance, resulting in the development and progression of NAFLD.

Given that NAFLD represents a complex disorder influenced by the interplay between genetic and environmental factors, multi-gene or SNP-SNP interaction studies may help discover the risk factors for NAFLD. Accordingly, we performed the MDR (12) and GMDR (20) to determine the potential SNP-SNP interactions between the 3 SNPs in the *SAMM50* gene. The analysis of the SNP-SNP interactions showed a strong interaction between *rs738491*, *rs2143571*, and *rs3761472* regarding susceptibility to NAFLD. As is described in Table 5, the results of the GMDR indicated that a synergistic relationship might exist between *rs738491*, *rs2143571*, and *rs3761472* in the *SAMM50* gene and the pathophysiology and genetic background of NAFLD. Additionally, a combination of *rs2143571* and *rs3761472* was the best model to predict the susceptibility to NAFLD compared to the single SNP alone.

However, several limitations existed in our study. First, the lack of liver biopsy is the main limitation of the present study. Liver biopsy is recognized as the gold standard in providing an accurate diagnosis of NAFLD; it is, however, expensive and not ethically feasible for uninvestigated subjects in epidemiological studies. We determined the hepatic fat content employing liver ultrasonography, which is widely utilized for the measurement of fatty liver disease (9) but cannot provide reliable quantitative information (21, 22). Moreover, liver ultrasonography cannot detect small changes in hepatic fat content over time, and its sensitivity and specificity are decreased in diagnosing obese NAFLD patients (23). Second, the underlying molecular mechanisms of the association and the SNP-SNP interactions should be explored by future basic and clinical studies.

We first demonstrated that genetic variants in *rs738491*, *rs2143571*, and *rs3761472* in the *SAMM50* gene created susceptibility to NAFLD in a Chinese Han population. The SNP-SNP interaction studies showed that a combination of the 3 SNPs in the *SAMM50* gene might have synergism to predict the predisposition to NAFLD. The present study will help identify high-risk populations and perform appropriate interventions. Meanwhile, it will provide new ideas for the prevention and treatment of NAFLD.

Authors' Contributions

Lizhen Chen, Man Jiang, and Yongning Xin conceived and designed the experiments. Zhonghua Lin collected subjects. Lizhen Chen and Linlin Lu performed the statistical analysis. Yongning Xin, Xiangjun Jiang, and Shiyong Xuan provided reagents, materials, and analysis tools. Lizhen Chen drafted and wrote the manuscript. Zhonghua Lin, Man Jiang, Linlin Lu, Haiying Zhang, Yongning Xin, and Xiangjun Jiang revised the manuscript. Shiyong Xuan approved the final version.

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