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ORIGINAL PAPER

Effect of *L3MBTL3/PTPN9* polymorphisms on risk to alcohol-induced ONFH in Chinese Han population

Jun Xiong[1] · Yi Niu[2] · Wei Liu[1] · Fan Zeng[1] · Jian-Fei Cheng[1] · Shi-Qiang Chen[1] · Xiang-Zhou Zeng[3]

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Abstract

Purpose Alcohol-induced osteonecrosis femoral head necrosis (ONFH) is a disease that seriously affects human health. Abnormal expression of *L3MBTL3/PTPN9* gene can cause a variety of human diseases. The purpose of this study is to investigate the effect of *L3MBTL3/PTPN9* gene polymorphism on the susceptibility of alcohol-induced ONFH in Chinese Han population.

Methods A total of 308 alcohol-induced ONFH patients and 425 healthy controls were enrolled in this case–control study. Alleles, genotypes, genetic models, haplotypes, and multifactor dimensionality reduction analyses (MDR) based on age-corrected by using odds ratio (OR) and 95% confidence interval (CI) were performed.

Results Our result revealed rs2068957 in the *L3MBTL3* gene increased the risk of alcohol ONFH under the recessive model after correction. Besides, we also found that rs75393192 in the *PTPN9* gene was a protective site in stratification over 40 years of age and stage. In stratified analysis of necrotic sites, we only found that rs2068957 was associated with increased susceptibility of alcohol-induced ONFH under the co-dominant model and recessive model. Haplotype "GC" in the block (rs76107647|rs10851882 in *PTPN9* gene) significantly decreased the susceptibility of alcoholic ONFH.

Conclusions Our results provide evidence that *L3MBTL3/PTPN9 polymorphisms* are associated with alcohol-induced ONFH risk in Chinese Han population.

Keywords *L3MBTL3/PTPN9* · Single-nucleotide polymorphisms · Alcohol-induced osteonecrosis femoral head necrosis · Chinese Han population

Introduction

Osteonecrosis of the femoral head (ONFH) is a very common disease in daily life. It is estimated that there are 20,000,000 ONFH patients in the world, of which alcohol-induced ONFH accounts for 20–45% of ONFH [1–3]. With alcohol into people's lives, the incidence of alcoholic ONFH is still rising. The risk of alcohol-induced ONFH is higher with the high alcohol intake and the longer drinking time [4]. Alcohol-induced ONFH has brought great damage to the patient's physiology and psychology and also brought a great burden to the society. A large number of literatures have reported that alcohol-induced ONFH is related to genetic factors. Okazaki et al. showed that *TLR4* signaling pathway was involved in the pathogenesis of alcohol-induced ONFH [5]. Yoon BH et al. pointed that alcohol-induced ONFH was associated with individual genetic susceptibility [6]. However, the exact pathogenesis and molecular mechanisms leading to the onset of the disease are still unclear.

Malignant brain tumor (MBT) family is a large family with *L3MBTL1* [7], *L3MBTL2* [8], *L3MBTL3, L3MBTL4*, and other members. Studies have shown that *L3MBTL1* and *L3MBTL2* are related to bone tissue related diseases. *L3MBTL3* (also known as *MBT1*) is located on the long arm

Jun Xiong and Yi Niu are co-first authors.

✉ Xiang-Zhou Zeng
zxzhyfy@126.com

[1] Department of Orthopedic Trauma, the Hainan Affiliated Hospital of Hainan Medical University, No. 19, Xiuhua Road, Haikou 570311, Hainan Province, China

[2] Department of Emergency and Critical Care Medicine, the Haikou Orthopedic and Diabetes Hospital of Shanghai Sixth People's Hospital, No. 3, Changxiu Road, Haikou 570300, Hainan Province, China

[3] Department of Pharmacology, School of Basic Medicine and Life Science, the Hainan Medical University, No. 3, Xueyuan Road, Haikou 571199, Hainan Province, China

of chromosome 6 and has 30 exons. It is a member of the transcriptional inhibitor family of chromatin interaction in *MBT* [9]. It can bind to methyl lysine in histone, further affecting the transcription and its abnormal regulation associated with many diseases. Seyede Zahra Nazari Mehrabani et al. showed that *L3MBTL3* gene polymorphism was related to the susceptibility of multiple sclerosis [10]. Research by Satoko Arai et al. showed that MBT-1 specifically regulated the maturation and progression of myeloid progenitor cells, and MBT-1 also showed that it effected bone marrow cell production by transiently increasing the expression of $p57^{KIP2}$ [11]. However, the association of *L3MBTL3* with alcoholic osteonecrosis has not been reported.

Protein tyrosine phosphatase acceptor 9 (*PTPN9*), also known as *PTP-MEG2*, is an important member of the protein tyrosine phosphatase family, and its N-terminal contains a phosphoinositol binding Sec14 homologous domain. Previous studies have shown that *PTPN9* is involved in the regulation of vesicle fusion and transport and then plays a crucial role in the physiological and pathological processes such as cell proliferation, differentiation, and migration [12]. Researches have shown that *PTPN9* is associated with a variety of human cancers, including hepatocellular carcinoma [13], colorectal cancer [14], cervical cancer [15], and breast cancer [16]. It has also been shown that *PTPN9* affects growth and expansion of erythroid cells [17] and embryonic development [18]. However, the relationship between *PTPN9* gene and alcohol-related ONFH is not clear.

As mentioned above, we speculate that the *L3MBTL3/PTPN9* gene is related to alcohol-induced ONFH. In this study, genotyping was used to explore the genetic association between *L3MBTL3/PTPN9* polymorphism and alcohol-related ONFH risk in Chinese Han population.

Methods

Research subjects

In this study, 308 patients with alcohol-induced ONFH were recruited, and 425 healthy control population samples were collected from the physical examination center of the same hospital during the same period. This study was approved by the ethics committee. Prior to the questionnaire and blood collection, all subjects signed informed consents.

Diagnosis and exclusion criteria The patient's intake of pure ethanol for more than 6 months should be greater than 400 ml/week (320 g/week, any alcoholic beverages); the patient was diagnosed with ONFH within 1 year after alcohol intake and the patient should not have other risk factors [19]. The combined results of bone scan [20], x-ray, and magnetic resonance imaging (MRI) were diagnosed as alcoholic ONFH. Patients with a clear history of direct trauma or chronic illnesses or possibly multiple causes were excluded. All healthy controls had a drinking habit, drinking more than 400 ml (320 g/week, any type of alcoholic beverage) per week for more than 6 months; however, they had never been diagnosed with alcohol-induced ONFH, hyperlipidemia, rheumatoid arthritis, syringomyelia, osteoporosis, decompression disease, cardiovascular disease, steroid use, smoking, etc.

Questionnaire investigation In this study, epidemiological questionnaire was used to investigate the subjects including general demographic characteristics (age, gender, height, weight, etc.), smoking, family genetic history, and other basic information.

Genotyping

The basis for our selection of candidate loci is based on haplotype data or genotype data [21, 22] and from the 1,000 Genome Projects (http://www.internationalgenome.org/) to select loci with a minor allele frequency (MAF) greater than 0.05 in the global population. By consulting a large number of documents and searching the database, we found that *L3MBTL3/PTPN9* gene was related to a variety of human diseases. However, the three single-nucleotide polymorphisms (SNPs) (rs1125970, rs4897367, rs2068957) in the *L3MBTL3* gene and the three SNPs (rs76107647, rs10851882, and rs75393192) in the *PTPN9* gene have not been reported before, and we used them as candidate sites for this study.

In the fasting state of the test population in the morning, 5 ml of peripheral venous blood was drawn into an anticoagulation tube containing EDTA and stored in a refrigerator at − 20 °C for later use. Then, according to the whole blood genomic DNA extraction kit (GoldMag Co. Ltd, Xi′an, China) [23], we extracted DNA from venous blood and measured the DNA concentration and purity by nanodrop 2000 ultraviolet spectrophotometer. We used Agena's online software (https://agenacx.com/online-tools/) to design the upstream and downstream primers and single-base extension primers for the selected six SNPs and used Agena MassARRAY system (Agena Bioscience, San Diego, CA, USA) for SNP genotyping, which was used in articles previously reported [24].

Statistical analyses

We used Microsoft Excel and SPSS 2.0 software to perform statistical analysis. Welch's *t* test was used to analyze whether there was statistical difference in the mean age between the case group and the control group. Furthermore, in order to ensure the reliability of the experimental results, an accurate test was performed to analyze whether the distribution of genotype frequency of each SNP site in the control group satisfied Hardy–Weinberg equilibrium (HWE) [25].

Afterwards, in order to clarify the relationship between studied sites and the susceptibility of alcoholic ONFH, we performed an unconditional logistic regression model analysis under the genetic models (co-dominant, dominant, recessive, and log-additive) through PLINK software (version 1.07) [26]. Then, we conducted stratification analysis on age, course of disease, necrotic site, and stage. We have also carried out linkage disequilibrium (LD) and haplotype analysis through the Haploview software package. Finally, to better understand the interaction of SNP–SNP in alcoholic ONFH risk, we conducted a multifactor dimensionality reduction (MDR) (version 3.0.2) analysis [27]. For all tests, a two-tailed p value < 0.05 is considered to have statistical significance. The sample size and the proportion between the experimental group and the control group were determined through G*Power 3.1.9.2 software [28]. After calculation, the total sample size of the experiment, the sample size of the control group, and the sample size of the case group were required to be greater than 400, 233, and 167, respectively. In our study, the total sample size, the sample size of the control group, and the sample size of the case group were 733, 308, and 425, respectively, so our sample size met the statistical requirements. At the same time, the calculated power of this study is 99.99%, which was fully in line with statistical significance.

Results

Subject characteristics

The basic information of all study participants is shown in Table 1. In this study, we recruited 308 alcoholic ONFH and 425 healthy controls. The average age of subjects was 43.4 ± 11.3 years in the case group and 42.7 ± 12.9 years in the control group. Three hundred eight patients presented with different distribution, according to necrotic sites [unilateral necrosis 65 (21%), bilateral necrosis 243 (79%)], phase [phase III + IV 218 (71%), phase I + II 90 (29%)], and course [greater than 22 months 103 (33%), less than or equal to 22 months 205 (67%)].

Relationships of L3MBTL3/PTPN9 polymorphisms with alcoholic ONFH predisposition

The basic information related to the study site and alcoholic ONFH is described in Table 2, including position, alleles (A/B), p HWE, and MAF (case and control). We analyzed the correlation between *L3MBTL3/PTPN9* polymorphisms and alcohol-induced ONFH risk in the allele model, and the results are shown in Table 2. However, we did not find any significant statistical difference between the SNPs and alcohol-induced ONFH risk ($p > 0.05$). The genetic model analyses showed that rs2068957 in the *L3MBTL3* gene increased the risk of alcohol-induced ONFH by 2.18 times under the co-dominant model (OR = 2.18; 95% CI, 1.01–4.72; $p = 0.048$) and 2.34 times under the recessive model (OR = 2.34; 95% CI, 1.09–5.02; $p = 0.030$) before correction and only 2.31 times under the recessive model (OR = 2.31; 95% CI, 1.07–4.97; $p = 0.032$) after correction. However, there was no significant statistical significance between the other loci and the risk of alcohol-induced ONFH in the four genetic models (co-dominant, dominant, recessive, and log-additive models) (Table 3).

Table 1 Comparison of characteristics between control group and alcohol-induced ONFH

Characteristics	Cases ($N = 308$)	Controls ($N = 425$)	p value
Age			0.487
>40 years	187 (61%)	246 (58%)	
≤40 years	121 (39%)	179 (42%)	
Mean ± SD	43.4 ± 11.3	42.7 ± 12.9	
Necrotic areas			
Unilateral necrosis	65 (21%)	0	
Bilateral necrosis	243 (79%)	0	
Phase			
Phase III + IV	218 (71%)	0	
Phase I + II	90 (29%)	0	
Course			
Greater than 22 months	103 (33%)	0	
Less than or equal to 22 months	205(67%)	0	

SD, standard deviation. p value was calculated from independent sample Student's t test; $p < 0.05$ indicates statistical significance

Table 2 Basic information of the 6 SNPs in *L3MBTL3/PTPN9* gene in this study

SNP_ID	Gene	Chr	Position	Allele A/B	MAF		*p* HWE	OR (95%CI)	*p* value
					Case	Control			
rs1125970	*L3MBTL3*	6	130,456,584	T/A	0.153	0.149	0.704	1.03(0.77–1.38)	0.832
rs4897367	*L3MBTL3*	6	130,458,152	T/C	0.112	0.100	0.786	1.14(0.81–1.59)	0.459
rs2068957	*L3MBTL3*	6	130,460,592	A/G	0.203	0.198	0.094	1.03(0.8–1.34)	0.803
rs76107647	*PTPN9*	15	75,827,187	G/A	0.110	0.128	0.124	0.84(0.61–1.16)	0.301
rs10851882	*PTPN9*	15	75,836,279	T/C	0.498	0.498	0.771	1.00(0.82–1.23)	0.978
rs75393192	*PTPN9*	15	75,870,551	C/T	0.070	0.076	0.495	0.91(0.61–1.35)	0.630

SNP, single-nucleotide polymorphism; *HWE*, Hardy–Weinberg equilibrium; *OR*, odds ratio; *95% CI*, 95% confidence interval

HWE *p* value obtained from Chi-squared test

p values were calculated from Chi-squared test regarding to the allele distribution frequencies among alcohol-induced ONFH patients and healthy controls

$p < 0.05$ indicates statistical significance

Stratified analysis

In order to better understand the relationship between the six loci studied and the susceptibility of alcohol-induced ONFH, we conducted four stratified analyses of age, stage, necrotic sites, and course of disease. In the age stratification analysis, we only found that rs75393192 in the *PTPN9* gene was related to the reduction of alcohol-induced ONFH when it was over 40 years old under the co-dominant model (OR = 0.54; 95% CI, 0.29–1.00; $p = 0.049$). When it was less than or equal to 40 years old, the six loci studied were not related to the susceptibility of alcohol-induced ONFH ($p > 0.05$). In the stage stratification analysis, we only found that rs75393192 was related to the reduction of alcohol-induced ONFH under the allele model (OR = 0.49; 95% CI, 0.26–0.93; $p = 0.025$) (Table 4). In the necrotic site stratification analysis, we only found that rs2068957 was increased to the susceptibility of alcohol-induced ONFH under the co-dominant model (OR = 2.26; 95% CI, 1.01–5.05, $p = 0.047$) and recessive model (OR = 2.45; 95% CI, 1.1–5.43; $p = 0.028$) (Table 5). In the course of disease stratification analysis, there was no significant correlation between the six loci studied and the susceptibility of alcohol-induced ONFH in four genetic models.

The association between L3MBTL3/PTPN9 haplotypes and alcoholic ONFH susceptibility

In the study of the relationship between the *L3MBTL3/PTPN9* haplotype and the susceptibility of alcoholic ONFH, we only found that the GC haplotype of

Table 3 Genetic model analyses of the SNP and the risk of alcohol-induced ONFH

SNP_ID	Model	Genotypes	Patients, *n* (%)	Controls, *n* (%)	Without adjustment		With adjustment	
					OR (95% CI)	p^a	OR (95% CI)	p^b
rs2068957	Co-dominant	G/G	201 (65%)	268 (63%)	1.00		1.00	
		G/A	89 (29%)	146 (34%)	0.81 (0.59–1.12)	0.205	0.81 (0.59–1.12)	0.208
		A/A	18 (6%)	11 (3%)	2.18 (1.01–4.72)	**0.048**	2.16 (1–4.67)	0.051
	Dominant	G/G	201 (65%)	268 (63%)	1.00		1.00	
		G/A-A/A	107 (35%)	157 (37%)	0.91 (0.67–1.23)	0.540	0.91 (0.67–1.23)	0.538
	Recessive	G/G-G/A	290 (94%)	414 (97%)	1.00		1.00	
		A/A	18 (6%)	11 (3%)	2.34 (1.09–5.02)	**0.030**	2.31 (1.07–4.97)	**0.032**
	Log-additive	-	-	-	1.03 (0.80–1.34)	0.803	1.03 (0.8–1.34)	0.816

SNP, single-nucleotide polymorphism; *OR*, odds ratio; *95% CI*, 95% confidence interval

p^a values were calculated by unconditional logistic regression analysis with the comparison between alcohol-induced ONFH and healthy controls

p^b values were calculated by unconditional logistic regression analysis with adjustments for age

Values in bold indicate a statistically significant SNP ($p < 0.05$)

Table 4 Stratified analysis of the age and stage on association between selected SNPs and alcohol-induced ONFH risk

SNP	Model	Genotype	>40		≤40		Stage	
			OR (95%CI)	*p* value	OR (95%CI)	*p* value	OR (95%CI)	*p* value
rs75393192	Allele	T	1.00		1.00		1.00	
		C	0.69 (0.4–1.22)	0.202	1.24 (0.69–2.21)	0.468	0.49 (0.26–0.93)	**0.025**
	Co-dominant	T/T	1.00		1.00		1.00	
		T/C	0.54 (0.29–1)	**0.049**	1.07 (0.54–2.1)	0.847	0.61 (0.29–1.29)	0.196
		C/C	-	0.999	5.04 (0.49–51.41)	0.173	0.27 (0.04–1.67)	0.159
	Dominant	T/T	1.00		1.00		1.00	
		T/C–C/C	0.6 (0.33–1.09)	0.093	1.21 (0.64–2.31)	0.559	0.55 (0.27–1.11)	0.093
	Recessive	T/T-T/C	1.00		1.00		1.00	
		C/C	-	0.999	4.99 (0.49–50.74)	0.175	0.29 (0.05–1.78)	0.182
	Log-additive	-	0.69 (0.39–1.21)	0.191	1.31 (0.74–2.32)	0.351	0.57 (0.32–1.03)	0.064

SNP, single-nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval

$p < 0.05$ indicates statistical significance

Values in bold indicate a statistically significant SNP ($p < 0.05$)

rs76107647|rs10851882 in *PTPN9* gene was related to the susceptibility of alcoholic ONFH (OR = 0.69; 95% CI, 0.49–0.98; $p = 0.040$ in crude analysis; OR = 0.67; 95% CI, 0.47–0.96, $p = 0.029$ in adjusted by age) (Table 6), and there was no statistical significance between the haplotype of rs1125970, rs4897367, and rs2068957 in *L3MBTL3* gene and rs75393192 in *PTPN9* gene and the susceptibility of alcoholic ONFH ($p > 0.05$).

MDR analysis for the effect of L3MBTL3/PTPN9 SNP–SNP interaction on alcoholic ONFH risk

In order to better understand the impact of *L3MBTL3/PTPN9* SNP–SNP interaction on the risk of ONFH, we conducted MDR analysis. Unfortunately, we did not find the best model for the impact of *L3MBTL3/PTPN9* SNP–SNP interaction on the risk of ONFH (Table 7, Supplementary Fig. 1).

Discussion

In this case–control study, we found that rs2068957 in the *L3MBTL3* gene was related to the susceptibility of alcoholic ONFH under the recessive model. After stratified analysis, we also found that rs75393192 in the *PTPN9* gene and rs2068957 in the *L3MBTL3* gene were relevant to the susceptibility of alcoholic ONFH. Haplotype "GC" had a certain relationship with reducing the susceptibility of alcoholic ONFH. These results suggest that the risk of alcoholic ONFH is associated with variants *L3MBTL3/PTPN9* gene in Chinese Han populations.

Alcoholic ONFH is a common hip disease, and it is relatively frequently disabled. Although much progress has been made in clinical manifestations and surgical treatment, further research is still needed. Intravascular thrombosis, abnormal lipid metabolism, and steroid sensitivity are considered to be the main causes of ONFH [29]. The *L3MBTL3* gene encodes a malignant brain tumor (MBT) family. Its function is a methyl lysine reader, which recognizes methyl lysine residues in the histone tail and is related to the suppression of gene expression. By consulting a large number of documents, we learned the encoded protein could regulate hematopoiesis [9, 30]. Therefore, we speculate that the abnormal expression of *L3MBTL3* may cause abnormalities in regulating hematopoiesis and further lead to the formation of intravascular thrombosis and eventually the formation of

Table 5 Stratified analysis of the necrotic areas on association between selected SNPs and alcohol-induced ONFH risk

SNP	Model	Genotype	With adjustment	
			OR (95% CI)	*p*
rs2068957	Allele	G	1.00	
		A	1.03 (0.78–1.36)	0.860
	Co-dominant	G/G	1.00	
		G/A	0.78 (0.55–1.11)	0.164
		A/A	2.26 (1.01–5.05)	**0.047**
	Dominant	G/G	1.00	
		G/A-A/A	0.88 (0.64–1.23)	0.468
	Recessive	G/G-G/A	1.00	
		A/A	2.45 (1.1–5.43)	**0.028**
	Log-additive	-	1.02 (0.77–1.35)	0.872

SNP, single-nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval

$p < 0.05$ indicates statistical significance

Values in bold indicate a statistically significant SNP ($p < 0.05$)

Table 6 Haplotype analysis for the effect of *L3MBTL3/PTPN9* gene haplotypes in course of disease stratified analysis on alcohol-induced ONFH risk

SNPs ID	Gene	Haplotype	Frequency		*p*	Crude analysis		Adjusted by age	
			Case	Control		OR (95% CI)	p^a	OR (95% CI)	p^b
rs76107647lrs10851882	*PTPN9*	GT	0.47	0.51	0.255	0.82 (0.59–1.15)	0.257	0.81 (0.57–1.14)	0.219
rs76107647lrs10851882	*PTPN9*	AC	0.14	0.10	0.152	1.45 (0.87–2.43)	0.156	1.49 (0.88–2.53)	0.139
rs76107647lrs10851882	*PTPN9*	GC	0.33	0.42	**0.037**	0.69 (0.49–0.98)	**0.040**	0.67 (0.47–0.96)	**0.029**

[a]*p* values were calculated by unconditional logistic regression. [b]*p* values were calculated by unconditional logistic regression adjusted for age
Values in bold indicate a statistically significant SNP ($p < 0.05$)

alcoholic ONFH. Studies have shown that Notch signaling pathway plays an important role in bone development and homeostasis, and serious bone diseases can be attributed to changes in Notch signaling pathway [31]. As a general modulator of Notch signaling pathway [32], *L3MBTL3* will cause abnormal expression of Notch signaling pathway, which will further affect bone development, osteoporosis, and alcoholic ONFH. Existing studies have shown that *L3MBTL3* gene polymorphisms are associated with various diseases, such as breast cancer [33], multiple sclerosis [10], and children's specific growth cycle [34]. However, the rs1125970, rs4897367, and rs2068957 in the *L3MBTL3* gene we studied have not been reported before. Our results also show that the *L3MBTL3* polymorphism is associated with the risk of alcoholic ONFH, which is also consistent with our previous hypothesis.

The protein encoded by *PTPN9* belongs to the family of protein tyrosine phosphatases (*PTP*) [35]. PTPs are considered to be signaling molecules that regulate various cellular processes, including cell growth, differentiation, mitotic cycle, and oncogenic transformation [36]. *PTPN9* is found to be activated by inositol polyphosphate peptides and is thought to be involved in the signaling events that regulates phagocytosis and has been reported to play an important role in promoting vesicle fusion of hematopoietic cells in endocrine fluid [35]. Studies have shown that *PTPN9* controls the expansion of erythroid cells [17] and regulates embryonic development [18]. *PTPN9* negatively regulates vascular endothelial growth factor receptor signaling and endothelial cell function, which is closely related to intravascular thrombosis [37]. Intravascular thrombosis is considered to be one of the main causes of ONFH [29]. Therefore, we speculate that the abnormality of *PTPN9* will cause the abnormality of hematopoietic function and red blood cells and then cause the formation of intravascular thrombosis. There are only two articles about *PTPN9* gene association analysis [38, 39], but three SNPs (rs76107647, rs10851882, and rs75393192) in the *PTPN9* gene we studied have not been reported before. Our research results showed that rs75393192 in the *PTPN9* gene was found to be associated with the risk of alcohol-reducing ONFH in a subgroup analysis older than 40 years and staging and the *PTPN9* gene GC haplotype was also associated with the risk of reducing alcoholic ONFH.

There are some potential limitations in this study. Firstly, this study has limitations of sample selection range and number. The sample size of this study is small, and a larger sample size is needed to verify the impact of *L3MBTL3/PTPN9* genetic polymorphism on risk of alcoholic ONFH. Secondly, the test population in this study is limited to the Chinese Han population, so other populations are needed to further verify the results of this study. Thirdly, this study only found that *L3MBTL3/PTPN9* polymorphisms were related to risk based on DNA level. Despite these limitations, our study is the first to demonstrate that *L3MBTL3/PTPN9* gene polymorphism is associated with alcoholic ONFH, which provides new insights into the association between *L3MBTL3/PTPN9* gene and alcoholic ONFH.

Table 7 MDR analysis of SNP–SNP interactions in relation with alcohol-induced ONFH risk

Model	Training Bal. Acc	Testing Bal. Acc	OR (95% CI)	CVC	Testing χ^2 value	*p* value
rs2068957	0.5273	0.5273	1.29 (0.47–3.51)	10/10	0.62	0.118
rs1125970,rs2068957	0.5335	0.5172	1.16 (0.44–3.03)	10/10	0.09	0.764
rs1125970,rs4897367,rs2068957	0.5335	0.5172	1.16 (0.44–3.03)	10/10	0.09	0.764

MDR, multifactor dimensionality reduction; *Bal. Acc.*, balanced accuracy; *CVC*, cross–validation consistency; *OR*, odds ratio; *CI*, confidence interval
p values were calculated using χ^2 tests
Bold indicated that $p < 0.05$ meant the data was statistically significant

This study provides a theoretical basis for further research on the function of *L3MBTL3/PTPN9* gene in the occurrence and development of alcoholic ONFH and provides a certain direction for screening high-risk groups of alcoholic ONFH.

Conclusion

In summary, our study showed that *L3MBTL3/PTPN9* polymorphism was related to the susceptibility of alcohol-induced ONFH in Chinese Han population. This study provides new insights for the identification, diagnosis, and treatment of alcohol-induced ONFH high-risk groups.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10072-021-05486-7.

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Author contribution Xiang-Zhou Zeng and Jun Xiong: conceived and designed the experiments.
Jun Xiong, Yi Niu, and Wei Liu: performed the experiments.
Jun Xiong and Fan Zeng: analyzed the data.
Jian-Fei Cheng: contributed reagents/materials/analysis tools.
Shi-Qiang Chen: prepared the figures and/or tables.
Xiang-Zhou Zeng and Jun Xiong: drafted the work or revised it critically for important content.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by the ethics committee of the Hainan Affiliated Hospital of Hainan Medical University. Prior to the questionnaire and blood collection, all subjects signed the informed consent.

Consent to participate All subjects were Han people who lived in and around Haikou, Hainan Province, China.

Consent for publication All authors have read and approved the manuscript.

Conflict of interest The authors declare no competing interests.

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