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# Significant association between *RETN* genetic polymorphisms and alcohol-induced osteonecrosis of femoral head

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## Abstract

**Background:** Alcohol-induced osteonecrosis of femoral head (ONFH) is a complex disease and genetic factors are one of the causes. The purpose of this study is to investigate the effects of *RETN* (resistin; OMIM: 605565) and *LDLR* (low density lipoprotein receptor; OMIM: 606945) polymorphisms on the risk of alcohol-induced ONFH in Chinese Han population.

**Methods:** A case–control study including 201 patients and 201 controls was designed. Seven single nucleotide polymorphisms (SNPs) in *RETN* gene and four SNPs in *LDLR* gene were genotyped using Agena MassARRAY platform. In allele model and genetic model, chi-square test and logistic regression were used to study the associations between these SNPs and ONFH susceptibility. In addition, the relationships between these SNPs, clinical phenotypes, and blood lipid level with one-way analysis of variance were analyzed.

**Results:** In the allele model, rs7408174 and rs3745369 in *RETN* were associated with increased risk of alcohol-induced ONFH, whereas rs34861192 and rs3219175 in *RETN* showed reduced risk of alcohol-induced ONFH. In the genetic model, rs7408174 was associated with increased risk of alcohol-induced ONFH in dominant model and log-additive model. Rs3745369 showed an increased risk in codominant model, recessive model, and log-additive model. Rs34861192 showed a decreased risk in codominant model, dominant model, and log-additive model, and rs3219175 showed a decreased risk in dominant model and log-additive model. The rs3745368 in *RETN* was associated with the clinical stage of the disease.

**Conclusion:** These results suggest that *RETN* genetic polymorphisms are associated with the susceptibility of alcohol-induced ONFH in Chinese Han population.

KEYWORDS

alcohol-induced osteonecrosis of femoral head, *LDLR*, *RETN*, single nucleotide polymorphisms

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# 1 | INTRODUCTION

Osteonecrosis of femoral head (ONFH) is a complicated disease in clinic and is usually divided into traumatic and nontraumatic types. Alcohol-induced ONFH caused by excessive alcohol intake over a long period of time is a type of nontraumatic ONFH. The etiology of alcohol-induced ONFH is complicated. Early diagnosis of this disease is difficult, and the complex pathological process is often manifested due to abnormal lipid metabolism and inflammation. Excessive alcohol drinking may result in dyslipidemia, abnormal differentiation of bone marrow mesenchymal stem cells (BMSCs) and bone metabolic disorders. Moreover, alcohol has a significant dose effect on bone homeostasis (Gaddini, Turner, Grant, & Iwaniec, 2016). However, we found in clinical work that only a portion of people who drank similar amounts of alcohol developed ONFH.

Some studies have suggested that the ONFH disease is caused by the interaction between genetic and environmental factors (Song et al., 2017; Wang, Azeddine, et al., 2018; Zhou, Qu, Lv, & Zhu, 2018). Therefore, genetic polymorphisms involved in alcohol metabolism, lipid metabolism, bone and circulatory homeostasis may lead to differences in susceptibility to alcohol-induced ONFH (Cui, Kaisaierjiang, Cao, Wu, & Lv, 2014; Hadjigeorgiou et al., 2008).

*RETN* (resistin; OMIM: 605565), located on chromosome 19, encodes resistin. Resistin affects bone metabolism and in vitro studies have shown that it can promote bone remodeling (Thommesen et al., 2006). Some scholars believe that there is a negative correlation between resistin content and bone density (Oh et al., 2005; Pedone et al., 2013; Zhang et al., 2010). Plasma resistin is also correlated with insulin resistance, lower HDL-C, and high hs-CRP (Osawa et al., 2007). Studies have shown that the polymorphisms of *RETN* have significant effect on plasma resistin concentration (Asano et al., 2010). In recent years, some scholars have found that the polymorphism of human *RETN* is also associated with osteoarthritis and rheumatoid arthritis (Hamalainen, Solovieva, Vehmas, Hirvonen, & Leino-Arjas, 2018; Junker et al., 2017; Wang, Tang, et al., 2018).

*LDLR* (low density lipoprotein receptor; OMIM: 606945) is located on chromosome 19, which plays a critical role in regulating the plasma cholesterol level. Mutations in *LDLR* result in elevated cholesterol (Hobbs, Brown, Russell, Davignon, & Goldstein, 1987). Cholesterol is one of the risk factors for osteoporosis and cholesterol metabolic disorders is detrimental to bone health (Li et al., 2018; Mandal, 2015). Alterations in the function of the *LDLR* affected bone development and homeostasis (Yang & Williams, 2017).

There are few studies on the association of *RETN* and *LDLR* with alcohol-induced ONFH. This work studies the association between *RETN* and *LDLR* genetic polymorphisms and the susceptibility of alcohol-induced ONFH in Chinese Han population, which can guide the identification of high-risk alcohol-induced ONFH patients.

# 2 | MATERIALS AND METHODS

## 2.1 | Ethics approval and consent to participate

This study was conducted under the approval of the Second Affiliated Hospital of Inner Mongolia Medical University of Inner Mongolia, China and Zhengzhou Traditional Chinese Medicine (TCM) Traumatology Hospital of Henan Province, China. Blood samples were collected at the time of initial diagnosis after informed consent was obtained from all participants.

## 2.2 | Subjects

All the 402 individuals including 201 cases and 201 controls were male and members of Chinese Han population living in Henan Province in China. Individuals who disagree to participate in this study were excluded.

The cases in our research satisfy the following criteria: (a) Patients should have a history of alcohol intake >400 ml/week (320 g/week, any type of alcoholic beverage) of pure ethanol for more than 6 months; (b) ONFH should be diagnosed within 1 year after the alcohol intake with this dose; (c) Patients should not have direct trauma and other risk factors (such as history of taking corticosteroids, cardiovascular diseases, congenital diseases, human immunodeficiency virus infection, diabetes mellitus, renal dysfunction, cancers, and familial hereditary diseases); (c) The diagnosis and staging of alcohol-induced ONFH was evaluated by X-ray, computed tomography(CT), nuclear magnetic resonance imaging (MRI); The selection criteria for control: (a) The age of the control group was matched with that of the case group; (b) The controls should have a history of alcohol intake >400 ml/week (320 g/week, any type of alcoholic beverage) of pure ethanol for more than 6 months; (c) No ONFH occurred; (d) Other factors were excluded (history of taking corticosteroids, cardiovascular diseases, congenital diseases, human immunodeficiency virus infection, diabetes mellitus, renal dysfunction, cancers, and familial hereditary diseases).

## 2.3 | SNP selection and genotyping

The GenBank reference sequence and version number: *RETN* (Reference Sequence and version number: NG_023447.1; accession: NG_023447), *LDLR* (Reference Sequence and version number: NG_009060.1; accession: NG_009060). All eleven SNPs in *RETN* and *LDLR* with minor allele frequencies >5% were selected from the 1,000 Genomes Project databases (http://www.internationalgenome.org/). Blood samples were collected in tubes containing ethylene diaminetetraacetic acid (EDTA) and stored at −80°C after centrifuging at 2,000 rpm for 10 min. Genomic DNA was extracted from the peripheral blood of the participants using the GoldMag whole blood genomic DNA purification kit (GoldMag Co. Ltd., Xi'an, China). DNA concentration was determined by using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA). The genotyping primers were designed with the Agena MassARRAY Assay Design 3.0 Software. Agena Typer 4.0 Software was used for managing the related data and the Agena MassARRAY RS1000 was used for genotyping.

**TABLE 1** Characteristics of the participants

| | Mean ± *SD* | | |
|---|---|---|---|
| **Variables** | **Cases (*n* = 201)** | **Controls (*n* = 201)** | ***p* value** |
| Age (years) | 42.68 ± 12.88 | 43.80 ± 8.38 | 0.302 |
| TC (mmol/L) | 4.65 ± 0.92 | 4.66 ± 0.88 | 0.938 |
| TG (mmol/L) | 1.89 ± 1.28 | 2.10 ± 1.12 | 0.084 |
| HDL-C (mmol/L) | 1.04 ± 0.24 | 1.08 ± 0.19 | 0.099 |
| LDL-C (mmol/L) | 2.73 ± 0.85 | 2.71 ± 0.74 | 0.869 |
| TC/HDL-C | 4.62 ± 1.15 | 4.38 ± 0.81 | 0.017* |
| TG/HDL-C | 1.98 ± 1.58 | 2.02 ± 1.14 | 0.759 |
| LDL-C/HDL-C | 2.70 ± 0.86 | 2.56 ± 0.72 | 0.086 |
| Clinical stages | | | |
| Stage II | 54 | | |
| Stage III | 89 | | |
| Stage IV | 58 | | |
| Hip lesions | | | |
| Unilateral | 44 | | |
| Bilateral | 157 | | |

*Note*: TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; LDL-C, high-density lipoprotein-cholesterol.
*p* value was calculated by Independent samples *t* test.
**p* < 0.05 indicates statistical significance.

## 2.4 | Statistical analyses

All statistical analyses were performed using Microsoft Excel, SPSS 19.0 (SPSS, Chicago, IL) and PLINK version 1.07 software. Two-sided *p*-values less than 0.05 were considered statistically significant. The alleles of cases and controls were tested by Chi-square test. The genotype frequencies were tested by logistic regression and the control group was compared with expected frequencies to test the deviations from Hardy–Weinberg equilibrium (HWE). Associations between SNPs and the risk of alcohol-induced ONFH were tested in four genetic models (codominant, dominant, recessive, and log-additive) using PLINK version 1.07 software and determined by unconditional logistic regression adjusted for age. Linkage disequilibrium among polymorphic sites was assessed with Haploview software package (version 4.2).

## 2.5 | Bioinformatics and expression analyses

The Genotype-Tissue Expression (GTEx) project provides a scientific resource to study SNPs and gene expression levels.

**TABLE 2** Basic information of candidate SNPs in this study

| | | | alleles | MAF | | *p* value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **Gene** | **Chromosome** | **A/B** | **case** | **control** | **for HWE** | **ORs** | **95% CI** | | ***p* value** |
| rs7408174 | *RETN* | 19 | C/T | 0.271 | 0.206 | 1.000 | 1.43 | 1.03 | 1.98 | 0.032* |
| rs34861192 | *RETN* | 19 | A/G | 0.135 | 0.199 | 0.826 | 0.63 | 0.43 | 0.92 | 0.016* |
| rs3219175 | *RETN* | 19 | A/G | 0.144 | 0.201 | 0.826 | 0.67 | 0.46 | 0.97 | 0.032* |
| rs3745367 | *RETN* | 19 | A/G | 0.373 | 0.423 | 0.885 | 0.81 | 0.61 | 1.08 | 0.150 |
| rs3745368 | *RETN* | 19 | A/G | 0.173 | 0.138 | 0.547 | 1.30 | 0.88 | 1.91 | 0.181 |
| rs3745369 | *RETN* | 19 | C/G | 0.381 | 0.313 | 0.254 | 1.35 | 1.01 | 1.81 | 0.045* |
| rs1477341 | *RETN* | 19 | A/T | 0.526 | 0.463 | 0.887 | 1.29 | 0.97 | 1.71 | 0.075 |
| rs12611067 | *LDLR* | 19 | T/G | 0.319 | 0.321 | 0.194 | 0.99 | 0.74 | 1.34 | 0.951 |
| rs14158 | *LDLR* | 19 | A/G | 0.400 | 0.388 | 0.882 | 1.05 | 0.79 | 1.40 | 0.718 |
| rs2738464 | *LDLR* | 19 | G/C | 0.278 | 0.294 | 0.125 | 0.93 | 0.68 | 1.26 | 0.630 |
| rs2738465 | *LDLR* | 19 | G/A | 0.485 | 0.495 | 0.480 | 0.96 | 0.73 | 1.27 | 0.778 |

*Note*: Reference Sequence and version number (*RETN*: NG_023447.1; *LDLR*: NG_009060.1).
Abbreviations: SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency.
*p* was calculated by Chi-squared test.
**p* < 0.05 indicates statistical significance.

**TABLE 3** Analysis of the association between SNPs and alcohol-induced ONFH risk in males

| SNP | Model | Genotype | case | control | OR (95% CI) | *p* value |
|---|---|---|---|---|---|---|
| rs7408174 | Codominant | T/T | 105 | 126 | 1.00 | 0.050 |
| | | C/T | 83 | 67 | 1.51 (1.00–2.29) | |
| | | C/C | 13 | 8 | 2.02 (0.80–5.08) | |
| | Dominant | T/T | 105 | 126 | 1.00 | 0.028* |
| | | C/T-C/C | 96 | 75 | 1.57 (1.05–2.34) | |
| | Recessive | T/T-C/T | 188 | 193 | 1.00 | 0.246 |
| | | C/C | 13 | 8 | 1.71 (0.69–4.23) | |
| | Log-additive | — | — | — | 1.47 (1.05–2.06) | 0.024* |
| rs34861192 | Codominant | G/G | 146 | 128 | 1.00 | 0.032* |
| | | A/G | 47 | 66 | 0.62 (0.39–0.96) | |
| | | A/A | 3 | 7 | 0.36 (0.09–1.43) | |
| | Dominant | G/G | 146 | 128 | 1.00 | 0.017* |
| | | A/G-A/A | 50 | 73 | 0.59 (0.38–0.91) | |
| | Recessive | G/G-G/A | 193 | 194 | 1.00 | 0.211 |
| | | A/A | 3 | 7 | 0.42 (0.11–1.64) | |
| | Log-additive | — | — | — | 0.61 (0.41–0.90) | 0.013* |
| rs3219175 | Codominant | G/G | 146 | 127 | 1.00 | 0.066 |
| | | A/G | 52 | 67 | 0.67 (0.43–1.03) | |
| | | A/A | 3 | 7 | 0.36 (0.09–1.42) | |
| | Dominant | G/G | 146 | 127 | 1.00 | 0.036* |
| | | A/G-A/A | 55 | 74 | 0.64 (0.42–0.97) | |
| | Recessive | G/G-G/A | 198 | 194 | 1.00 | 0.197 |
| | | A/A | 3 | 7 | 0.41 (0.10–1.60) | |
| | Log-additive | — | — | — | 0.65 (0.44–0.95) | 0.025* |
| rs3745369 | Codominant | G/G | 80 | 91 | 1.00 | 0.007* |
| | | C/G | 80 | 94 | 0.97 (0.63–1.48) | |
| | | C/C | 34 | 16 | 2.50 (1.28–4.90) | |
| | Dominant | G/G | 80 | 91 | 1.00 | 0.403 |
| | | C/G-C/C | 114 | 110 | 1.19 (0.80–1.77) | |
| | Recessive | G/G-G/C | 160 | 185 | 1.00 | 0.004* |
| | | C/C | 34 | 16 | 2.55 (1.35–4.81) | |
| | Log-additive | — | — | — | 1.36 (1.01–1.82) | 0.042* |

Abbreviations: SNP, single nucleotide polymorphism; OR odds ratio; CI, confidence interval.
*p* value adjusted for age was calculated by logistic regression.
*$p < 0.05$ indicates statistical significance.

In this research, online database (http://www.gtexportal.org/) was used to investigate the association between the 11 selected SNPs and the expression of two genes.

## 3 | RESULTS

This study involved 402 male subjects as shown in Table 1, including 201 cases and 201 controls. The mean ages were 42.68 ± 12.88 years for the cases and 43.80 ± 8.38 years for the controls. In the case group, there were 54 cases of stage II, 89 cases of stage III, 58 cases of stage IV, 44 cases of unilateral side and 157 cases of bilateral side. No significant differences in the distributions of age, TC, TG, HDL-C, LDL-C, TG/HDL-C, and LDL-C/HDL-C between the cases and the controls were observed from the statistical analysis. However, there was a significant difference in TC/HDL-C between the cases and the controls.

The basic information of all SNPs is shown in Table 2. The genotype distributions were in Hardy–Weinberg

**TABLE 4** The association of genotypes in *RETN* and *LDLR* genes with the clinical phenotypes

| | | | Hip lesions | | | Clinical stages | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP** | **genotype** | **Unilateral** | **Bilateral** | ***p*** | **Stage II** | **Stage III** | **Stage IV** | ***p*** |
| *RETN* | rs7408174 | CC | 5 | 8 | 0.268 | 2 | 7 | 4 | 0.884 |
| | | CT | 19 | 64 | | 22 | 36 | 25 | |
| | | TT | 20 | 85 | | 30 | 46 | 29 | |
| | rs34861192 | AA | 0 | 3 | 0.436 | 1 | 2 | 0 | 0.698 |
| | | AG | 8 | 39 | | 14 | 18 | 15 | |
| | | GG | 34 | 112 | | 38 | 68 | 40 | |
| | rs3219175 | AA | 0 | 3 | 0.543 | 1 | 2 | 0 | 0.489 |
| | | AG | 10 | 42 | | 14 | 19 | 19 | |
| | | GG | 34 | 112 | | 39 | 68 | 39 | |
| | rs3745367 | AA | 3 | 23 | 0.217 | 7 | 11 | 8 | 0.338 |
| | | AG | 20 | 78 | | 32 | 38 | 28 | |
| | | GG | 21 | 56 | | 15 | 40 | 22 | |
| | rs3745368 | AA | 3 | 4 | 0.396 | 5 | 1 | 1 | 0.022* |
| | | AG | 12 | 43 | | 10 | 31 | 14 | |
| | | GG | 29 | 109 | | 39 | 56 | 43 | |
| | rs3745369 | CC | 5 | 29 | 0.547 | 10 | 14 | 10 | 0.976 |
| | | CG | 19 | 61 | | 20 | 35 | 25 | |
| | | GG | 18 | 62 | | 22 | 36 | 22 | |
| | rs1477341 | AA | 14 | 40 | 0.243 | 13 | 21 | 20 | 0.125 |
| | | AT | 15 | 76 | | 31 | 39 | 21 | |
| | | TT | 12 | 32 | | 7 | 21 | 16 | |
| *LDLR* | rs12611067 | GG | 20 | 68 | 0.739 | 27 | 40 | 21 | 0.306 |
| | | TG | 19 | 72 | | 19 | 41 | 31 | |
| | | TT | 5 | 12 | | 6 | 5 | 6 | |
| | rs14158 | AA | 9 | 20 | 0.409 | 9 | 8 | 12 | 0.158 |
| | | AG | 20 | 83 | | 23 | 49 | 31 | |
| | | GG | 15 | 54 | | 22 | 32 | 15 | |
| | rs2738464 | CC | 27 | 72 | 0.19 | 28 | 40 | 31 | 0.784 |
| | | GC | 14 | 71 | | 23 | 38 | 24 | |
| | | GG | 2 | 10 | | 3 | 7 | 2 | |
| | rs2738465 | AA | 13 | 37 | 0.489 | 15 | 18 | 17 | 0.621 |
| | | GA | 24 | 83 | | 26 | 50 | 31 | |
| | | GG | 7 | 37 | | 13 | 21 | 10 | |

*Note*: Reference Sequence and version number (*RETN*: NG_023447.1; *LDLR*: NG_009060.1).
*p* value was calculated by Chi-squared test.
**p* < 0.05 indicates statistical significance.

equilibrium for the case and control groups ($p > 0.05$). Two SNPs, rs7408174 and rs3745369 in *RETN*, were associated with the increased risk of alcohol-induced ONFH (OR = 1.43, 95% CI: 1.03–1.98, $p = 0.032$; OR = 1.35, 95% CI: 1.01–1.81, $p = 0.045$). On the other hand, rs34861192 and rs3219175 in *RETN* showed reduced risk (OR = 0.63, 95% CI: 0.43–0.92; $p = 0.016$; OR = 0.67, 95% CI: 0.46–0.97; $p = 0.032$) of alcohol-induced ONFH.

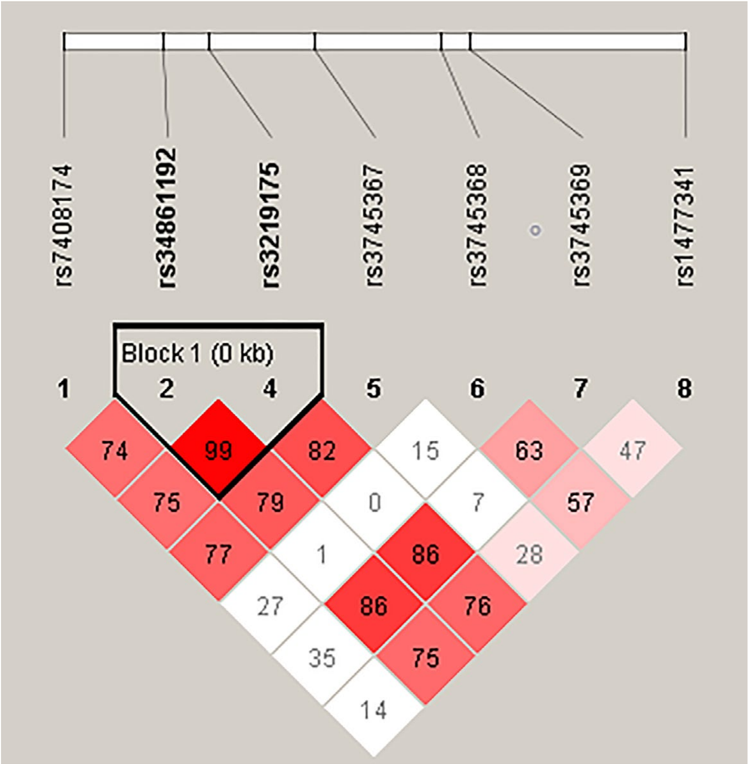
Genetic models were used to compare the SNP genotypes and the risk of alcohol-induced ONFH. The results of logistic regression analysis for each genetic model are shown in Table 3. Four SNPs in *RETN* had strong associations with alcohol-induced ONFH in genetic models after they were adjusted by age. It was discovered that rs7408174 was associated with increased risk of alcohol-induced ONFH in dominant model (OR = 1.57, 95% CI: 1.05–2.34, $p = 0.028$) and log-additive

**TABLE 5** Comparison of lipids levels between each genotype

| SNP | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | TC/HDL-C | TG/HDL-C | LDL-C/HDL-C |
|---|---|---|---|---|---|---|---|
| rs7408174 | | | | | | | |
| CC (*n* = 13) | 4.49 ± 0.88 | 1.76 ± 0.89 | 1.02 ± 0.26 | 2.66 ± 0.87 | 4.52 ± 0.85 | 1.88 ± 1.12 | 2.61 ± 0.74 |
| CT (*n* = 83) | 4.66 ± 0.95 | 2.07 ± 1.60 | 1.03 ± 0.22 | 2.75 ± 0.94 | 4.64 ± 1.08 | 2.19 ± 1.99 | 2.74 ± 0.90 |
| TT (*n* = 105) | 4.66 ± 0.91 | 1.77 ± 1.01 | 1.06 ± 0.25 | 2.72 ± 0.78 | 4.61 ± 1.25 | 1.82 ± 1.21 | 2.68 ± 0.85 |
| *p* | 0.821 | 0.247 | 0.688 | 0.923 | 0.934 | 0.289 | 0.828 |
| rs34861192 | | | | | | | |
| AA (*n* = 3) | 4.68 ± 0.48 | 1.37 ± 0.42 | 1.08 ± 0.14 | 2.30 ± 0.85 | 4.36 ± 0.23 | 1.32 ± 0.56 | 2.22 ± 1.01 |
| AG (*n* = 47) | 4.67 ± 1.02 | 2.02 ± 1.73 | 1.07 ± 0.26 | 2.78 ± 0.91 | 4.52 ± 1.25 | 2.08 ± 2.08 | 2.67 ± 0.92 |
| GG (*n* = 146) | 4.65 ± 0.91 | 1.86 ± 1.12 | 1.04 ± 0.23 | 2.73 ± 0.84 | 4.64 ± 1.14 | 1.94 ± 1.38 | 2.72 ± 0.85 |
| *p* | 0.992 | 0.59 | 0.619 | 0.627 | 0.765 | 0.68 | 0.589 |
| rs3219175 | | | | | | | |
| AA (*n* = 3) | 4.68 ± 0.48 | 1.37 ± 0.42 | 1.08 ± 0.14 | 2.30 ± 0.85 | 4.36 ± 0.23 | 1.32 ± 0.56 | 2.22 ± 1.01 |
| AG (*n* = 52) | 4.68 ± 1.00 | 1.96 ± 1.59 | 1.07 ± 0.26 | 2.74 ± 0.89 | 4.58 ± 1.26 | 2.01 ± 1.89 | 2.65 ± 0.89 |
| GG (*n* = 146) | 4.64 ± 0.90 | 1.88 ± 1.17 | 1.04 ± 0.23 | 2.73 ± 0.84 | 4.63 ± 1.13 | 1.98 ± 1.47 | 2.73 ± 0.85 |
| *p* | 0.952 | 0.718 | 0.720 | 0.677 | 0.886 | 0.760 | 0.546 |
| rs3745368 | | | | | | | |
| AA (*n* = 7) | 4.53 ± 1.00 | 1.46 ± 0.73 | 1.02 ± 0.40 | 2.67 ± 0.89 | 4.85 ± 1.47 | 1.72 ± 1.31 | 2.79 ± 0.79 |
| AG (*n* = 55) | 4.51 ± 0.81 | 1.74 ± 1.09 | 1.03 ± 0.18 | 2.70 ± 0.82 | 4.50 ± 1.02 | 1.81 ± 1.30 | 2.67 ± 0.76 |
| GG (*n* = 138) | 4.72 ± 0.96 | 1.97 ± 1.37 | 1.05 ± 0.25 | 2.75 ± 0.87 | 4.65 ± 1.19 | 2.05 ± 1.69 | 2.70 ± 0.91 |
| *p* | 0.326 | 0.355 | 0.727 | 0.940 | 0.628 | 0.578 | 0.931 |
| rs3745369 | | | | | | | |
| CC (*n* = 34) | 4.53 ± 0.68 | 1.60 ± 0.87 | 1.04 ± 0.26 | 2.74 ± 0.63 | 4.56 ± 1.08 | 1.68 ± 1.03 | 2.74 ± 0.71 |
| CG (*n* = 80) | 4.58 ± 1.00 | 1.96 ± 1.30 | 1.02 ± 0.22 | 2.65 ± 0.89 | 4.61 ± 1.17 | 2.09 ± 1.66 | 2.64 ± 0.83 |
| GG (*n* = 80) | 4.76 ± 0.94 | 1.84 ± 1.20 | 1.07 ± 0.25 | 2.77 ± 0.92 | 4.64 ± 1.20 | 1.90 ± 1.42 | 2.72 ± 0.96 |
| *p* | 0.364 | 0.345 | 0.548 | 0.662 | 0.942 | 0.366 | 0.798 |

Abbreviations: TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; LDL-C, high-density lipoprotein-cholesterol.

**FIGURE 1** Haplotype block map for the seven SNPs in the *RETN* gene. Block 1 includes rs34861192 and rs3219175



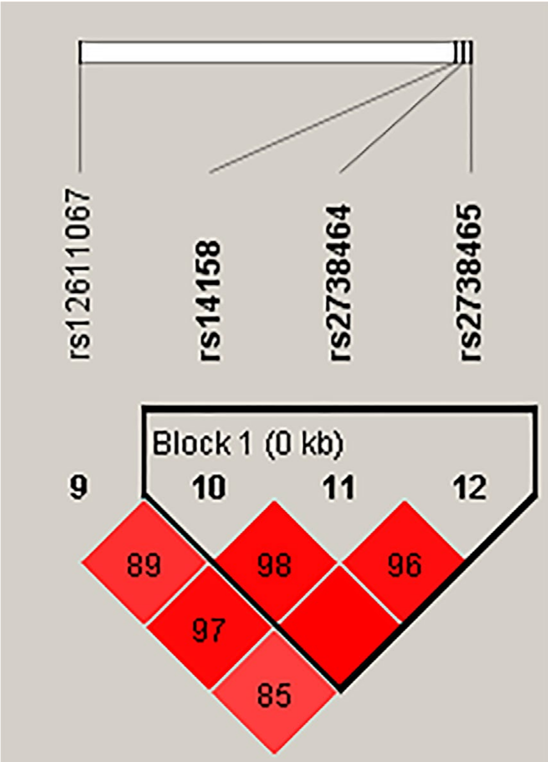




**FIGURE 2** Haplotype block map for the four SNPs in the *LDLR* gene. Block 1 includes rs14158, rs2738464, and rs2738465

model (OR = 1.47, 95% CI: 1.05–2.06, $p = 0.024$). On the other hand, rs3219175 showed a decreased risk in dominant model (OR = 0.64, 95% CI: 0.42–0.97, $p = 0.036$)

**TABLE 6** GTEx results for three SNPs in genes expression in the most relevant tissue

| SNP | Gene | Effect | *p*-value | Tissue |
|---|---|---|---|---|
| rs34861192 | *RETN* | 1.00 | 4.00E-14 | Whole-Blood |
| rs34861192 | *RETN* | 1.00 | 6.40E-08 | Muscle-Skeletal |
| rs3219175 | *RETN* | 0.84 | 1.60E-14 | Whole-Blood |
| rs3219175 | *RETN* | 1.00 | 1.90E-09 | Muscle-Skeletal |
| rs2738464 | *LDLR* | −0.26 | 3.40E-05 | Muscle-Skeletal |

and log-additive model (OR = 0.65, 95% CI: 0.44–0.95, $p = 0.025$). In the other two SNPs, rs34861192 showed a reduced risk in codominant model (AG: OR = 0.62, 95% CI: 0.39–0.96, $p = 0.032$), dominant model (OR = 0.59, 95% CI: 0.38–0.91, $p = 0.017$), and log-additive model (OR = 0.61, 95% CI: 0.41–0.90, $p = 0.013$). Rs3745369 showed an increased risk in codominant model (CC: OR = 2.50, 95% CI: 1.28–4.90, $p = 0.007$), recessive model (CC: OR = 2.55, 95% CI: 1.35–4.81, $p = 0.004$), and log-additive model (OR = 1.36, 95% CI: 1.01–1.82, $p = 0.042$).

Correlation analysis between the genotypes and hip lesions, as well as clinical stages are shown in Table 4. The rs3745368 in *RETN* shows association with the clinical stages ($p = 0.022$). Comparisons of lipid levels between each genotype are shown in Table 5. The blood lipid levels of different SNP genotypes were compared by Analysis of Variance (ANOVA), but no difference was found.

The Linkage analysis showed that two SNPs (rs34861192, rs3219175) in *RETN* (Figure 1) and three SNPs (rs14158, rs2738464, rs2738465) in *LDLR* exhibited significant linkage disequilibrium (Figure 2).

In Table 6, the risk alleles of rs34861192 ($p = 4.0 \times 10^{-14}$, $p = 6.4 \times 10^{-8}$) and rs3219175 ($p = 1.6 \times 10^{-14}$, $p = 1.9 \times 10^{-9}$) were associated with increased expression of *RETN* gene in the whole-blood and muscle-skeletal. In contrast, rs2738464 ($p = 3.4 \times 10^{-5}$) was associated with decreased expression of *LDLR* gene in muscle-skeletal.

## 4 | DISCUSSION

In this research, it was discovered that *RETN* genetic polymorphisms were associated with alcohol-induced ONFH risk among Chinese Han individuals. The rs3745368 was associated with the stages of the disease, and more patients with AA genotype were in Stage II than those in Stage III and Stage IV. However, more patients with AG and GG genotypes were in stage III. The SNPs (rs7408174, rs34861192, and rs3219175) are located in the upstream of *RETN* and rs3745369 is located in the downstream. The rs34861192 is associated with the level of serum insulin, glycemic index and cholesterol (Zhou, Chen, Ji, Luo, & Luo, 2018). The cholesterol of the case group was measured but no association with this SNP was found. In orthopedic diseases, individuals with the C allele of the SNP rs7408174 and the AG or A allele of the SNP rs3219175 are at a higher risk of developing rheumatoid arthritis compared with wild-type (Wang, Tang, et al., 2018). Plasma resistin level is strongly affected by rs34861192, rs3219175, and rs3745368 (Asano et al., 2010; Nakatochi et al., 2015). Using GTEx portal, *RETN* and *LDLR* expressions in different genotype individuals were compared and it was found that the risk alleles of rs34861192 and rs3219175 were associated with increased expression of *RETN* gene in the whole-blood and muscle-skeletal. We observed from Tables 2 and 3 that the number of GG genotypes in rs34861192 and rs3219175 in the case group was significantly higher than that in the control group, while the number of AG/AA genotypes was lower than that in the control group. The SNPs rs34861192 and rs3219175 located in the promoter region of *RETN* were associated with resistin levels. Moreover, the number of minor alleles of the two SNPs was negatively associated with DNAm level at cg02346997 (Nakatochi et al., 2015). We also discovered these two minor alleles reduce the risk of ONFH. Therefore, we hypothesized that different genotypes in these two SNPs affected the expression of resistin in bones, which then had an impact on the occurrence of ONFH. It was also found that the risk alleles of rs2738464 decreased the expression of *LDLR* in muscle-skeletal, but no significant difference was observed between this genotype and alcohol-induced ONFH.

By comparing the blood lipid levels between the case group and the control group, we discovered that TC/HDL-C and LDL-C/HDL-C in the case groups were much higher than those in the control group, which showed the disorder of lipid metabolism in alcohol-induced ONFH. This result may be related to the decrease of HDL-C in the case groups and studies have found that resistin affects HDL-C levels (Osawa et al., 2007). To identify the effect of the five SNPs on the metabolic disorder of alcohol-induced ONFH, we examined blood lipid levels and analyzed their associations. However, no significant association was found due to two possible reasons: (a) Dietary differences in the subjects may have an impact on lipid levels; (b) For this analysis, only 201 cases of the population were studied, which resulted in a small number of each genotype, which may affect the results.

## 5 | CONCLUSION

In summary, this study suggested that polymorphisms of *RETN* were associated with the alcohol-induced ONFH. The SNPs (rs7408174, rs34861192, rs3219175, and rs3745369) in *RETN* were associated with the risk of alcohol-induced ONFH. The rs3745368 was associated with the stage of the disease. The levels of TC/HDL-C in the case group was significantly lower than that in the control group. This study provide new insights to facilitate early diagnosis and early prevention of ONFH, as well as for new candidate gene studies. The sample size will be increased to stud the mechanism of *RETN* action in our future research.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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