**MMP20 Single-Nucleotide Polymorphisms Correlate with Susceptibility to Alcohol-Induced Osteonecrosis of the Femoral Head in Chinese Males**

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**Background:** Alcohol-induced osteonecrosis of the femoral head (ONFH) is caused by the interaction of genetic and environmental factors. Genetic variations of matrix metalloproteinase (MMP) system are associated with ONFH development and progression. In this study, we aimed to evaluate the relationships between MMP20 gene polymorphisms and the risk of alcohol-induced ONFH in Chinese Han males.

**Material/Methods:** In this case-control study, genotypes of 14 selected SNPs in the MMP20 gene were assayed using MassARRAY in 299 male cases with alcohol-induced ONFH and in 197 healthy males. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the influence of gene polymorphism on occurrence of alcohol-induced ONFH by allelic model analysis, genotype model analysis and haplotype analysis.

**Results:** After allelic model analysis, the minimum alleles of rs10895322, rs1784424, rs3781788, and rs1573954 correlated with an increased risk of alcohol-induced ONFH ($P<0.05$). Genetic model analysis revealed significant associations of 9 SNPs with alcohol-induced ONFH occurrence even after adjustment for age ($P<0.05$): 2 protective SNPs (rs1711423 and rs1784418) and 7 high-risk SNPs (rs10895322, rs1784424, rs3781788, rs7126560, rs1573954, rs1711399, and rs2292730). Moreover, 8 SNPs showed a statistically significant association with different clinical phenotypes ($P<0.05$). Beyond that, haplotype “CGTTCGA” in MMP20 was discovered to correlate with a 1.63-fold increased risk of alcohol-induced ONFH (OR: 1.63, 95% CI: 1.15–2.30, $P=0.0058$).

**Conclusions:** Our data sheds new light on the associations of MMP20 gene polymorphisms with alcohol-induced ONFH predisposition in Chinese Han males.

**MeSH Keywords:** Genetic Association Studies • Matrix Metalloproteinase 20 • Osteonecrosis • Polymorphism, Genetic

Full-text PDF: [https://www.medscimonit.com/abstract/index/idArt/913918](https://www.medscimonit.com/abstract/index/idArt/913918)
Background

Alcohol-induced osteonecrosis of the femoral head (alcohol-induced ONFH) caused by excessive and chronic alcohol consumption is an intractable orthopedic disease that is characterized by the progressive collapse of the femur head and loss of hip joint function. Although there are a variety of clinical treatments, the prevalence of alcohol-induced ONFH and resulting disability in the Chinese population continues to increase, particularly among young men. Data demonstrate that 30.7% of ONFH cases in China are alcohol induced [1]. For these reasons, identifying risk factors and taking primary prevention measures are necessary to prevent alcohol-induced ONFH.

Recent research into the molecular biology and genetic underpinnings of alcohol-induced ONFH has provided new information about gene polymorphisms, which has opened up novel avenues of research to uncover the risk factors of this condition [2]. In the past few years, genome-wide association studies (GWAS) have identified several susceptibility genes such as APOA1, PAI-1, SREBF1, VEGF MTHFR, and TFPI that are associated with ONFH [3–8]. Chondrocytes from a femoral head with osteonecrosis lose their phenotype during ONFH, revealed associated with ONFH [3–8]. Chondrocytes from a femoral head with osteonecrosis lose their phenotype during ONFH, revealed by reducing of extracellular matrix production in glycosaminoglycan (GAG) and type 2 collagen excretion [9]. In addition, our previous studies demonstrated that NOS3, ABCB, IL23R, APOA1, APOB, TIMP2, MMP2, MMP3, and MMP8 single-nucleotide polymorphisms were related to the risk of alcohol-induced ONFH and clinical outcomes or other clinical characteristics in the Chinese population [10–13]. Although multiple gene variants have been proposed as risk factors for alcohol-induced ONFH, the molecular etiology and pathogenesis have remained indistinct.

The matrix metalloproteinase-20 (MMP20) gene is located in an MMP cluster with 7 other MMPs at chromosomal location 11q22.3. MMP20, which has been well established as an essential participant in amelogenesis, is expressed in ameloblasts and odontoblasts during dental enamel development and is responsible for cleaving and removing most of the protein components of the extracellular enamel matrix such as amelogenin (the most abundant extracellular matrix (ECM) protein in developing enamel) thus facilitating enamel mineralization [14,15]. MMP20 is a relatively newly identified member of the MMP family and was also named “human enamelysin” because it was originally thought that MMP20 expression was restricted to enamel. In recent years, the expression of MMP20 has also been reported in other tissues. A study in 2015 confirmed the expression of MMP20 in the retina and RPE/choroid [16]. Krauss et al. [17] investigated the expression of MMP20 in 3 major human tumor entities: colon, breast, and lung tumors. They found that MMP20 was identified at both the mRNA and the protein level in breast MCF-7, colon HT-29, and lung A549 cell lines. Thus, they concluded that MMP20 was a potential new candidate for tumor diagnosis or therapy.

In this case-control study, 14 SNPs on the MMP20 gene were selected to shed light on possible correlations with the occurrence of alcohol-induced ONFH and the clinical phenotypes in a Chinese male population.

Material and Methods

Study participants

There were 496 males (299 patients with alcohol-induced ONFH and 197 healthy males) among Han Chinese, sequentially enrolled in this study. All of the patients were selected randomly from the Zhengzhou Traditional Chinese Medicine Traumatology Hospital without restriction of disease severity. The patients were diagnosed with ONFH after using plain radiographs in stage II, III, and IV of the Ficat Classification system. Patients with traumatic osteonecrosis and other hip diseases were excluded. Alcohol-induced osteonecrosis was defined by a history of consumption of more than 400 mL of alcohol per week [18]. As well, enrolled patients had not received systemic treatment before the blood samples used for the study were collected. Control study participants were genetically irrelevant healthy males who were enrolled from Zhengzhou Medical Center and lived in or near Zhengzhou without hip pain, without lesions found on hip joint plain radiographs, and without relationship to the enrolled patients. Those who had a chronic metabolic disorder or who needed steroid treatment were also excluded from this study.

A normative questionnaire was used to collect personal information. Our research was approved by the ethics committee of Zhengzhou Traditional Chinese Medicine Traumatology Hospital and The Second Affiliated Hospital of Inner Mongolia Medical University (No. YKD2016138). Signed informed consent documents were obtained from all candidates.

SNP selection and genotyping

A total of 14 SNPs were selected from the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp) and the 1000 Genomes Project databases (http://www.internationalgenome.org/) with minor allele frequencies (MAFs) >5% in the 1000 Genomes Chinese Han Beijing population. 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 using a Nanodrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA). Primers for amplification process and single base extension reactions were designed using the Assay Design Suite.
SNP genotyping was performed by Agena MassARRAY iPLEX (Agena Bioscience, San Diego, CA, USA). Genotyping results were output by Agena Bioscience TYPER version 4.0 software.

Statistical analysis

SPSS 19.0 (version 19.0, Chicago, IL, USA) was used to do the statistical analysis. All the P-values were 2-sided, and P<0.05 was considered to be statistically significant. The t-test were used to compare the age difference of cases and controls. An exact test was used to assess the variation in each SNP genotype frequency from the Hardy-Weinberg equilibrium (HWE) in the control participants before analysis. Differences in SNP genotype and allele distribution between cases and controls were compared by the \( \chi^2 \) test/Wald test. Four models including the codominant, dominant, recessive, and log-additive model were used to assess the association between each genotype and the risk of alcohol-induced ONFH. Finally, both the SHEsis software platform (http://analysis.bio-x.cn/myAnalysis.php) and the Haploview software (version 4.2) were used to estimate the linkage disequilibrium (LD) patterns and construct haplotypes. The risk associated with individual genotypes and allele was calculated as the odds ratio (OR) with their 95% confidence interval (95% CI) based on logistic regression model analysis with an adjustment for age.

Results

Altogether, 299 eligible cases with alcohol-induced ONFH (mean age ±SD: 43.24±13.07) and 197 controls (mean age ±SD: 49.71±8.85) were recruited for our study. All of the individuals were males. The results of t-tests showed that the cases and controls were not matched for age (P<0.05), so we adjusted the age factor in the subsequent analysis (Table 1).

The detailed information about the 14 selected SNPs on the MMP20 gene including band, alleles, minor allele frequency (MAF), and HWE results. The P-values of alleles were calculated by chi-square test. All SNPs did not deviate from HWE (P>0.05). Comparing the differences in MAF distributions between cases and controls, we found that 4 SNPs may significantly correlate with the risk of alcohol-induced ONFH under the allele model (rs10895322G/A, OR=1.422, 95% CI=1.069–1.892, P=0.015; rs1784424T/G, OR=1.368, 95% CI=1.059–1.767, P=0.016; rs3781788T/C, OR=1.345, 95% CI=1.031–1.755, P=0.029; rs1573954C/T, OR=1.328, 95% CI=1.028–1.716, P=0.030) (Table 2).

Subsequently, by unconditional logistic-regression analysis, we further identified the correlation between selected SNPs and alcohol-induced ONFH risk under 4 gene models (codominant, dominant, recessive, and log-additive). Meanwhile, all results were adjusted by age (Table 3). Rs10895322 and rs1784424 notably increased the risk of alcohol-induced ONFH under all of the gene models, including codominant, dominant, recessive,
and log-additive. Rs3781788 also revealed an increased risk under both the dominant and the additive model. Meanwhile, both rs7126560 and rs2292730 revealed an increased risk under the recessive model, while rs1784410 exhibited a decreased risk under both models (recessive and log-additive). Rs1711399 revealed an increased risk under the dominant model. In addition, rs1711437 showed a decreased effect under the recessive and log-additive models, and rs1784418 also showed a decreased risk under both the dominant model and the log-additive model.

Next, we completed the correlation analysis of alleles and genotypes of SNPs with the clinical phenotypes (unilateral or bilateral hip lesions, >12 months or ≤12 months course, and clinical stages of alcohol-induced ONFH), respectively (Table 4). Regarding rs10895322, some significant association was found between the G allele and the patients with different clinical phenotypes. In patients who had bilateral hip lesions, >12 months course, and/or stage II/III alcohol-induced ONFH, the G allele was a risk factor. Likewise, the rs3781788 T allele showed the same results. Among patients who had bilateral hip lesions, >12 months course, and/or stage III alcohol-induced ONFH, the G allele of rs1784424 showed significantly increased risk, and the G allele of rs1711423 showed significantly decreased risk, relative to controls. In addition, the allele distribution showed a higher frequency of the rs1573954 C allele in the patients with unilateral hip lesions, ≤12 months course, and/or stage II alcohol-induced ONFH. Rs1784418 showed a decreased risk in patients with >12 months course or/and stage III of alcohol-induced ONFH. Regarding rs7126560 and rs2292730, these 2 SNPs only revealed the increased risk for >12 months course and stage IV alcohol-induced ONFH, respectively.

Finally, using parameter D', we detected the extent of linkage disequilibrium between SNPs, and then determined the haplotype LD block according to the control group data. We observed that 2 blocks existed strong linkage disequilibrium in MMP20 (Figure 1). Block 1 included 8 SNPs: rs1711437, rs10895322, rs1784424, rs3781788, rs1711423, rs17174327, rs1784418, and rs7126560; block 2 includes 2 SNPs: rs1784410 and rs1711399. In block 1, compared with the “TATCGCTG” wild-type, the “CGGTTCCA” haplotype was found to be associated with an increased alcohol-induced ONFH risk after adjustment for age (OR=1.63, 95% CI=1.15–2.30, P=0.0058) (Table 5). However, we did not find any association between alcohol-induced ONFH and the haplotype of block 2.

### Discussion

In this study, we have attempted to show the correlation between MMP20 polymorphisms and alcohol-induced ONFH and the different clinical phenotypes in Chinese Han males. Nine susceptibility SNPs in the MMP20 gene revealed a statistically significant association with the alcohol-induced ONFH occurrence: 2 protective SNPs (rs1711423 and rs1784418) and 7 high-risk
Table 3. Genotypic model analysis of relationship between SNPs and alcohol-induced ONFH risk adjusted for age.

| SNP ID   | Model     | Genotype | Control | Case   | OR (95% CI)  | P-value | AIC  | BIC  |
|----------|-----------|----------|---------|--------|--------------|---------|------|------|
| rs10895322 | Codominant | AA       | 109 (55.9%) | 136 (46%) | 1 | 0.018* | 625.9 | 642.6 |
|          |           | GA       | 73 (37.4%)  | 127 (42.9%) | 1.44 (0.97–2.14) |         |      |      |
|          |           | GG       | 13 (6.7%)   | 33 (11.2%) | 2.47 (1.21–5.04) |         |      |      |
|          | Dominant  | AA       | 109 (55.9%) | 136 (46%) | 1 | 0.016* | 626.1 | 638.7 |
|          |           | GA-GG    | 86 (44.1%)  | 160 (54%) | 1.58 (1.09–2.31) |         |      |      |
|          | Recessive | AA-GA    | 182 (93.3%) | 263 (88.8%) | 1 | 0.029* | 627.1 | 639.7 |
|          |           | GG       | 13 (6.7%)   | 33 (11.2%) | 2.10 (1.05–4.19) |         |      |      |
|          | Log-additive | –     | –      | –      | 1.51 (1.13–2.03) | 0.0051* | 624  | 636.6 |
| rs1784424 | Codominant | TT       | 48 (24.5%)  | 52 (17.4%) | 1 | 0.018* | 629.8 | 646.6 |
|          |           | GT       | 101 (51.5%) | 150 (50.2%) | 1.43 (0.88–2.33) |         |      |      |
|          |           | GG       | 47 (24%)    | 97 (32.4%) | 2.18 (1.26–3.77) |         |      |      |
|          | Dominant  | TT       | 48 (24.5%)  | 52 (17.4%) | 1 | 0.032* | 631.2 | 643.8 |
|          |           | GT-GG    | 148 (75.5%) | 247 (82.6%) | 1.66 (1.05–2.63) |         |      |      |
|          | Recessive | TT-GT    | 149 (76%)   | 207 (69%)  | 1 | 0.014* | 629.9 | 642.5 |
|          |           | GG       | 47 (24%)    | 97 (32.4%) | 1.69 (1.10–2.58) |         |      |      |
|          | Log-additive | –     | –      | –      | 1.51 (1.13–2.03) | 0.0051* | 624  | 646.6 |
| rs3781788 | Codominant | CC       | 89 (45.4%)  | 104 (34.8%) | 1 | 0.072 |       |      |      |
|          |           | CT       | 83 (42.4%)  | 149 (49.8%) | 1.53 (1.02–2.29) |         |      |      |
|          |           | TT       | 24 (12.2%)  | 46 (15.4%) | 1.66 (0.92–2.99) |         |      |      |
|          | Dominant  | CC       | 89 (45.4%)  | 104 (34.8%) | 1 | 0.023* | 630.7 | 643.3 |
|          |           | CT-TT    | 107 (54.6%) | 195 (65.2%) | 1.56 (1.06–2.28) |         |      |      |
|          | Recessive | CC-CT    | 172 (87.8%) | 253 (84.6%) | 1 | 0.310 |       |      |      |
|          |           | TT       | 24 (12.2%)  | 46 (15.4%) | 1.33 (0.77–2.29) |         |      |      |
|          | Log-additive | –     | –      | –      | 1.35 (1.02–1.78) | 0.032* | 631.3 | 643.9 |
| rs1711423 | Codominant | TT       | 51 (26%)    | 97 (32.4%) | 1 | 0.052 |       |      |      |
|          |           | GT       | 99 (50.5%)  | 150 (50.2%) | 0.74 (0.48–1.16) |         |      |      |
|          |           | GG       | 46 (23.5%)  | 52 (17.4%) | 0.51 (0.30–0.88) |         |      |      |
|          | Dominant  | TT       | 51 (26%)    | 97 (32.4%) | 1 | 0.057 |       |      |      |
|          |           | GT-GG    | 145 (74%)   | 202 (67.6%) | 0.67 (0.44–1.02) |         |      |      |
|          | Recessive | TT-GT    | 150 (76.5%) | 247 (82.6%) | 1 | 0.042* | 631.7 | 644.3 |
|          |           | GG       | 46 (23.5%)  | 52 (17.4%) | 0.62 (0.39–0.98) |         |      |      |
|          | Log-additive | –     | –      | –      | 0.72 (0.55–0.94) | 0.016* | 630  | 642.6 |
| rs1784418 | Codominant | CC       | 50 (25.4%)  | 96 (32.1%) | 1 | 0.066 |       |      |      |
|          |           | CT       | 102 (51.8%) | 150 (50.2%) | 0.71 (0.45–1.10) |         |      |      |
|          |           | TT       | 45 (22.8%)  | 53 (17.7%) | 0.53 (0.31–0.91) |         |      |      |
|          | Dominant  | CC       | 50 (25.4%)  | 96 (32.1%) | 1 | 0.043* | 632.8 | 645.4 |
|          |           | CT-TT    | 147 (74.6%) | 203 (67.9%) | 0.65 (0.43–1.00) |         |      |      |
|          | Recessive | CC-CT    | 152 (77.2%) | 246 (82.3%) | 1 | 0.082 |       |      |      |
|          |           | TT       | 45 (22.8%)  | 53 (17.7%) | 0.66 (0.41–1.05) |         |      |      |
|          | Log-additive | –     | –      | –      | 0.73 (0.55–0.95) | 0.020* | 631.5 | 644.1 |
SNPs (rs10895322, rs1784424, rs3781788, rs7126560, rs1573954, rs1711399, and rs2292730). Among these SNPs, 8 SNPs showed a statistically significant association with different clinical phenotypes. In particular, the haplotype “CGGTTCCA” in MMP20 was also found to be correlated with a 1.63-fold increased risk of alcohol-induced ONFH. As far as we know, this is the first report to reveal the correlation between these loci and ONFH susceptibility.

MMPs devastate the cartilaginous extracellular matrix (ECM) production during chondrogenesis [19,20] and ONFH [9]. And ECM, including GAG and collagen type 2, are commodities not only in normal chondrocytes but also in chondrocytic differentiation [21]. MMP20 may inhibit the synthesis of ECM of chondrocytes in the femoral heads under osteonecrosis. But studies on these risk loci are relatively rare. A recent GWAS reported

| SNP ID   | Model    | Genotype | Control | Case | OR (95% CI) | P-value | AIC   | BIC   |
|----------|----------|----------|---------|------|-------------|---------|-------|-------|
| rs7126560 | Codominant | GG       | 85 (43.1%) | 104 (35.1%) | 1 | 0.130 | 633 | 649.8 |
|          |          | AG       | 89 (45.2%) | 145 (49%) | 1.35 (0.90–2.02) |         |       |       |
|          |          | AA       | 23 (11.7%) | 47 (15.9%) | 1.73 (0.95–3.13) |         |       |       |
|          | Dominant  | GG       | 85 (43.1%) | 104 (35.1%) | 1 | 0.068 | 631.7 | 644.2 |
|          |          | AG-AA    | 112 (56.9%) | 192 (64.9%) | 1.43 (0.97–2.09) |         |       |       |
|          | Recessive | GG-AG    | 174 (88.3%) | 249 (84.1%) | 1 | 0.170 | 633.1 | 645.7 |
|          |          | AA       | 23 (11.7%) | 47 (15.9%) | 1.47 (0.84–2.54) |         |       |       |
| rs1573954 | Codominant | TT       | 48 (24.6%) | 90 (30.1%) | 1 | 0.050 | 628.4 | 645.2 |
|          |          | GT       | 100 (51%) | 142 (47.5%) | 1.02 (0.67–1.57) |         |       |       |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.78 (1.02–3.10) |         |       |       |
|          | Dominant  | TT       | 48 (24.6%) | 90 (30.1%) | 1 | 0.390 | 631.9 | 648.7 |
|          |          | GT-GG    | 132 (67.3%) | 209 (69.9%) | 1.19 (0.80–1.79) |         |       |       |
|          | Recessive | TT-GT    | 153 (78.5%) | 206 (69.1%) | 1 | 0.021* | 629.9 | 644.2 |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.75 (1.08–2.85) |         |       |       |
| rs1711399 | Codominant | TT       | 64 (32.6%) | 90 (30.1%) | 1 | 0.071 | 631.9 | 648.7 |
|          |          | GT       | 100 (51%) | 142 (47.5%) | 1.02 (0.67–1.57) |         |       |       |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.78 (1.02–3.10) |         |       |       |
|          | Dominant  | TT       | 64 (32.6%) | 90 (30.1%) | 1 | 0.390 | 634.4 | 647.1 |
|          |          | GT-GG    | 132 (67.3%) | 209 (69.9%) | 1.19 (0.80–1.79) |         |       |       |
|          | Recessive | TT-GT    | 153 (78.5%) | 206 (69.1%) | 1 | 0.021* | 629.9 | 644.2 |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.75 (1.08–2.85) |         |       |       |
| rs2292730 | Codominant | GG       | 106 (53.8%) | 140 (46.8%) | 1 | 0.120 | 634.7 | 651.5 |
|          |          | GA       | 82 (41.6%) | 141 (47.2%) | 1.39 (0.94–2.04) |         |       |       |
|          |          | AA       | 9 (4.6%) | 19 (6%) | 1.94 (0.81–4.66) |         |       |       |
|          | Dominant  | GG       | 106 (53.8%) | 140 (46.8%) | 1 | 0.058 | 633.3 | 645.9 |
|          |          | GA-AA    | 91 (46.2%) | 159 (53.2%) | 1.44 (0.99–2.09) |         |       |       |
|          | Recessive | GG-GA    | 188 (95.4%) | 281 (94%) | 1 | 0.240 | 635.5 | 648.1 |
|          |          | AA       | 9 (4.6%) | 19 (6%) | 1.66 (0.71–3.90) |         |       |       |
|          | Log-additive | –       | –       | –       | – | 1.29 (0.99–1.68) | 0.063 | 631.7 | 644.3 |
| rs1573954 | Codominant | TT       | 64 (32.6%) | 90 (30.1%) | 1 | 0.071 | 631.9 | 648.7 |
|          |          | GT       | 100 (51%) | 142 (47.5%) | 1.02 (0.67–1.57) |         |       |       |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.78 (1.02–3.10) |         |       |       |
|          | Dominant  | TT       | 64 (32.6%) | 90 (30.1%) | 1 | 0.390 | 634.4 | 647.1 |
|          |          | GT-GG    | 132 (67.3%) | 209 (69.9%) | 1.19 (0.80–1.79) |         |       |       |
|          | Recessive | TT-GT    | 153 (78.5%) | 206 (69.1%) | 1 | 0.021* | 629.9 | 644.2 |
|          |          | GG       | 32 (16.3%) | 67 (22.4%) | 1.75 (1.08–2.85) |         |       |       |
|          | Log-additive | –       | –       | –       | – | 1.29 (0.99–1.68) | 0.063 | 631.7 | 644.3 |

*P<0.05 indicates statistical significance. *P values were calculated by Wald test by unconditional logistic regression adjusted for age. Bold values indicate a significant difference.
### Table 4. Stratification analysis for association of MMP20 gene polymorphism with the clinical phenotypes of alcohol-induced ONFH.

| SNPs | Genotype (%) | Allele (%) |
|------|--------------|------------|
|      | Controls     |            |
|      | Unilateral   |            |
|      | Bilateral    |            |
|      | Stage II     |            |
|      | Stage III    |            |
|      | Stage IV     |            |
|      | >12 months   |            |
|      | ≤12 months   |            |

**rs2292730**

| Genotype (%) | G    | A    | P    | G    | A    |
|--------------|------|------|------|------|------|
| GG           | 106  | 82   | 9    | 4.60 | 294  | 100 (25.4) |
| GA           | 32   | 32   | 6    | 6.60 | 0.320 | 96 (68.6)  |
| AA           | 108  | 109  | 12   | 5.20 | 0.190 | 325 (71.0) |
| Stage II     | 32   | 39   | 3    | 4.00 | 0.320 | 103 (69.6) |
| Stage III    | 66   | 51   | 4    | 3.30 | 0.780 | 183 (75.6) |
| Stage IV     | 42   | 51   | 11   | 10.60 | 0.023* | 135 (64.9) |
| >12 months   | 76   | 67   | 8    | 5.30 | 0.710 | 219 (72.5) |
| ≤12 months   | 64   | 74   | 10   | 6.80 | 0.043* | 202 (68.2) |

**rs1784410**

| Genotype (%) | G    | A    | P    | G    | A    |
|--------------|------|------|------|------|------|
| AA           | 65   | 97   | 35   | 17.8 | 227  | 167 (42.4) |
| GA           | 25   | 30   | 15   | 21.4 | 0.710 | 80 (57.1)  |
| CC           | 84   | 109  | 35   | 15.3 | 0.310 | 277 (60.7) |
| Stage II     | 25   | 32   | 16   | 21.9 | 0.880 | 82 (56.2)  |
| Stage III    | 45   | 65   | 11   | 9.1  | 0.012* | 155 (64.0) |
| Stage IV     | 39   | 42   | 23   | 22.1 | 0.350 | 120 (57.7) |
| >12 months   | 57   | 71   | 22   | 14.7 | 0.520 | 185 (61.7) |
| ≤12 months   | 52   | 68   | 28   | 18.9 | 0.570 | 172 (58.1) |

**rs1711399**

| Genotype (%) | G    | A    | P    | G    | A    |
|--------------|------|------|------|------|------|
| TT           | 64   | 100  | 32   | 16.3 | 228  | 164 (41.8) |
| GT           | 22   | 31   | 17   | 24.3 | 0.230 | 75 (53.6)  |
| GG           | 68   | 111  | 50   | 21.8 | 0.120 | 247 (53.9) |
| Stage II     | 19   | 41   | 14   | 18.9 | 0.450 | 79 (53.4)  |
| Stage III    | 32   | 60   | 29   | 24.0 | 0.013* | 124 (51.2) |
| Stage IV     | 39   | 41   | 24   | 23.1 | 0.120 | 119 (57.2) |
| >12 months   | 43   | 70   | 38   | 25.2 | 0.088 | 156 (51.7) |
| ≤12 months   | 47   | 72   | 29   | 19.6 | 0.280 | 166 (56.1) |

**rs1711437**

| Genotype (%) | G    | A    | P    | G    | A    |
|--------------|------|------|------|------|------|
| CC           | 67   | 95   | 33   | 16.9 | 229  | 161 (41.3) |
| TC           | 26   | 30   | 14   | 20.0 | 0.800 | 82 (58.6)  |
| TT           | 87   | 107  | 31   | 13.8 | 0.230 | 281 (62.4) |
| Stage II     | 28   | 38   | 12   | 16.4 | 0.540 | 89 (61.0)  |
| Stage III    | 46   | 65   | 10   | 8.30 | 0.005* | 157 (64.9) |
| Stage IV     | 39   | 39   | 23   | 22.8 | 0.240 | 117 (57.9) |
| >12 months   | 59   | 69   | 21   | 14.1 | 0.480 | 187 (62.8) |
| ≤12 months   | 54   | 68   | 24   | 16.4 | 0.440 | 176 (60.3) |

Note: C = Control, T = Test; *p < 0.05; 95% CI = 95% confidence interval.
## Table 4 continued. Stratification analysis for association of MMP20 gene polymorphism with the clinical phenotypes of alcohol-induced ONFH.

| SNPs   | Genotype (%) | Allele (%) |
|--------|--------------|------------|
|        | rs10895322   |            |
| Controls | 109 (55.9) | 73 (37.4) | 13 (6.70) | 291 (74.6) | 99 (25.4) |
| Unilateral | 34 (49.3) | 26 (37.7) | 9 (13.0) | 0.110 | 94 (68.1) | 44 (31.9) |
| Bilateral | 102 (44.9) | 101 (44.5) | 24 (10.6) | 0.022* | 305 (67.2) | 149 (32.8) |
| Stage II | 30 (41.1) | 36 (49.3) | 7 (9.60) | 0.049* | 96 (65.8) | 50 (34.2) |
| Stage III | 54 (45.0) | 51 (42.5) | 15 (12.5) | 0.007* | 159 (66.3) | 81 (33.8) |
| Stage IV | 52 (50.5) | 40 (38.8) | 11 (10.7) | 0.410 | 144 (69.9) | 62 (30.1) |
| >12 months | 63 (41.7) | 69 (45.7) | 19 (12.6) | 0.016* | 195 (64.6) | 107 (35.4) |
| ≤12 months | 73 (50.3) | 58 (40.0) | 14 (9.70) | 0.092 | 204 (70.3) | 86 (29.7) |
|        | rs1784424    |            |
| Controls | 48 (24.5) | 101 (51.5) | 47 (24.0) | 197 (50.3) | 195 (49.7) |
| Unilateral | 36 (15.7) | 117 (51.1) | 76 (33.2) | 0.006* | 189 (41.3) | 269 (58.7) |
| Bilateral | 13 (17.6) | 35 (47.3) | 26 (35.1) | 0.035* | 61 (41.2) | 87 (58.8) |
| Stage II | 14 (11.7) | 65 (54.2) | 41 (34.2) | 0.001* | 93 (38.8) | 147 (61.3) |
| Stage III | 24 (23.1) | 50 (48.1) | 30 (28.9) | 0.650 | 98 (47.1) | 110 (52.9) |
| Stage IV | 25 (16.6) | 73 (48.3) | 53 (35.1) | 0.031* | 123 (40.7) | 179 (59.3) |
| >12 months | 16 (15.4) | 27 (17.9) | 77 (52.4) | 0.060 | 129 (43.9) | 165 (56.1) |
| ≤12 months | 36 (24.3) | 71 (46.0) | 44 (29.7) | 0.069 | 129 (43.9) | 165 (56.1) |
|        | rs3781788    |            |
| Controls | 89 (45.4) | 83 (42.4) | 24 (12.2) | 261 (66.6) | 131 (33.4) |
| Unilateral | 27 (39.1) | 29 (42.0) | 13 (18.8) | 0.340 | 83 (60.1) | 55 (39.9) |
| Bilateral | 76 (33.2) | 120 (52.4) | 33 (14.4) | 0.028* | 272 (59.4) | 186 (40.6) |
| Stage II | 23 (31.1) | 37 (50.0) | 14 (18.9) | 0.061 | 83 (56.1) | 65 (43.9) |
| Stage III | 36 (30.0) | 68 (56.7) | 16 (13.3) | 0.023* | 140 (58.3) | 100 (41.7) |
| Stage IV | 44 (42.3) | 44 (42.3) | 16 (15.4) | 0.720 | 132 (63.5) | 76 (36.5) |
| >12 months | 50 (31.3) | 74 (49.0) | 27 (17.9) | 0.048* | 174 (57.6) | 128 (42.4) |
| ≤12 months | 53 (36.0) | 75 (51.0) | 19 (12.9) | 0.310 | 181 (61.6) | 113 (38.4) |
|        | rs1711823    |            |
| Controls | 51 (26.0) | 99 (50.5) | 46 (23.5) | 201 (51.3) | 191 (48.7) |
| Unilateral | 23 (32.9) | 31 (44.3) | 16 (22.9) | 0.560 | 77 (55.0) | 63 (45.0) |
| Bilateral | 74 (32.3) | 119 (52.0) | 36 (15.7) | 0.021* | 267 (58.3) | 191 (41.7) |
| Stage II | 25 (33.8) | 34 (46.0) | 15 (20.3) | 0.140 | 84 (56.8) | 64 (43.2) |
| Stage III | 42 (34.7) | 65 (53.7) | 14 (11.6) | 0.001* | 149 (61.6) | 93 (38.4) |
| Stage IV | 30 (28.9) | 51 (49.0) | 23 (22.1) | 0.860 | 111 (53.4) | 97 (46.6) |
| >12 months | 52 (34.4) | 75 (49.7) | 24 (15.9) | 0.081 | 179 (59.3) | 123 (40.7) |
| ≤12 months | 45 (30.4) | 75 (50.7) | 28 (19.8) | 0.120 | 165 (55.7) | 131 (44.3) |
Table 4 continued. Stratification analysis for association of MMP20 gene polymorphism with the clinical phenotypes of alcohol-induced ONFH.

| SNPs       | Genotype (%) | Allele (%) |
|------------|--------------|------------|
| rs1784418  | C  | T  | P  | OR | 95% CI  |
| Controls   | 50 (25.4) | 102 (51.8) | 45 (22.8) | 202 (51.3) | 192 (48.7) | 0.280 | 79 (56.4) | 61 (43.6) | 0.294 | 0.81 | 0.55 | 1.20 |
| Unilateral | 25 (35.7) | 29 (41.4) | 16 (22.9) | 0.044* | 263 (57.4) | 195 (42.6) | 0.072 | 0.78 | 0.60 | 1.02 |
| Bilateral  | 71 (31.0) | 121 (52.8) | 37 (16.2) | 0.001* | 147 (60.7) | 95 (39.3) | 0.020* | 0.68 | 0.49 | 0.94 |
| Stage II   | 25 (33.8) | 34 (46.0) | 15 (20.3) | 0.150 | 84 (56.8) | 64 (43.2) | 0.254* | 0.80 | 0.55 | 1.17 |
| Stage III  | 40 (33.1) | 67 (55.4) | 14 (11.6) | 0.001* | 147 (60.7) | 95 (39.3) | 0.020* | 0.68 | 0.49 | 0.94 |
| >12 months | 52 (34.4) | 74 (49.0) | 25 (16.6) | 0.096 | 178 (58.9) | 124 (41.1) | 0.044* | 0.73 | 0.54 | 0.99 |
| ≤12 months | 44 (29.7) | 76 (51.4) | 28 (18.9) | 0.140 | 164 (55.4) | 132 (44.6) | 0.281 | 0.85 | 0.60 | 1.29 |
| rs7126560  | G  | A  | P  | OR | 95% CI  |
| Controls   | 85 (43.1) | 89 (45.2) | 23 (11.7) | 259 (65.7) | 135 (34.3) | 0.220 | 83 (59.3) | 57 (40.7) | 0.172 | 1.32 | 0.89 | 1.96 |
| Unilateral | 27 (38.6) | 29 (41.4) | 14 (20.0) | 0.100 | 270 (59.7) | 182 (40.3) | 0.072 | 1.29 | 0.98 | 1.71 |
| Bilateral  | 77 (34.1) | 116 (51.3) | 33 (14.6) | 0.089 | 84 (56.8) | 64 (43.2) | 0.053 | 1.46 | 0.99 | 2.15 |
| Stage II   | 23 (31.1) | 38 (51.4) | 13 (17.6) | 0.051 | 141 (58.3) | 101 (41.7) | 0.058 | 1.37 | 0.99 | 1.91 |
| Stage III  | 37 (30.6) | 67 (55.4) | 17 (14.1) | 0.440 | 128 (63.4) | 74 (36.6) | 0.566 | 1.11 | 0.78 | 1.58 |
| >12 months | 51 (34.2) | 72 (48.3) | 26 (17.4) | 0.130 | 174 (58.4) | 124 (41.6) | 0.048* | 1.37 | 1.00 | 1.86 |
| ≤12 months | 55 (36.0) | 73 (49.7) | 21 (14.3) | 0.350 | 179 (60.9) | 115 (39.1) | 0.191 | 1.23 | 0.90 | 1.69 |
| rs1573954  | T  | C  | P  | OR | 95% CI  |
| Controls   | 48 (24.6) | 105 (53.9) | 42 (21.5) | 201 (51.5) | 189 (48.5) | 0.046* | 57 (41.3) | 81 (58.7) | 0.039* | 1.51 | 1.02 | 2.24 |
| Unilateral | 13 (18.8) | 31 (44.9) | 25 (36.2) | 0.082 | 206 (45.2) | 250 (54.8) | 0.065 | 1.29 | 0.98 | 1.69 |
| Bilateral  | 45 (19.7) | 116 (50.9) | 67 (29.4) | 0.051 | 141 (58.3) | 101 (41.7) | 0.058 | 1.37 | 0.99 | 1.91 |
| Stage II   | 12 (16.2) | 31 (41.9) | 31 (41.9) | 0.005* | 55 (37.2) | 93 (62.8) | 0.003* | 1.80 | 1.22 | 2.65 |
| Stage III  | 18 (15.0) | 69 (57.5) | 33 (27.5) | 0.010* | 105 (43.8) | 135 (56.3) | 0.058 | 1.37 | 0.99 | 1.89 |
| >12 months | 30 (19.9) | 76 (50.3) | 45 (29.8) | 0.130 | 136 (45.0) | 166 (55.0) | 0.090 | 1.30 | 0.96 | 1.75 |
| ≤12 months | 28 (19.2) | 71 (48.6) | 47 (32.2) | 0.084 | 127 (43.5) | 165 (56.5) | 0.037* | 1.38 | 1.02 | 1.87 |

* P<0.05 indicates statistical significance. ^ P values were calculated by Wald test adjusted for age; b P was calculated by Pearson chi-squared test. Bold values indicate a significant difference.

A susceptibility locus (rs10895322) for 11q-deletion neuroblastoma, and 11q deletion is an inversely correlated prognostic factor in neuroblastoma [22]. In 2015, another GWAS identified association between rs10895322 and neovascular lesion size in age-related macular degeneration (AMD), which suggests the possibility of using MMP20 as a novel target to control the growth of choroidal neovascularization (CNV) after the onset of AMD [16]. Similarly, in this study, we found that rs10895322 may be a high-risk factor of alcohol-induced ONFH, implying that the risk attributed to rs10895322 in ONFH and neuroblastoma may be mediated through a comparable angiogenesis process that is regulated by MMP20.

In a family-based association study, Jeremias et al. [23] concluded that rs1711399 and rs1711423 were associated with the susceptibility to molar-incisor hypomineralization (MIH). Our study showed that rs1711399 played a risk-heightening role in the occurrence of alcohol-induced ONFH, while rs1711423 may be a protective SNP. We hold the opinion that there are genetic differences between the 2 diseases.
In our study, rs1784418 was also a protective SNP concerning the occurrence of alcohol-induced ONFH in both dominant and log-additive genetic models. In 2012, Tannure et al. [24] speculated that rs1784418 C>T may be involved with caries susceptibility in primarily Caucasian patients with poor oral health habits. A similar study by Filho et al. in 2016 explored the association of rs1784418 C>T and dental caries experience. Their conclusion is that the rs1784418 T allele can potentially be used as a marker for lower caries risk in certain populations [25]. The latest report from Brazil by Antunes et al. [26] suggested that MMP20 rs1784418 C>T may contribute to the occurrence of white spot lesions in the primary dentition. The aforementioned conclusions are consistent with our results; though the function of rs1784418 is unknown, there is a possibility that the variant affects the transcription of MMP20.

In the study by Han et al. [27], they analyzed the correlations between polymorphisms of rs1711437 (G>A) and kidney transplantation outcomes in 235 transplant recipients. The conclusions suggest that MMP20 polymorphisms may influence the long-term outcome of kidney allografts. Beyond that, the MMP20 gene also has been shown to be related to kidney aging by Wheeler et al. [28]. They showed that the A allele at rs1711437 was associated with high glomerular filtration rate (GFR). However, our study found no relationship between this locus and alcohol-induced ONFH. The study by Wang et al. [29] in 2015 suggested that MMP20 rs2292730, rs12278250, and rs9787933 might be associated with ovarian cancer risk in a group of 417 ovarian cancer cases and 417 controls. In contrast to this conclusion, a comprehensive meta-analysis by Zhu and Sun [30] in 2017 found that these 3 loci might not be associated with ovarian cancer risk. Our present study only indicated that rs2292730 is a risk SNP of alcohol-induced ONFH.

### Table 5. The haplotype frequencies of MMP20 polymorphisms and their association with alcohol-induced ONFH risk.

| Haplotype | Freq | OR* (95% CI) | P* |
|-----------|------|--------------|----|
| rs1711437 | 0.377| 1            | -- |
| rs1711423 | 0.278| 1.63 (1.15–2.30) | 0.006* |
| rs1784424 | 0.088| 1.68 (1.00–2.82) | 0.051 |
| rs3781788 | 0.074| 0.89 (0.51–1.53) | 0.670 |
| rs17174327| 0.062| 1.14 (0.63–2.07) | 0.670 |
| rs1784418 | 0.055| 0.79 (0.44–1.43) | 0.440 |

* P<0.05 indicates statistical significance. * Adjusted by age. Bold values indicate a significant difference.
among these 3 SNPs. In the study by Kanna et al. [31], genetic polymorphisms of MMP20 (rs17099008) were significantly associated with Modic changes, which are vertebral endplate signal changes predominantly observed in the lumbar spine. Our study did not include this site. It is necessary to conduct larger-scale, multicenter, and high-quality studies in the future.

Up to now, the other 4 high-risk sites (rs1784424, rs3781788, rs7126560, and rs1573954) have not been described in other diseases. Collectively, our study provides insight into the pathogenesis of alcohol-induced ONFH. Although this study had an adequately sufficient statistical power, some intrinsic limitations may still exist. First, our study did not perform a functional study of these SNPs. To clarify the function of MMP20 in alcohol-induced ONFH, SNPs functional study is essential. Second, the sample capacity was relatively small; a larger sample capacity will be more persuasive. Finally, participants’ ethnicity was limited to the Han Chinese population. Hence, the associations identified in this study should be confirmed in further studies with other ethnic groups.

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Conclusions

We demonstrated in this study that MMP20 polymorphisms can play both risk-heightening and risk-mitigating roles and that MMP20 may serve as a marker to predict the occurrence of alcohol-induced ONFH. These findings were in agreement with the multifactorial etiology of alcohol-induced ONFH. However, because of the lack of functional studies and the fact that most intronic SNPs are nonfunctional, these results more likely suggest that other coding SNP(s) tagged by these positive SNPs might be causal to alcohol-induced ONFH.

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Conflict of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [Chemical Abstracts/CAS]
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