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Original Article

Association of *MMPs/TIMPs* polymorphism with alcohol-induced osteonecrosis of femoral head in the Chinese Han population

Junyu Chen[1], Yongchang Guo[2], Tianbo Jin[3,4], Jian Li[2], Jieli Du[1], Yuju Cao[2], Jianzhong Wang[5]

[1]Inner Mongolia Medical University, Hohhot 010030, Inner Mongolia, China; [2]Zhengzhou Traditional Chinese Medicine Traumatology Hospital, Zhengzhou 450016, Henan, China; [3]National Engineering Research Center for Miniaturized Detection System, Xi'an 710069, Shaanxi, China; [4]The College of Life Sciences Northwest University, Xi'an 710069, China; [5]Department of Orthopedics and Traumatology, The 2th Affiliated Hospital of Inner Mongolia University, Hohhot 010030, Inner Mongolia, China

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Abstract: Alcohol-induced osteonecrosis of the femoral head (alcohol-induced ONFH) makes up almost one-third of the total proportion of non-traumatic ONFH. Polymorphism of matrix metalloproteinase (*MMP*) or tissue inhibitors matrix metalloproteinase (*TIMP*) is related to bone metabolic disorders. This study aimed to explore whether *MMPs/TIMPs* polymorphisms influenced alcohol-induced ONFH risk in the Chinese Han population. We selected 300 patients and 308 controls, with 18 single nucleotide polymorphisms (SNPs) successfully genotyped, and evaluated the association using the chi-squared test, Fisher's exact test, *t*-test, and genetic model analyses. We found that rs4789936 on *TIMP-2* (OR = 0.75; 95% CI = 0.58-0.96; *P* = 0.025) decreased alcohol-induced ONFH risk under the allele model. After adjusting for age and gender, rs4789936was significant in the dominant (OR = 0.63; 95% CI = 0.43-0.93; *P* = 0.018), overdominant (OR = 0.66; 95% CI = 0.45-0.98; *P* = 0.038), and log-additive (OR = 0.72; 95% CI = 0.53-0.97; *P* = 0.033) model. Furthermore, rs2277698 was significant in the overdominant model (OR = 0.66; 95% CI = 0.44-1.00; *P* = 0.049), which indicated the two SNPs had a protective function in decreasing alcohol-induced ONFH risk. In conclusion, rs4789936 and rs2277698 on *TIMP-2* significantly decreased alcohol-induced ONFH risk in the Chinese Han population. Our study attempts to offer an objective basis for alcohol-induced ONFH clinical prevention.

Keywords: *MMPs/TIMPs*, alcohol-induced ONFH, SNP, osteoblast, osteoclast

Introduction

Osteonecrosis of the femoral head (ONFH) is also called aseptic or anemic necrosis of the femoral head. It is a condition of partial insufficient blood supplementation caused by several complicated reasons, ONFH leads to osteocyteischemia, necrosis, bone trabecula breakage, and femoral head collapse [1]. This disease is widely believed to be an orthopedic refractory disease and is usually divided into two types, traumatic and non-traumatic ONFH.

Alcohol-induced osteonecrosis of the femoral head (alcohol-induced ONFH) is a type of non-traumatic ONFH caused by excessive alcoholin take over a long period of time. The increased intake of alcohol causes dyslipidemia, bone marrow stroma (BMSC) prosoplasia, and bone metabolic disorder. It affects bone resorption and remodeling, and finally causes osteonecrosis of the femoral head [2, 3]. Patients with this disease are characterized by hip pains, sick limb shortening and myophagism, claudication, tenderness and limited joint mobility (Thomas). Widely known hypotheses about alcohol-induced ONFH pathomechanism include lipid metabolism disorder, fat embolism and local intravascular coagulation theory, bone cell steatosis doctrine, high-pressure theory in the bone, theory of osteoporosis, and nitric oxide and tumor necrosis factor theory. However, the straight forward pathogenesis of alcohol-induced ONFH remains unclear. If no treatment is implemented in the pathological process, at least 70% of patients will eventually experience femoral head collapse and the destruction of the hip [4]. Recently, with the continual

in-depth research of molecular biology, cytobiology, and genetics about alcohol-induced ONFH, gene polymorphism has been considered to have a strong relationship with this disease. This discovery has opened up a new direction for us to examine the development and information onalcohol-induced ONFH.

Several genome-wide association studies determined that genes ApoA1, ApoB, PAI-1, ADH2, VEGF, MTHFR, CYP3A4, MTHFR, ANXA and TFPIare associated with alcohol-induced ONFH [1, 5-9]. These studies illustrated the heredity of the disease, but information reported on gene polymorphism and alcohol-induced ONFH is still scarce. MMPs and TIMPs belong to two kinds of proteases involved in the degradation of all organizations of the extracellular matrix of the human body, including osseous tissue. Previous studies identified *MMPs/TIMPs* are associated with several diseases, such as coronary heart disease [10], human cancer [11], fibrosis bronchiectasis [12] and glaucoma [13], among others. Any type of ONFH involves changes in bone transformation, bone resorption and remodeling, imbalance of *MMPs/TIMPs* caused by degradation of the bone matrix and bone damage. This system also affects the differentiation and function of BMSCs to act on the process of bone transformation and reconstruction. However, the relationship between this system and alcohol-induced ONFH has been less systematically researched. Our study aims to explore the possible correlations between polymorphisms of *MMPs/TIMPs* and alcohol-induced ONFH in the Chinese Han population.

Experimental section

Ethics review committee statement

This investigation followed the principles of the Declaration on Helsinki of the World Medical Association and obtained permission from the Ethics Committee of Zhengzhou Traditional Chinese Medicine Traumatology Hospital. All participants signed informed consent forms and were notified for our case-control study.

Research subjects

A sample of 608 individuals including 300 patients and 308 healthy controls, were selected consecutively from Zhengzhou Traditional Chinese Medicine Traumatology Hospital up to January 2016 from September 2014 for our case-control study. Alcohol-induced ONFH was diagnosed on the basis of clinical manifestations, such as hip pain, activity limitation of hip, lower limb muscle atrophy of the sick side, or through image ological examination such as higher density shadows, rupture of the joint surface, or bumpiness and narrowness of the hip joint. Notably, magnetic resonance imaging (MRI) was used to make a definite diagnosis for patients without X-ray changed. The exclusion criteria were as follows: (1) Individuals who did not agree to take part in this study. (2) Those who did not satisfy the diagnostic criteria of alcohol-induced ONFH or patients diagnosed with traumatic osteonecrosis or other hip diseases. (3) Those suffering from serious primary diseases and who required steroid treatment for replacement (4) Those affected by drugs that cause liver disease or dyslipidemia.

A total of 308 healthy controls were selected from September 2014 to January 2016 in Zhengzhou Traditional Chinese Medicine Traumatology Hospital through physical examination. All individuals who overused steroids or had a chronic metabolic disease in the heart, kidney, or liver were excluded. All participants signed informed consent forms.

SNP site selection

A total of 18 SNPs on four genes in the *MMPs/TIMPs* system, including five SNPs on *MMP-1*, three SNPs on *MMP-9*, six SNPs on *TIMP-2*, and four SNPs on *TIMP-3*, were selected for this study with a minor allele frequency (MAF) higher than 5% in the HapMap Chinese Han Beijing (CHB) population. We prepared genomic DNA from peripheral blood samples using a genomic DNA purification kit (GoldMag, China), and the blood was stored with a condition of -20°C. The concentration of DNA was measured through spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). We used the SequenomMassARRAY Assay Design 4.0 software to project the Multiplexed SNP MassEXTENDED assay [14], and Sequenom MassARRAYRS 1000 was used to perform *MMPs/TIMPs* SNP genotyping using the standard protocol.

Statistical analysis

Statistical analysis of our case-control study was performed using the SPSS19.0 statistical

Table 1. Basic SNP information summary of all the individuals in our study

Gene	SNP ID	Position	Band	Alleles A[a]/B	MAF Case	MAF Control	Role	HWE-*P*	OR (95% CI)	*P*[b] value
MMP1	rs5854	102660874	11q22.2	A/G	0.10	0.09	3' UTR	0.490	1.18 (0.80-1.73)	0.404
MMP1	rs2071230	102660959	11q22.2	G/A	0.19	0.19	3' UTR	0.272	0.99 (0.74-1.32)	0.947
MMP1	rs2239008	102661080	11q22.2	G/A	0.48	0.48	3' UTR	0.568	1.00 (0.80-1.26)	0.970
MMP1	rs470215	102661099	11q22.2	C/T	0.10	0.09	3' UTR	0.490	1.18 (0.80-1.73)	0.404
MMP1	rs2071232	102665669	11q22.2	T/C	0.47	0.46	Intron	0.732	1.02 (0.82-1.28)	0.842
MMP9	rs3918249	44638136	20q13.12	T/C	0.29	0.31	Intron	0.895	0.89 (0.69-1.13)	0.341
MMP9	rs2274755	44639692	20q13.12	T/G	0.12	0.15	Intron (boundary)	0.265	0.80 (0.57-1.11)	0.183
MMP9	rs3918254	44640391	20q13.12	T/C	0.19	0.20	Intron (boundary)	0.368	0.95 (0.71-1.26)	0.723
TIMP2	rs2277698	76867017	17q25.3	T/C	0.21	0.22	Coding exon	0.503	0.92 (0.70-1.21)	0.552
TIMP2	rs2009196	76870581	17q25.3	C/G	0.40	0.39	Intron	0.720	1.03 (0.82-1.30)	0.791
TIMP2	rs7342880	76874512	17q25.3	A/C	0.17	0.13	Intron	1.000	1.34 (0.97-1.84)	0.071
TIMP2	rs11654470	76877331	17q25.3	C/T	0.23	0.26	Intron	0.461	0.85 (0.66-1.11)	0.228
TIMP2	rs2003241	76885117	17q25.3	C/T	0.16	0.16	Intron	0.287	0.99 (0.73-1.35)	0.969
TIMP2	rs4789936	76897974	17q25.3	T/C	0.25	0.31	Intron	1.000	0.75 (0.58-0.96)	0.025*
TIMP3	rs715572	33234931	22q12.3	A/G	0.33	0.35	Intron	0.618	0.93 (0.74-1.18)	0.562
TIMP3	rs8136803	33237112	22q12.3	T/G	0.04	0.05	Intron	1.000	0.95 (0.55-1.64)	0.858
TIMP3	rs9609643	33251059	22q12.3	A/G	0.14	0.14	Intron	0.648	0.94 (0.68-1.30)	0.695
TIMP3	rs11547635	33253292	22q12.3	T/C	0.34	0.34	Coding exon	0.900	1.01 (0.80-1.28)	0.929

SNP, Single nucleotide polymorphism; MAF, Minor allele frequency; HWE, Hardy-Weinberg equilibrium; ORs, Odds ratios; CI, Confidence interval; [a]Minor allele, [b]*P* were adjusted by gender and age, **P*<0.05, statistical significance.

software (SPSS, Chicago, IL) and Microsoft Excel. *P* values were all two-sided and we considered $P \leq 0.05$ to be statistically significant. A chi-squared test was conducted to examine the SNP genotype frequencies in our case and control groups [15]. Then the Hardy-Weinberg equilibrium (HWE) was used to verify the genotype frequency of the control group. The constructed 95% confidence intervals (95% CIs) and odds ratios (ORs) were tested using unconditional logistic regression analysis [16] with adjustments for age and gender. PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) was used by four models (dominant, recessive, codominant and log-additive models) to evaluate the association between SNPs and alcohol-induced ONFH risk. Lastly, linkage disequilibrium structure was examined using Haploview 4.2.

Results

Our case-control study selected 608 individuals, including 300 alcohol-induced ONFH patients with a mean age of 43.29 ± 13.084 and 308 healthy controls with a mean age of 49.47 ± 7.973. We researched for 18 SNPs on eight genes. The basic information of all SNPs, including position, band, alleles, MAF, ORs, 95% CI and *P* values, is enumerated in **Table 1**. The primer message, which is presented in the Supplementary Table 1, was obtained through the SequenomMassARRAY Assay Design 4.0 software [14]. The Hardy-Weinberg equilibrium (HWE) was used to verify the genotype frequency of the control group. The *P* values showed none of the 18 SNPs had a significant departure from the HWE.

In accordance with the results in **Table 1**, rs4789936 on *TIMP-2* with a minor allele T decreased alcohol-induced ONFH risk (OR = 0.75; 95% CI = 0.58-0.96; *P* = 0.025). A logistic test was used to assess the association between alcohol-induced ONFH risk and SNPs in the *MMPs/TIMPs* system with five models, including dominant, recessive, codominant, overdominant, and log-additive models, as shown in **Table 2**. Three SNPs on *TIMP-2* significantly decreased alcohol-induced ONFH risk: rs4789936 was significant in the dominant (OR = 0.69; 95% CI = 0.50-0.95; *P* = 0.023) and log-additive (OR = 0.75; 95% CI = 0.59-0.97; *P* = 0.027) models; rs11654470 was conspicuous in the overdominant model (OR = 0.68; 95% CI = 0.48-0.95; *P* = 0.022); and rs2277698 was positive in the codominant (OR = 0.68; 95% CI = 0.48-0.97; *P* = 0.025) and overdominant

Table 2. Analysis of the association between SNPs and alcohol-induced ONFH risk (based on logistical tests)

Gene	SNP_ID	Model	Genotype	Control	Case	OR (95% CI)	*P*-value	AIC	BIC
TIMP-2	rs4789936	Codominant	C/C	149 (48.4%)	170 (57.6%)	1	0.071	836.4	849.6
			C/T	130 (42.2%)	104 (35.2%)	0.70 (0.50-0.98)			
			T/T	29 (9.4%)	21 (7.1%)	0.63 (0.35-1.16)			
		Dominant	C/C	149 (48.4%)	170 (57.6%)	1	0.023*	834.5	843.3
			C/T-T/T	159 (51.6%)	125 (42.4%)	0.69 (0.50-0.95)			
		Recessive	C/C-C/T	279 (90.6%)	274 (92.9%)	1	0.31	838.6	847.4
			T/T	29 (9.4%)	21 (7.1%)	0.74 (0.41-1.32)			
		Overdominant	C/C-T/T	178 (57.8%)	191 (64.8%)	1	0.08	836.6	845.4
			C/T	130 (42.2%)	104 (35.2%)	0.75 (0.54-1.04)			
		Log-additive	---	---	---	0.75 (0.59-0.97)	0.027*	834.8	843.6
	rs11654470	Codominant	T/T	166 (53.9%)	184 (61.3%)	1	0.068	843.4	856.6
			T/C	124 (40.3%)	94 (31.3%)	0.68 (0.49-0.96)			
			C/C	18 (5.8%)	22 (7.3%)	1.10 (0.57-2.13)			
		Dominant	T/T	166 (53.9%)	184 (61.3%)	1	0.063	843.3	852.1
			T/C-C/C	142 (46.1%)	116 (38.7%)	0.74 (0.53-1.02)			
		Recessive	T/T-T/C	290 (94.2%)	278 (92.7%)	1	0.46	846.2	855
			C/C	18 (5.8%)	22 (7.3%)	1.27 (0.67-2.43)			
		Overdominant	T/T-C/C	184 (59.7%)	206 (68.7%)	1	0.022*	841.5	850.3
			T/C	124 (40.3%)	94 (31.3%)	0.68 (0.48-0.95)			
		Log-additive	---	---	---	0.86 (0.66-1.11)	0.23	845.4	854.2
	rs2277698	Codominant	C/C	184 (60.1%)	198 (66%)	1	0.025*	838.7	851.9
			C/T	110 (36%)	81 (27%)	0.68 (0.48-0.97)			
			T/T	12 (3.9%)	21 (7%)	1.63 (0.78-3.40)			
		Dominant	C/C	184 (60.1%)	198 (66%)	1	0.13	841.8	850.6
			C/T-T/T	122 (39.9%)	102 (34%)	0.78 (0.56-1.08)			
		Recessive	C/C-C/T	294 (96.1%)	279 (93%)	1	0.093	841.2	850
			T/T	12 (3.9%)	21 (7%)	1.84 (0.89-3.82)			
		Overdominant	C/C-T/T	196 (64%)	219 (73%)	1	0.018*	838.4	847.2
			C/T	110 (36%)	81 (27%)	0.66 (0.47-0.93)			
		Log-additive	---	---	---	0.92 (0.71-1.21)	0.56	843.7	852.5

ORs, Odds ratios; AIC, Akaike's Information criterion; BIC, Bayesian Information criterion. *P* value was calculated with logistic analysis. **P*<0.05, statistical significance.

(OR = 0.66; 95% CI = 0.47-0.93; *P* = 0.018) models. **Table 3** indicates that two SNPs on *TIMP-2* had a strong protective function against alcohol-induced ONFH after adjusting for age and gender: rs4789936 was significant in the dominant (OR = 0.63; 95% CI = 0.43-0.93; *P* = 0.018), overdominant (OR = 0.66; 95% CI = 0.45-0.98; *P* = 0.038) and log-additive (OR = 0.72; 95% CI = 0.53-0.97; *P* = 0.033) models; and rs2277698 was significant in the overdominant model (OR = 0.66; 95% CI = 0.44-1.00; *P* = 0.049).

Figure 1 illustrates that one block in *MMP-9* contained three SNPs that had an interaction. Haplotype analysis was verified using chi-square and logistic tests (**Table 4**). In the **Figure 2** we found there was no haplotype association between the SNPs in TIMP-2. Results showed that three SNPs rs3918249, rs2274755 and rs3918254 on *MMP-9* gene had a significant linkage disequilibrium. **Table 4** indicates that allele T in rs3918249, G in rs2274755, and C in rs3918254 obtained a significant result (OR = 0.69; 95% CI = 0.49-0.96; *P* = 0.029) after adjusting for age and gender.

Discussion

In our current study, we found that two SNPs rs4789936 and rs2277698 on *TIMP-2* had significant differences in the alcohol-induced ONFH patients and in the control group. Thus, this finding indicates that they play a preventive

Table 3. Analysis of the association between SNPs and alcohol-induced ONFH risk (adjusted for age and gender)

Gene	SNP_ID	Model	Genotype	Control	Case	OR (95% CI)	*P*-value	AIC	BIC
TIMP-2	rs4789936	Codominant	C/C	149 (48.4%)	170 (57.6%)	1	0.061	627.2	649.2
			C/T	130 (42.2%)	104 (35.2%)	0.63 (0.42-0.94)			
			T/T	29 (9.4%)	21 (7.1%)	0.65 (0.31-1.35)			
		Dominant	C/C	149 (48.4%)	170 (57.6%)	1	0.018*	625.2	642.8
			C/T-T/T	159 (51.6%)	125 (42.4%)	0.63 (0.43-0.93)			
		Recessive	C/C-C/T	279 (90.6%)	274 (92.9%)	1	0.52	630.4	648
			T/T	29 (9.4%)	21 (7.1%)	0.79 (0.39-1.61)			
		Overdominant	C/C-T/T	178 (57.8%)	191 (64.8%)	1	0.038*	626.5	644.1
			C/T	130 (42.2%)	104 (35.2%)	0.66 (0.45-0.98)			
		Log-additive	---	---	---	0.72 (0.53-0.97)	0.033*	626.3	643.9
	rs2277698	Codominant	C/C	184 (60.1%)	198 (66%)	1	0.065	629.2	651.2
			C/T	110 (36%)	81 (27%)	0.69 (0.46-1.05)			
			T/T	12 (3.9%)	21 (7%)	1.79 (0.71-4.55)			
		Dominant	C/C	184 (60.1%)	198 (66%)	1	0.24	631.3	648.9
			C/T-T/T	122 (39.9%)	102 (34%)	0.79 (0.53-1.17)			
		Recessive	C/C-C/T	294 (96.1%)	279 (93%)	1	0.12	630.2	647.9
			T/T	12 (3.9%)	21 (7%)	2.02 (0.80-5.08)			
		Overdominant	C/C-T/T	196 (64%)	219 (73%)	1	0.049*	628.8	646.4
			C/T	110 (36%)	81 (27%)	0.66 (0.44-1.00)			
		Log-additive	---	---	---	0.94 (0.68-1.29)	0.7	632.5	650.1

ORs, Odds ratios; AIC, Akaike's Information criterion; BIC, Bayesian Information criterion. *P* value was adjusted by age and gender; **P*<0.05, statistical significance.

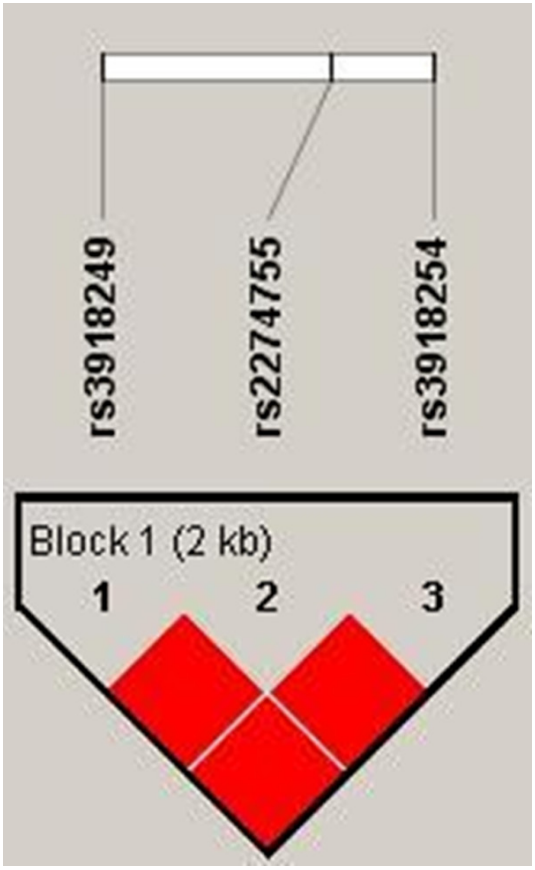

Figure 1. Linkage disequilibrium (LD) analysis of the SNPs on *MMP-9*. Bright red is a significant LD displayed by standard colors schemes. Parameters r2 and D' were used to analyze LD pattern.

role against this disease. *MMPs/TIMPs* are a system constituted by *MMPs* and *TIMPs*. Until now, scientists have found more than 20 different kinds of purified *MMPs*, and only for kinds of *TIMPs*. *MMP* sare a group of proteases that could degrade the extracellular matrix, and *TIMPs* are the natural inhibitors of *MMPs* that have an antagonistic action in the function of *MMPs*. On the one hand, *MMPs/TIMPs* participate in the development and rebuilding of all the body tissue, especially in the process of bone metabolism. On the other hand, they play a role in the pathology of several diseases, such as inflammatory diseases, lipid metabolic disorders, and cancer [11-13].

Under physiological conditions, human bone metabolism is a complex process that has two important parts: bone resorption and bone formation. Bone metabolism includes the interaction among bone cells, the interaction among hemopoietic cells, and the stroma cells in bone

Table 4. Haplotype association with response (n = 608, adjusted by gender and age)

	rs3918249	rs2274755	rs3918254	Freq	OR (95% CI)	*P*-value
1	C	G	C	0.3717	1	---
2	T	G	C	0.2993	0.69 (0.49-0.96)	0.029*
3	C	G	T	0.1941	0.77 (0.52-1.15)	0.2
4	C	T	C	0.1349	0.65 (0.42-1.00)	0.05

Global haplotype association *P*-value: 0.084. ORs, Odds ratios; rs3918249, rs2274755 and rs3918254 were on *MMP-9*. **P*<0.05, statistical significance.

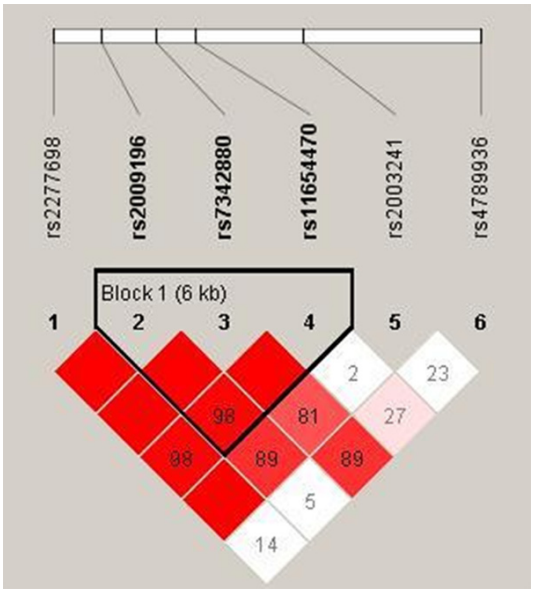

Figure 2. Linkage disequilibrium (LD) analysis of the SNPs on TIMP-2. Bright red is a significant LD displayed by standard colors schemes. Parameters r2 and D' were used to analyze LD pattern.

marrow. Two kinds of cells differentiated by BMSCs play a key role in the process of bone metabolism: one consists of osteoclasts that participate in bone resorption and the other consists of osteoblasts that act in the compound bone matrix. In the beginning, the osteoclasts involved in bone resorption are mostly-activated. Sensitized osteoclasts dissolve the matrix cells andmove the calcium inside out before initiating bone resorption. Afterwards, osteocytes are formed on the surface of bone resorption sites. Then, osteoblasts compounded the non-mineralized bone matrix and transport calcium to the zone of calcification at the same time. Finally, calcium and phosphorus ionsaccumulate in the bone matrix, cause bone matrix calcification, and then form bone tissue. During the process of bone metabolism, a certain amount of bone tissue is absorbed every day. A large amount of bone tissue is compounded, and the amount is absorbed and compounded to maintain a dynamic balance. Once the dynamic balance is broken, orthopedic diseases can occur.

Alcohol-induced ONFH is a type of bone metabolic disorder disease mainly caused by the chronic, consumption of large amounts of alcohol. Macroscopic causes include the defectively osteogenic ability of BMSCs or the metabolic disorder mostly caused by the imbalance between osteoblasts and osteoclasts. Previous studies determined that the osteogenic ability of BMSCs is not defective in alcohol-induced ONFH patients [17, 18]. Thus, our research focused on the factors that influence bone metabolic disorder. Bone metabolism consists of bone resorption and bone formation. The precondition of bone resorption is osteoclast activation. Osteoblast secrete *MMPs* and activate osteoclasts by secreting *MMPs*. *TIMPs* are natural inhibitors of *MMPs*. First, *MMPs* participate in bone matrix degradation directly by promoting the migration of osteoclasts. Then, the osteoclasts secrete acidoid to dissolve minerals and degrade the bone matrix. However, with the presence of *TIMPs*, bone resorption caused by osteoclasts is significantly inhibited. Second, *MMPs/TIMPs* play a role in the regulation and control of osteoblast proliferation and apoptosis. *MMP-2* secretion indicates osteoblast maturation. *MMP-1* is the key enzyme for bone surface collagen. The ratio of *TIMP-1/MMP-9* even affects bone resorption. Several scientists considered that the membrane-type *MMPs* in osteoblast maintained cells survived in the differentiation process of matrix cells to osteocytes and ensured the survival of mature osteocytes by disturbing *TIMPs* [19]. Third, *MMPs/TIMPs* regulate and control osteoblast differentiation and bone resorption of osteoclasts to complete the reparation of the lesion bone tissue [20]. Fourth, the proportion of *MMPs/TIMPs* is highly controlled in the process of osteogenesis and is involved in bony remodeling [21].

Furthermore, polymorphism of *MMPs/TIMPs* is important in several bone metabolic disor-

ders. In osteoarthritis (OA) patients, the enzymatic activity of *MMP-2* and *MMP-9* was significantly stronger than controls in serum and synovia. However, *TIMPs* increased negligibly and caused an imbalance between *MMPs* and *TIMPs*, thus leading to increased bone degradation [22]. A study by Bord found that osteoclasts in normal new-born bone expressed a certain amount of *TIMP-1* continuity, but pathologic and ectopic bone never or scarcely expressed *TIMP-1* [23]. The study by Mattot found *TIMP-2* played a regulation function in the level of transcription but and at a posttranscriptional level [24]. In conclusion, our study projected 18 SNPs on *MMPs/TIMPs* and investigated the association of polymorphisms of *MMPS/TIMPs* with alcohol-induced ONFH patients. We found two SNPs, rs4789936 and rs2277698 on *TIMP-2* gene with polymorphism significantly decreased the risk of alcohol-induced ONFH. The mutation of rs4789936 and rs2277698 on *TIMP-2* might adjust the *TIMP-2* amounts, regulate the balance of *MMPs/TIMPs* system, and inhibit the overexpression of *MMPs* in pathological bone tissue of alcohol-induced ONFH. The mutations also resisted osteoblast differentiation decrease and adipocyte differentiation increase caused by BMSC prosoplasia in over-drinking patients by regulating the balance of *MMPs/TIMPs*, prevented bone metabolic disorders, and played a protective function in alcohol-induced ONFH.

Nevertheless, our research has several limitations. The common variants usually had weaker stratification than the rare variants, and population-specific was common [25]. Moreover, all of our participants were from the Chinese Han population. Thus, larger samples and other populations should be examined to confirm our results and conclusion.

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Disclosure of conflict of interest

None.

Authors' contribution

Jianzhong Wang, Yuju Cao and Tianbo Jin conceived and designed this experiment. Junyu Chen completed the experiments and wrote of the manuscript. Yongchang Guo analyzed the data. Jieli Du and Jian Li gathered the samples. The final manuscript was read and approved by all authors.

Abbreviations

MMP, matrix metalloproteinase; *TIMP*, tissue inhibitor of matrix metalloproteinases; alcohol-induced ONFH, alcohol-induced osteonecrosis of femoral head; BMSC, bone marrow stroma cell; CIs, confidence intervals; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; ORs, odds ratios; SNPs, single nucleotide polymorphisms.

Address correspondence to: Dr. Jianzhong Wang, Department of Orthopedics and Traumatology, The 2th Affiliated Hospital of Inner Mongolia University, #1 Yingfang Road, Hohhot 010030, Inner Mongolia, China. Tel: +864716351235; Fax: +86-4716351235; E-mail: wangjianzhongWJ@163.com; Yuju Cao, Zhengzhou Traditional Chinese Medicine Traumatology Hospital, #1266 East Hanghai Road, Zhengzhou 450016, Henan, China. Tel: +86 13203882927; Fax: +86 13203882927; E-mail: caoyuju@126.com

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