

# Association between genetic polymorphisms of MMP8 and the risk of steroid-induced osteonecrosis of the femoral head in the population of northern China

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## Abstract

**Background:** Steroid-induced osteonecrosis of the femoral head (ONFH) is the most common clinical nontraumatic ONFH. Once ONFH occurs, it seriously reduces patients' quality of life. The matrix metalloproteinase/tissue inhibitor of metalloproteinase (MMP/TIMP) system was found to play a significant role in the development of ONFH. The aim of this study was to identify the associations between 7 genes selected from the MMP/TIMP system and steroid-induced ONFH.

**Methods:** We genotyped 34 single-nucleotide polymorphisms (SNPs) of 7 genes selected from the MMP/TIMP system in a case-control study with 285 cases of steroid-induced ONFH and 308 healthy controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using the chi-squared test, genetic model analysis, haplotype analysis, and stratification analysis.

**Results:** We found that the minor alleles of rs1940475 and rs11225395 in *MMP8* were associated with a 1.32-fold increased risk of steroid-induced ONFH in the allelic model analysis ( $P=0.021$  and  $0.022$ , respectively). In the genetic model analysis, we found that rs3740938, rs2012390, rs1940475, and rs11225395 were associated with an increased risk of steroid-induced ONFH. In further stratification analysis, rs3740938 and rs2012390 displayed a significantly increased risk of steroid-induced ONFH in females under the dominant (rs3740938, OR=2.69, 95% CI: 1.50–4.83,  $P=0.001$ ; rs2012390, OR=2.30, 95% CI: 1.31–4.03,  $P=0.012$ ) and additive (rs3740938, OR=2.02, 95% CI: 1.24–3.29,  $P=0.010$ ; rs2012390, OR=1.77, 95% CI: 1.12–2.80,  $P=0.047$ ) models. In addition, haplotype "AGTCA" of *MMP8* was found to be associated with a 1.40-fold increased risk of steroid-induced ONFH (95% CI: 1.04–1.88,  $P=0.025$ ).

**Conclusion:** Our results verify that genetic variants of *MMP8* contribute to steroid-induced ONFH susceptibility in the population of northern China. In addition, we found that gender differences might interact with *MMP8* polymorphisms to contribute to the overall susceptibility to steroid-induced ONFH.

**Abbreviations:** CI = confidence interval, HWE = Hardy-Weinberg equilibrium, MMPs = matrix metalloproteinases, OA = osteoarthritis, ONFH = osteonecrosis of the femoral head, OR = odds ratio, SNPs = single-nucleotide polymorphisms, TIMPs = tissue inhibitors of metalloproteinases.

**Keywords:** association study, *MMP8*, single-nucleotide polymorphism, steroid-induced osteonecrosis of the femoral head

## 1. Introduction

Osteonecrosis of the femoral head (ONFH) is a common disease encountered in clinical work. With the wide use of glucocorticoids for the treatment of rheumatic, autoimmune, and hematopoietic-system diseases, steroid-induced ONFH has

become the most common type of clinical nontraumatic ONFH. ONFH occurs in 39% of patients with severe acute respiratory syndrome within a few months of treatment with glucocorticoid therapy.<sup>[1]</sup> Once the ONFH occurs, it seriously reduces the patients' quality of life and is difficult to reverse. At present, there are several hypotheses about the causes of steroid-induced

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ONFH, including a hypercoagulable state,<sup>[2]</sup> intraosseous pressure changes,<sup>[3]</sup> fat embolism,<sup>[4]</sup> bone-cell apoptosis,<sup>[5,6]</sup> and circulatory impairment,<sup>[7]</sup> but the exact pathogenesis is still unknown. Recently, associated studies revealed genetic polymorphisms of enzymes contributing to individual differences in steroid metabolism, steroid receptors, and transport proteins in patients with steroid-induced ONFH.<sup>[8,9]</sup> All of these results suggest that genetic factors might play an important role in the development of steroid-induced ONFH.

Matrix metalloproteinases (MMPs), a family of zinc-dependent proteolytic enzymes, are expressed by stromal cells, infiltrating inflammatory cells, cancer cells, and osteoclasts. MMPs play central roles in morphogenesis, wound healing, tissue repair, and remodeling in response to injury.<sup>[10]</sup> Tissue inhibitors of metalloproteinases (TIMPs), a family of natural inhibitors of both MMPs and pro-MMPs, inhibit the activities of MMPs by creating crystal structures in TIMP–MMP complexes.<sup>[11,12]</sup> Previous studies<sup>[13–15]</sup> reported that genetic variations of the MMP/TIMP system could cause the abnormal activation of osteoclasts, which might result in further ONFH. MMP-1 and MMP-3 are the key enzymes of collagen formation on the bone surface, which can promote the reabsorption of bone, leading to bone repair.<sup>[16–18]</sup> MMP-2, -9, and -13 might act on osteoblast and osteoclast behavior and development in bone growth.<sup>[11,12]</sup> MMP-1, -2, -7, and -9 are involved in the development of metastatic prostate cancer in bone.<sup>[20]</sup> TIMP-1 and -2 can stimulate the bone-resorbing activity of osteoclasts by inhibiting the responsible MMPs, such as MMP-2 and -9, or the activation of the MAPK pathway.<sup>[21]</sup> MMP-8 was expressed intensely in the process of osteoblast development into osteocytes.<sup>[22]</sup>

Recently, numerous studies have reported associations between genes and steroid-induced ONFH. However, there is little insight into the association between gene polymorphisms in the MMP/TIMP system and steroid-induced ONFH. In the present study, we selected 34 single-nucleotide polymorphisms (SNPs) of 7 genes from the MMP/TIMP system that have been previously reported to be associated with the risk of steroid-induced ONFH or of diseases with pathogenesis similar to that of steroid-induced ONFH. Our study aims to determine the relationship between genetic polymorphisms of the TIMP/MMP system and the risk of steroid-induced ONFH as well as to explore the interaction between the TIMP/MMP system and gender factors for the risk of steroid-induced ONFH.

## 2. Materials and methods

### 2.1. Study participants

A total of 285 patients with steroid-induced ONFH were recruited between September 2014 and January 2016 at Zhengzhou Traditional Chinese Medicine Traumatology Hospital. ONFH was diagnosed based on evidence of osteonecrosis on anteroposterior and frog-view X-rays of both hips and/or magnetic resonance imaging.<sup>[23]</sup> Steroid-induced ONFH was defined by a history of a mean daily dose  $\geq 16.6$  mg or a high-dose steroid impulsion therapy for more than 1 week.<sup>[24–26]</sup> We used detailed exclusion criteria: patients who did not meet the diagnostic criteria of steroid-induced ONFH or had traumatic ONFH, dislocation of the hip joint, or other hip diseases; patients who drank more than 400 mL ethanol per week; patients who had significant familial hereditary disease; and patients who did not agree to participate in the study.

Simultaneously, a total of 308 healthy, unrelated controls were recruited based on a medical examination at the Zhengzhou

Traditional Chinese Medicine Traumatology Hospital. All of the chosen subjects were Han Chinese and lived in Zhengzhou city or the surrounding area. The controls did not have steroid-induced ONFH or other related diseases. Individuals with excessive use of corticosteroids, alcohol consumption, or significant familial hereditary disease were excluded.

Our study protocol adhered to the principles of the Declaration of Helsinki and was reviewed and approved by the Ethical Committee of Zhengzhou Traditional Chinese Medicine Traumatology Hospital. Informed consent was obtained from all candidate subjects.

### 2.2. SNPs selection and genotyping

All 34 SNPs had minor allele frequencies  $>5\%$  in the HapMap Chinese Han Beijing population and were previously shown to be associated with the risk of steroid-induced ONFH or of diseases with pathogenesis similar to that of steroid-induced ONFH. Blood samples were collected in EDTA tubes and stored at  $-80^{\circ}\text{C}$  after centrifugation at 2000 rpm for 10 minutes. The GoldMag extraction method (GoldMag Co. Ltd, Xi'an, China) was used to extract genomic DNA from whole blood. The DNA quantity was evaluated by spectrometry (DU530UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA). The Sequenom MassARRAY Assay Design 3.0 Software was used to design a Multiplexed SNP MassEXTEND assay (Sequenom, Inc., San Diego, CA). A Sequenom MassARRAY RS1000 was used to perform the SNP genotyping according to the manufacturer's protocol.

### 2.3. Statistical analyses

All of the statistical analyses were performed with the SPSS 19.0 software for Windows (SPSS, Chicago, IL). Allele and genotype frequencies were obtained by direct counts. The genotype frequencies of each SNP among the control subjects were checked for Hardy–Weinberg equilibrium (HWE) before analysis. In the present study, all  $P$  values were 2-sided, and  $P \leq 0.05$  was considered to have statistical significance. The allele and genotype frequencies among the cases and controls were calculated by chi-squared test/Fisher exact test. Associations between the genotype polymorphisms and the risk of steroid-induced ONFH were evaluated under different genetic models (codominant, dominant, recessive, overdominant, and log-additive). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis with an adjustment for gender and age. Then, we further analyzed the association between genotype and the risk of steroid-induced ONFH using each of the genetic models stratified by sex, and we tested the  $P$  values using Wald test. Finally, the linkage disequilibrium and haplotype construction were assessed using the Haploview software package (version 4.2) which developed and maintained by Dr. Mark Daly's lab at the MIT/Harvard Broad Institute, Massachusetts, United States and the SHEsis software platform (<http://www.nhgg.org/analysis/>).

## 3. Results

### 3.1. Study population

The distributions of sex and age among the cases and controls are shown in Table 1. A total of 285 cases (112 females and 173 males) and 308 controls (111 females and 197 males) were recruited for our study. The mean age of the patients was  $41.88 \pm$

**Table 1**  
**Characteristics of cases and controls in this study.**

Variable (s)	Case (n=285)	Control (n=308)	P
Sex (N, %)			0.413*
Male	173 (60.7)	197 (64.0)	
Female	112 (39.3)	111 (36.0)	
Age, years (mean ± SD)	41.88 ± 12.79	49.47 ± 7.97	<0.001†

P ≤ 0.05 indicates statistical significance.

\* Two-sided chi-squared test.

† Independent samples t test.

12.79 years and that of the controls was 49.47 ± 7.97 years. The mean age between patients and controls was not matched in present study, and we would adjust the factor in the followed analysis. The groups were well matched by sex (P=0.413).

**3.2. Hardy–Weinberg equilibrium and SNP alleles**

The basic information about all the SNPs including gene, band, position, alleles, and HWE results are presented in Table 2. All of

the 34 tag SNPs were in HWE among the control subjects (P > 0.05). Two SNPs were found to be significantly associated with an increased risk of steroid-induced ONFH (rs1940475T/C, OR=1.32, 95% CI: 1.04–1.67; rs11225395A/G, OR=1.32, 95% CI: 1.04–1.67).

**3.3. Association between MMP8 and the risk of steroid-induced ONFH**

Genetic models (codominant, dominant, recessive, overdominant, and additive) and the genotype frequencies were used to further identify the associations between the SNPs and the risk of steroid-induced ONFH (Table 3). The results showed that rs1940475 significantly increased the risk of steroid-induced ONFH under the recessive model (OR=1.67, 95% CI: 1.01–2.76, P=0.042) and the additive model (OR=1.36, 95% CI: 1.06–1.76, P=0.016) and that rs11225395 exhibited an increased risk under both the recessive model (OR=1.70, 95% CI: 1.03–2.81, P=0.037) and the additive model (OR=1.34, 95% CI: 1.04–1.73, P=0.023). Meanwhile, rs3740938 showed a negative effect on steroid-induced ONFH under the

**Table 2**  
**Allele frequencies in cases and controls and OR estimates for steroid-induced ONFH.**

SNP_ID	Gene	Position	Alleles A/B	MAF		P value for WHE test*	ORs	95% CI		P‡
				Case	Control					
rs14983	MMP7	11q22.2	A/G	0.251	0.261	0.883	0.95	0.73	1.23	0.679
rs17352054	MMP7	11q22.2	C/A	0.112	0.127	1.000	0.87	0.61	1.24	0.447
rs10502001	MMP7	11q22.2	T/C	0.254	0.262	0.883	0.96	0.74	1.25	0.758
rs11568818	MMP7	11q22.2	C/T	0.088	0.078	0.704	1.14	0.75	1.72	0.540
rs17098318	MMP7	11q22.2	A/G	0.086	0.076	0.692	1.14	0.75	1.73	0.542
rs3740938	MMP8	11q22.2	A/G	0.260	0.213	1.000	1.29	0.99	1.69	0.061
rs2012390	MMP8	11q22.2	G/A	0.295	0.245	0.878	1.29	1.00	1.66	0.054
rs1940475	MMP8	11q22.2	T/C	0.412	0.347	0.618	1.32	1.04	1.67	0.021‡
rs11225394	MMP8	11q22.2	T/C	0.116	0.091	0.147	1.31	0.90	1.92	0.162
rs11225395	MMP8	11q22.2	A/G	0.407	0.343	0.704	1.32	1.04	1.67	0.022‡
rs5854	MMP1	11q22.2	A/G	0.088	0.088	0.490	1.00	0.67	1.50	0.997
rs2071230	MMP1	11q22.2	G/A	0.181	0.193	0.272	0.92	0.69	1.23	0.582
rs2239008	MMP1	11q22.2	G/A	0.505	0.477	0.568	1.12	0.89	1.40	0.335
rs470215	MMP1	11q22.2	C/T	0.088	0.088	0.490	1.00	0.67	1.50	0.997
rs2071232	MMP1	11q22.2	T/C	0.496	0.464	0.732	1.14	0.91	1.43	0.267
rs639752	MMP3	11q22.2	C/A	0.325	0.341	0.376	0.93	0.73	1.18	0.551
rs650108	MMP3	11q22.2	G/A	0.391	0.423	0.079	0.87	0.69	1.10	0.260
rs520540	MMP3	11q22.2	A/G	0.325	0.341	0.376	0.93	0.73	1.18	0.551
rs646910	MMP3	11q22.2	A/T	0.067	0.081	1.000	0.81	0.52	1.25	0.341
rs602128	MMP3	11q22.2	A/G	0.326	0.339	0.523	0.94	0.74	1.20	0.635
rs679620	MMP3	11q22.2	T/C	0.324	0.343	0.527	0.92	0.72	1.17	0.498
rs678815	MMP3	11q22.2	G/C	0.325	0.341	0.376	0.93	0.73	1.18	0.551
rs522616	MMP3	11q22.2	C/T	0.398	0.365	0.902	1.15	0.91	1.46	0.237
rs2277698	TIMP2	17q25.3	T/C	0.198	0.219	0.503	0.88	0.67	1.17	0.382
rs2009196	TIMP2	17q25.3	C/G	0.391	0.389	0.720	1.01	0.80	1.27	0.944
rs7342880	TIMP2	17q25.3	A/C	0.165	0.130	1.000	1.32	0.96	1.83	0.088
rs11654470	TIMP2	17q25.3	C/T	0.226	0.260	0.461	0.83	0.64	1.09	0.180
rs2003241	TIMP2	17q25.3	C/T	0.165	0.157	0.287	1.06	0.78	1.44	0.727
rs4789936	TIMP2	17q25.3	T/C	0.254	0.305	1.000	0.77	0.60	1.01	0.056
rs9619311	SYN3	22q12.3	C/T	0.088	0.075	1.000	1.19	0.78	1.81	0.410
rs715572	TIMP3	22q12.3	A/G	0.353	0.347	0.618	1.02	0.81	1.30	0.850
rs8136803	TIMP3	22q12.3	T/G	0.039	0.045	1.000	0.84	0.48	1.49	0.557
rs9609643	TIMP3	22q12.3	A/G	0.139	0.144	0.648	0.95	0.69	1.32	0.772
rs11547635	TIMP3	22q12.3	T/C	0.346	0.341	0.900	1.02	0.80	1.30	0.865

95% CI = 95% confidence interval, HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, OR = odds ratio, SNP = single-nucleotide polymorphism.

\* P was calculated by exact test.

† P was calculated by Pearson chi-squared test.

‡ P ≤ 0.05 indicates statistical significance.

**Table 3**

**Genotypic model analysis of the relationship between SNPs and the risk of steroid-induced ONFH.**

SNP_ID	Model	Genotype	Group = control	Group = hormone	OR (95% CI)	P	AIC	BIC
rs3740938	Codominant	G/G	190 (61.9%)	159 (55.8%)	1	0.110	753.6	775.5
		G/A	103 (33.5%)	104 (36.5%)	1.38 (0.95–1.99)			
		A/A	14 (4.6%)	22 (7.7%)	1.81 (0.85–3.85)			
	Dominant	G/G	190 (61.9%)	159 (55.8%)	1	0.045*	752.1	769.6
		G/A-A/A	117 (38.1%)	126 (44.2%)	1.43 (1.01–2.04)			
	Recessive	G/G-G/A	293 (95.4%)	263 (92.3%)	1	0.210	754.5	772
		A/A	14 (4.6%)	22 (7.7%)	1.61 (0.77–3.36)			
	Overdominant	G/G-A/A	204 (66.5%)	181 (63.5%)	1	0.150	754	771.6
		G/A	103 (33.5%)	104 (36.5%)	1.30 (0.91–1.88)			
	Log-additive	–	–	–	–	1.36 (1.02–1.82)	0.034*	751.6
–		–	–	–	–	0.046*	752.7	774.7
rs2012390	Codominant	A/A	176 (57.1%)	141 (49.5%)	1	0.013*	750.7	768.3
		A/G	113 (36.7%)	120 (42.1%)	1.56 (1.08–2.26)			
		G/G	19 (6.2%)	24 (8.4%)	1.51 (0.75–3.01)			
	Dominant	A/A	176 (57.1%)	141 (49.5%)	1	0.013*	750.7	768.3
		A/G-G/G	132 (42.9%)	144 (50.5%)	1.55 (1.10–2.21)			
	Recessive	A/A-A/G	289 (93.8%)	261 (91.6%)	1	0.520	756.5	774
		G/G	19 (6.2%)	24 (8.4%)	1.24 (0.63–2.44)			
	Overdominant	A/A-G/G	195 (63.3%)	165 (57.9%)	1	0.028*	752.1	769.6
		A/G	113 (36.7%)	120 (42.1%)	1.49 (1.04–2.13)			
	Log-additive	–	–	–	–	1.37 (1.04–1.82)	0.025*	751.9
–		–	–	–	–	0.052	753	774.9
rs1940475	Codominant	C/C	129 (41.9%)	101 (35.4%)	1	0.056	753.3	770.8
		C/T	144 (46.8%)	133 (46.7%)	1.29 (0.89–1.88)			
		T/T	35 (11.4%)	51 (17.9%)	1.93 (1.12–3.32)			
	Dominant	C/C	129 (41.9%)	101 (35.4%)	1	0.056	753.3	770.8
		C/T-T/T	179 (58.1%)	184 (64.6%)	1.41 (0.99–2.01)			
	Recessive	C/C-C/T	273 (88.6%)	234 (82.1%)	1	0.042*	752.8	770.3
		T/T	35 (11.4%)	51 (17.9%)	1.67 (1.01–2.76)			
	Overdominant	C/C-T/T	164 (53.2%)	152 (53.3%)	1	0.650	756.7	774.2
		C/T	144 (46.8%)	133 (46.7%)	1.08 (0.77–1.53)			
	Log-additive	–	–	–	–	1.36 (1.06–1.76)	0.016*	751.2
–		–	–	–	–	0.063	753.4	775.3
rs11225395	Codominant	G/G	131 (42.5%)	104 (36.5%)	1	0.090	754	771.6
		A/G	143 (46.4%)	130 (45.6%)	1.23 (0.85–1.78)			
		A/A	34 (11%)	51 (17.9%)	1.91 (1.11–3.28)			
	Dominant	G/G	131 (42.5%)	104 (36.5%)	1	0.090	754	771.6
		A/G-A/A	177 (57.5%)	181 (63.5%)	1.36 (0.95–1.93)			
	Recessive	G/G-A/G	274 (89%)	234 (82.1%)	1	0.037*	752.5	770.1
		A/A	34 (11%)	51 (17.9%)	1.70 (1.03–2.81)			
	Overdominant	G/G-A/A	165 (53.6%)	155 (54.4%)	1	0.830	756.9	774.4
		A/G	143 (46.4%)	130 (45.6%)	1.04 (0.74–1.46)			
	Log-additive	–	–	–	–	1.34 (1.04–1.73)	0.023*	751.8

P values were calculated by Wald test by unconditional logistic regression adjusted for age and gender.

\* P ≤ 0.05 indicates statistical significance.

dominant model (OR = 1.43, 95% CI: 1.01–2.04, P = 0.045) and the additive model (OR = 1.36, 95% CI: 1.02–1.82, P = 0.034). In addition, rs2012390 was found to be associated with an increased risk of steroid-induced ONFH under all of the genetic models including the codominant (OR = 1.56, 95% CI: 1.08–2.26, P = 0.046), dominant (OR = 1.55, 95% CI: 1.10–2.21, P = 0.013), overdominant (OR = 1.49, 95% CI: 1.04–2.13, P = 0.028), and additive (OR = 1.37, 95% CI: 1.04–1.82, P = 0.025) models. Collectively, these results, adjusted by age and gender, showed that 4 SNPs of *MMP8* (rs3740938, rs2012390, rs1940475, and rs11225395) had a negative effect on steroid-induced ONFH.

**3.4. Stratified analysis of *MMP8* polymorphisms by sex and the risk of steroid-induced ONFH**

The results of the stratified analysis of the effects of rs3740938 and rs2012390 on steroid-induced ONFH are shown in Table 4. In the stratified analysis, there was no association between

rs3740938 or rs2012390 and the risk of steroid-induced ONFH among males, whereas the associations between those SNPs and the risk of steroid-induced ONFH among females under the dominant model (rs3740938, OR = 2.69, 95% CI: 1.50–4.83, P = 0.001; rs2012390, OR = 2.30, 95% CI: 1.31–4.03, P = 0.012) and the additive model (rs3740938, OR = 2.02, 95% CI: 1.24–3.29, P = 0.010; rs2012390, OR = 1.77, 95% CI: 1.12–2.80, P = 0.047) were even stronger than those in the nonstratified analysis.

**3.5. Association of *MMP8* haplotypes with the risk of steroid-induced ONFH**

Finally, the linkage disequilibrium and haplotype construction were detected and evaluated. One block of *MMP8* SNPs (Fig. 1) comprising rs3740938, rs2012390, rs1940475, rs11225394, and rs11225395 was found by haplotype analysis. Compared with the “GACCG” wild-type, the “AGTCA” haplotype was found to be associated with an increased risk

**Table 4**  
**Stratified analysis of MMP8 polymorphisms by sex and risk of steroid-induced ONFH.**

Model	Genotype	Male				Female				
		Case	Control	OR 95% CI	P*	Case	Control	OR 95% CI	P*	P†
Dominant	rs3740938									
	G/G	97 (56.1%)	106 (54.1%)	1	0.710	62 (55.4%)	84 (75.7%)	1	0.001‡	0.001‡
	G/A-A/A	76 (43.9%)	90 (45.9%)	0.92 (0.57–1.46)		50 (44.6%)	27 (24.3%)	2.69 (1.50–4.83)		
Recessive	G/G-G/A	157 (90.8%)	187 (95.4%)	1	0.270	106 (94.6%)	106 (95.5%)	1	0.740	0.722
	A/A	16 (9.2%)	9 (4.6%)	1.72 (0.65–4.57)		6 (5.4%)	5 (4.5%)	1.23 (0.36–4.18)		
Log-additive	–	–	–	1.03 (0.70–1.50)	0.890	–	–	2.02 (1.24–3.29)	0.004‡	0.010‡
Dominant	rs2012390									
	A/A	83 (48%)	99 (50.2%)	1	0.640	58 (51.8%)	77 (69.4%)	1	0.003‡	0.012‡
	A/G-G/G	90 (52%)	98 (49.8%)	1.12 (0.70–1.78)		54 (48.2%)	34 (30.6%)	2.30 (1.31–4.03)		
Recessive	A/A-A/G	156 (90.2%)	184 (93.4%)	1	0.660	105 (93.8%)	105 (94.6%)	1	0.810	0.875
	G/G	17 (9.8%)	13 (6.6%)	1.22 (0.50–2.95)		7 (6.2%)	6 (5.4%)	1.15 (0.37–3.57)		
Log-additive	–	–	–	1.11 (0.77–1.61)	0.580	–	–	1.77 (1.12–2.80)	0.013‡	0.047‡

\* P values were calculated from 2-sided chi-squared test adjusted for age.

† P values were calculated from Wald test.

‡ P < 0.05 indicates statistical significance.

of steroid-induced ONFH (OR = 1.40, 95% CI: 1.04–1.88, P = 0.025; Table 5).

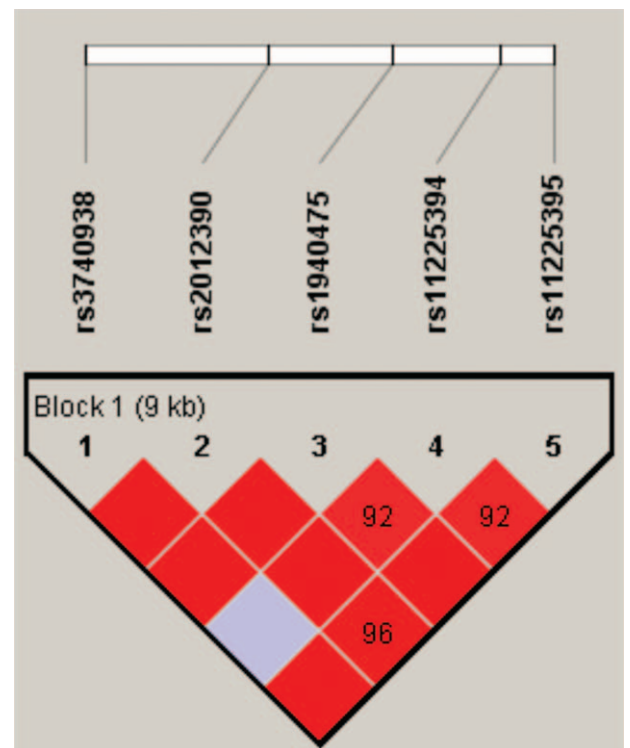
#### 4. Discussion

In the present case–control study, we investigated the associations between 34 SNPs of the MMP/TIMP system and the risk of steroid-induced ONFH. We demonstrated that *MMP8* genetic polymorphisms (rs3740938, rs2012390, rs1940475, and rs11225395) are associated with an increased risk of steroid-induced ONFH in the population of northern China. We found that gender differences might interact with *MMP8* polymorphisms to affect the development of steroid-induced ONFH. We also found that the “AGTCA” haplotype of *MMP8* was associated with a 1.40-fold increased risk of steroid-induced ONFH.

MMP-8, also known as collagenase-2 or neutrophil collagenase, can be expressed by a wide variety of cells such as maturing neutrophils, peripheral neutrophils, endothelial cells, and chondrocytes. MMP-8 plays an important role in a wide range of inflammatory disorders.<sup>[27]</sup> MMP-8 initiates the first step of collagen degradation by disrupting triple helical fibrillar collagen.<sup>[28]</sup> Billingham et al<sup>[29]</sup> suggested that increased expression of MMP-8 would have an effect on the cleavage of type II collagen in human cartilage of osteoarthritis (OA). MMP-8 also possesses proteolytic activity on several matrix proteins, particularly some nonmatrix proteins such as angiotensin I. Fang et al<sup>[30]</sup> reported that MMP-8 can cleave angiotensin I and convert it to angiotensin II, which in turn induces the endothelial cell expression of platelet or endothelial cell adhesion molecule-1 to promote angiogenesis. Hence, we suppose that polymorphisms of MMP-8 might have an effect on the inflammatory response or the circulatory impairment of the femoral head, leading further to steroid-induced ONFH.

Of the 4 SNPs in *MMP8* that were associated with increased risk of steroid-induced ONFH, rs3740938 and rs1940475 were located in exon coding regions; rs2012390 was located in an intron; and rs11225395 was located in a promoter region. It is possible that rs1940475 has an effect on proMMP-8 activation by a glutamate-to-lysine substitution at amino acid residue 87 located in the propeptide of MMP-8.<sup>[31]</sup> The rs11225395 polymorphism has been shown by functional analysis to have

an effect on MMP-8 promoter activity.<sup>[31]</sup> The rs1940475 and rs11225395 polymorphisms have been reported to have an association with atherosclerosis. Ye<sup>[32]</sup> conjectured that those SNPs might represent the same genetic signal, which arises from both the influence of rs11225395, located in the promoter region (position—799), on MMP-8 expression and the effect of rs1940475, located in the coding region (Glu87Lys), on MMP-8 zymogen activation, because the 2 SNPs were in strong linkage disequilibrium in Chinese and Japanese populations. In our study, rs1940475 and rs11225395 were found to have a



**Figure 1.** Haplotype block map for SNPs of the *MMP-8* gene. Linkage disequilibrium plots containing 5 SNPs from *MMP-8*. Red squares display statistically significant associations between a pair of SNPs, as measured by *D'*; darker shades of red indicate higher *D'*.

**Table 5****The haplotype frequencies of MMP8 polymorphisms and their association with the risk of steroid-induced ONFH.**

Haplotype	rs3740938	rs2012390	rs1940475	rs11225394	rs11225395	Freq	Without adjustment		With adjustment	
							OR (95% CI)	P*	OR (95% CI)	P†
1	G	A	C	C	G	0.6189	1	–	1	–
2	A	G	T	C	A	0.236	1.32 (1.01–1.73)	0.046	1.40 (1.04–1.88)	0.025‡
3	G	A	T	T	A	0.1096	1.26 (0.86–1.86)	0.240	1.22 (0.81–1.85)	0.340
4	G	G	T	C	A	0.0279	1.29 (0.65–2.59)	0.470	1.24 (0.59–2.60)	0.570
Rare	‡	‡	‡	‡	‡	0.0076	0.60 (0.15–2.46)	0.480	0.95 (0.21–4.36)	0.950

\* P values were calculated by unconditional logistic regression.

† P values were calculated by unconditional logistic regression adjusted for age and gender.

‡ P &lt; 0.05 indicates statistical significance.

significantly increased association with the risk of steroid-induced ONFH in both the allele model and the genetic models. We suppose that the rs1940475 and rs11225395 polymorphisms of *MMP8* might have a negative effect on the circulatory impairment of the femoral head as previous studies suggested that rs1940475 and rs11225395 have a functional role in *MMP8* expression.<sup>[33–35]</sup>

Previously, a GWAS study conducted in Spain investigated the associations between rs3740938, rs2012390, and rs1940475 and OA, and rs1940475 showed a suggestive association with knee OA.<sup>[36]</sup> In our study, rs3740938 and rs2012390 were associated with a significantly increased risk of steroid-induced ONFH under the genetic models. In addition, further stratification analysis showed that the associations between rs3740938 and rs2012390 and the risk of steroid-induced ONFH were significantly stronger in females than in males, which provides the first evidence of interactive effects between rs3740938 and rs2012390 and gender on the risk of steroid-induced ONFH.

To our knowledge, our study provides for the first time evidence of associations between the 4 SNPs (rs3740938, rs2012390, rs1940475, and rs11225395) of *MMP8* from the *MMP/TIMP* system and the risk of steroid-induced ONFH. Although there are important discoveries revealed in our study, our study has some limitations. First, our study does not include an analysis of biological functions, which will be crucial for elucidating the role of *MMP8* in steroid-induced ONFH. Second, steroid-induced ONFH can be classified into different clinical stages for further analysis. Third, the participants in our study were all Han Chinese individuals recruited from the Zhengzhou Traditional Chinese Medicine Traumatology Hospital, which might involve a selection bias. Fourth, we used a hospital-based case–control design, which may involve selection bias. Lastly, the sample size was relatively small after stratification by sex, which might convert the positive findings into negative results. A larger case–control study is expected to circumvent those problems, which could make our conclusions more powerful.

## 5. Conclusion

To sum up, we have confirmed for the first time that 4 susceptible SNPs (rs3740938, rs2012390, rs1940475, and rs11225395) of *MMP8* from the *MMP/TIMP* system exhibit a significant association with increased risk of steroid-induced ONFH in the population of northern China. Further functional studies and larger population-based studies are needed to confirm our results.

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