Association between Genetic Polymorphisms of *MIR3142HG* and the Risk of Steroid-Induced Osteonecrosis of the Femoral Head in the Population of Northern China

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Keywords

Steroid-induced osteonecrosis of the femoral head · *MIR3142HG* · Genetic polymorphisms · Case-control study

Abstract

Background: Steroid-induced osteonecrosis of the femoral head (ONFH) is aseptic necrosis of the femoral head caused by glucocorticoid use. Once necrotic femoral head necrosis occurs, it irreversibly affects the quality of life seriously. Studies have shown that the susceptibility to steroid-induced ONFH is likely to be related to the variation of miRNA-coding genes. Therefore, this study aimed to investigate the effect of *MIR3142HG* on steroid-induced ONFH. **Methods:** Agena MassARRAY was used to genotype *MIR3142HG* gene rs1582417, rs2431689, rs7727155, and rs17057846 in 199 patients and 725 healthy people. A genetic model and haplotype analysis were used to evaluate the relationship between the *MIR3142HG* polymorphism and the risk of steroid-induced ONFH. The odds ratio and 95% confidence intervals were obtained through logistic regression to assess the influence of gene polymorphisms on the occurrence of ste-

roid-induced ONFH. **Results:** The consequences show that rs7727115 is a protective factor, it could reduce the risk of steroid-induced ONFH, and rs1582417 could increase the risk of steroid-induced ONFH. In the genetic model, rs1582417 was associated with increased risk of alcohol-induced ONFH in dominant model and log-additive model. rs7727115 showed a decreased risk in codominant model, dominant model, and log-additive model. In addition, rs2431689 is related to HDL-C ($p = 0.012$) and ApoA1 ($p = 0.010$) levels, and rs17057846 ($p = 0.024$) is related to ApoB levels. The linkage analysis indicated 3 single-nucleotide polymorphisms (rs2431689, rs7727115, and rs17057846) in *MIR3142HG* with significant chain imbalance. In addition, haplotype “GGG” of *MIR3142HG* was found out and is harmful for steroid-induced ONFH. **Conclusion:** Our results first confirm that the genetic polymorphism of *MIR3142HG* is associated with steroid-induced ONFH susceptibility in Chinese Han population.

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Introduction

With the widespread, clinical use of hormone drugs, the reports of steroid-induced osteonecrosis of the femoral head (ONFH) at home and abroad are gradually increasing. Steroid-induced ONFH is a serious concomitant hormone widely recognized in clinical practice disease, high disability rate, and seriously affects the physical and mental health of patients. In China, long-term or high-dose corticosteroids are the most common cause of nontraumatic femoral head necrosis [1]. The pathogenesis is currently unclear, and studies have shown that the genome changes in epigenetics are closely related to its occurrence and development, including genetic polymorphism and methylation changes.

Neuroimmune diseases and rheumatic diseases often need to be treated with glucocorticoids. Glucocorticoids can cause adverse reactions such as edema, high sodium and low potassium, high blood sugar, centripetal obesity, and infection. Steroid-induced ONFH is the most common one of the severe adverse reactions. Abnormal glucocorticoid metabolism, transporter receptor and target, lipid metabolism disorder, coagulation and fibrinolysis dysfunction, inflammatory response, and osteoporosis are the important pathogenesis of steroid-induced ONFH. Glucocorticoids act on osteoblasts, osteoclasts, and osteocytes, reducing fluid in the interstitial tubules of bone, blood supply and strength of bone, and causing damage [2]. However, not all glucocorticoids are used for treatment. All patients had femoral head necrosis, suggesting that there may be other risk factors or individual differences in the occurrence of steroid-induced ONFH. Current research has shown that the pathogenesis and genes of the disease single-nucleotide polymorphisms (SNPs) are related to gene mutations. Steroid-induced ONFH may be a polygenic disease, such as lipid metabolism-related genes (ApoA and ApoB), drug metabolizing enzymes (CYP), drug transporters (*ABCB1* and *ABCG2*), and drug target molecules (NR3C1). [3–5] For example, MMP-8, MMP-9, MMP-14, and TIMP4 [6–9] have been confirmed to be associated with steroid-induced ONFH risk.

MiRNAs are regulatory short-stranded non-coding RNAs found in eukaryotes [9]. MiRNAs have been shown to participate in many biological and pathological processes, as well as tissue development [10]. The primary targeted pathways of miRNA with differential expression are mostly related to osteogenic differentiation, bone metabolism, and vascular pathways. SNPs in miRNAs-encoding genes may alter the expression or function of their

host miRNAs and are thus associated with cancer-related risks [11, 12]. *MIR3142HG*, also known as the *MIR146A* host gene, is reported that *MIR3142HG* can produce miR-146a [13]. MiR-146a is located on chromosome 5Q34 and inhibits tumor necrosis factor receptor-associated factor 6 and interleukin-1 receptor-associated kinase 1 by targeting. It can reduce the production of tumor necrosis factor- α , interleukin-1 β , interleukin-6, and other cytokines in the nuclear protein factor κ B pathway, achieve the negative feedback regulation of innate immunity, and participate in inflammation, immune response regulation, cell proliferation and apoptosis, and other pathophysiological processes. Therefore, miR-146a is closely related to the pathological process of tissue necrosis.

To our knowledge, the contact between *MIR3142HG* polymorphism and the risk of steroid-induced ONFH has not been studied previously. Therefore, we designed a study to evaluate the *MIR3142HG* gene polymorphism at the allele, genotype, and haplotype interface for the development of the steroid-induced ONFH in the population of Northern China.

Materials and Methods

Study Participants

From 2018 to 2020, a total of 924 subjects participated in this study including 199 consecutive recruited steroid-induced ONFH and 725 healthy controls at the Second Affiliated Hospital of Inner Mongolia Medical University. The control is based on the physical examination enrollment group, without steroid-induced ONFH or other related diseases. This study was approved by the Hospital Ethics Committee. Informed consent of all participants was collected.

All patients were diagnosed with hormonal femoral head necrosis in accordance with the relevant standards established by the International Bone Circulation Research Association. Inclusion criteria for the case group were long-term use of steroids, more than 16 mg per day, or high-dose steroid impulsive treatment appears after more than 1 week. The patient presented with relevant clinical manifestations, such as hip pain, lower limb muscle atrophy, claudication, etc. [6]. All outcome measures were confirmed as femur head necrosis by MRI X-ray bone scan or MRI. None of the patients had a history of alcohol consumption, chronic metabolic history, tumor history, hip trauma history, or infection history. The patient had complete clinical data and no history of other treatments. Our exclusion criteria are patients who did not strictly meet the diagnostic criteria for hormonal cerebral hemorrhage and the diagnostic criteria for traumatic cerebral hemorrhage compared to other hip diseases and those with weekly alcohol intake of more than 400 mL patients. The healthy control group is defined according to the following criteria: no symptoms of hip-joint disease and no history of thromboembolism. People with severe chronic diseases and other significant family genetic diseases will be excluded.

Table 1. Characteristics of the participants

Variables	Cases (n = 199)	Controls (n = 725)	p value
Sex, N			
Male	116	424	0.961 ^a
Female	83	301	
Age, years (mean ± SD)	42.21±12.890	42.15±11.854	0.332 ^b
Clinical stages			
Stage I and II	46		
Stage III and IV	153		
Hip lesions			
Unilateral	142		
Bilateral	55		
Course of disease, month			
More than 25 months	67		
25 months or less	132		

SD, standard deviation. $p < 0.05$ indicates statistical significance. ^a p value was calculated by χ^2 test. ^b p value was calculated by independent-samples t test.

Genotyping Assay

In our study, blood samples of steroid-induced ONFH patients and normal control group were collected to analyze the difference of *miR3142HG* SNP in the blood of steroid-induced ONFH patients and normal control group and its correlation with clinical data, so as to study the application of *miR3142HG* in the diagnosis of steroid-induced ONFH. rs1582417, rs2431689, rs7727115, and rs17057846 in the *MIR3142HG* gene were further selected. Blood samples were taken in EDTA tubes and centrifuged at 2,000 rpm for 10 min. Blood samples are stored at -80°C for future experiments. Genomic DNA was extracted from whole blood of steroid-induced ONFH patients and healthy controls, using the GoldMag-Mini Purification Kit (GoldMag Co. Ltd., Xian City, China). In this experiment, MassARRAY flight mass spectrometer was mainly used for genotyping detection, including PCR amplification, SAP purification, single base extension, resin purification, and chip mass spectrometry. The genotyping of 4 SNPs (rs1582417, rs2431689, rs7727115, and rs17057846) was put into practice on the Agena MassARRAY platform (San Diego, CA, USA). And data management and analysis were performed using the Sequenom TYPER software.

Statistical Analyses

Results were presented as the mean $\pm$ standard deviation. All data were statistically analyzed using SPSS19.0. An exact test was used to assess the variation in each SNP genotype frequency from the Hardy-Weinberg equilibrium in the control participants before analysis. The difference in age between the 2 groups was analyzed by T test or T' test, and the functional analysis of SNP on SONFH susceptibility was performed by logistic regression analysis. We used the χ^2 test/Fisher's exact tests to compare the allelic and genotypic frequencies of cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using logistic regression analysis with adjustments for age and gender. Associations between the genotype polymorphisms and the risk of steroid-induced ONFH were evaluated under different genetic models using PLINK version 1.07 software. Finally, linkage disequilibrium among polymorphic sites was assessed with Haploview software package (version 4.2). p values < 0.05 were considered statistically significant.

Results

A total of 924 people participated in this study of 199 patients (83 female, 116 male) and 725 healthy controls (301 female, 424 male). The mean ages were 42.21 ± 12.890 years for the cases and 41.15 ± 11.854 years for the controls. The information of the steroid-induced ONFH patients and healthy participants were shown in Table 1. There was no gender match between the patients and healthy participants in this study, and we will adjust this factor in the subsequent analysis.

Four SNPs (rs1582417, rs2431689, rs7727115, and rs17057846) of *MIR3142HG* gene were selected for this case-control study. The basic information about all the SNPs, including alleles, minor allele frequency, OR, 95% CI, and Hardy-Weinberg equilibrium results are presented in Table 2. It was found that rs1582417 (OR = 1.29, 95% CI = 1.03–1.61, $p = 0.028$) was related to an increased risk of steroid-induced ONFH. On the other hand, rs7727115 (OR = 0.74, 95% CI = 0.57–0.95, $p = 0.017$) was related to a reduced risk of steroid-induced ONFH.

Subsequently, genetic models were used to compare the relationship between SNP genotypes and steroid-induced ONFH risk. As illustrated in Table 3, rs1582417 in the *MIR3142HG* gene showed an increased risk of steroid-induced ONFH in the dominant model (OR = 1.46, 95% CI: 1.04–2.04, $p = 0.027$). At the same time, rs1582417 showed a negative effect on steroid-induced ONFH under the log-additive model (OR = 1.29, 95% CI: 1.03–1.62, $p = 0.027$). Moreover, rs7727115 was found to be associated with a reduced risk of steroid-induced ONFH under the log-additive model (OR = 0.73, 95% CI: 0.57–0.94,

Table 2. Basic information of candidate SNPs of *MIR3142HG* gene in this study

SNP ID	Gene	Band	Alleles A/B	MAF		HWE- <i>p</i> value ^a	OR (95% CI)	<i>p</i> value ^b
				case	control			
rs1582417	<i>MIR3142HG</i>	5	A/G	0.427	0.367	0.810	1.29 (1.03–1.61)	0.028*
rs2431689	<i>MIR3142HG</i>	5	A/G	0.126	0.149	0.555	0.83 (0.60–1.15)	0.262
rs7727115	<i>MIR3142</i>	5	G/T	0.260	0.323	0.865	0.74 (0.57–0.95)	0.017*
rs17057846	<i>MIR3142HG</i>	5	A/G	0.214	0.206	0.572	1.04 (0.80–1.37)	0.758

SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; 95% CI, 95% confidence interval; MAF, minor allele frequency. *p* values were calculated by χ^2 test. * *p* < 0.05 indicates statistical significance.

Table 3. Genotypic model analysis of relationship between SNPs and steroid-induced ONFH

SNP ID	Model	Genotype	Control	Case	Without adjustment		With adjustment	
					OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
rs1582417	Codominant	GG	292	63	1		1	
		GA	333	102	1.42 (1.00–2.02)	0.050	1.42 (0.99–2.01)	0.053
		AA	99	34	1.60 (0.99–2.56)	0.055	1.60 (0.99–2.56)	0.053
	Dominant	GG	292	63	1		1	
		GA-AA	432	136	1.46 (1.05–2.04)	0.026*	1.46 (1.04–2.04)	0.027*
	Recessive	AA-GA	625	165	1		1	
		GG	99	34	1.30 (0.85–1.99)	0.226	1.31 (0.86–2.00)	0.214
	Log-additive	–	–	–	1.29 (1.03–1.62)	0.028*	1.29 (1.03–1.62)	0.027*
rs7727115	Codominant	TT	331	107	1		1	
		TG	320	79	0.76 (0.55–1.06)	0.108	0.76 (0.55–1.06)	0.103
		GG	74	12	0.50 (0.26–0.96)	0.037*	0.50 (0.26–0.96)	0.037*
	Dominant	TT	331	107	1		1	
		TG-GG	394	91	0.71 (0.52–0.98)	0.037*	0.71 (0.52–0.98)	0.035*
	Recessive	TT-TG	651	186	1		1	
		GG	74	12	0.57 (0.30–1.07)	0.079	0.57 (0.30–1.07)	0.079
	Log-additive	–	–	–	0.73 (0.57–0.95)	0.017*	0.73 (0.57–0.94)	0.016*

SNP, single-nucleotide polymorphism; ONFH, osteonecrosis of the femoral head; OR, odds ratio; 95% CI, 95% confidence interval. *p* value adjusted for age was calculated by logistic regression. * *p* < 0.05 indicates statistical significance.

p = 0.016), codominant model (OR = 0.50, 95% CI: 0.26–0.96, *p* = 0.037), and dominant model (OR = 0.71, 95% CI: 0.52–0.98, *p* = 0.035).

Stratified analysis by age, hip lesions classification, and gender was performed. The results show that in the male group, the *MIR3142HG* rs1582417 GA-AA genotype was significantly associated with increased risk of steroid-induced ONFH in the dominant model (OR = 2.13, 95% CI: 1.35–3.37, *p* = 0.001) and in the codominant model (GA:OR = 2.10, 95% CI: 1.30–3.40, *p* = 0.003;

AA:OR = 2.24, 95% CI: 1.20–4.16, *p* = 0.012) and in the log-additive model (OR = 1.56, 95% CI: 1.16–2.09, *p* = 0.003). In the female group, the *MIR3142HG* rs17057846 AA genotype accelerated the risk of steroid-induced ONFH in the codominant model (OR = 3.57, 95% CI: 1.30–9.80, *p* = 0.013) and in the recessive model (OR = 3.37, 95% CI: 1.25–9.06, *p* = 0.016). We observed that rs17057846 shows association with unilateral and bilateral lesion of the hip joint in the codominant model (OR = 2.41, 95% CI: 1.14–5.09, *p* = 0.021) and in the dom-

Table 4. Analysis of the relationship between *MIR3142HG* gene polymorphism and steroid-induced ONFH subgroups

Subgroup analysis	SNP	Model	Genotype	Control, <i>n</i> (%)	Case, <i>n</i> (%)	OR (95% CI)	<i>p</i> value
Male	rs1582417	Codominant	GG	182 (42.9)	30 (25.9)	1	
			GA	182 (42.9)	64 (55.1)	2.10 (1.30–3.40)	0.003*
			AA	60 (14.2)	22 (19)	2.24 (1.20–4.16)	0.012*
		Dominant	GG	182 (42.9)	30 (26)	1	
			GA-AA	242 (57.1)	86 (74)	2.13 (1.35–3.37)	0.001*
		Recessive	GG-GA	364 (85.8)	94 (81)	1	
			AA	60 (14.2)	22 (19.0)	1.44 (0.84–2.48)	0.184
		Log-additive				1.56 (1.16–2.09)	0.003*
Female	rs17057846	Codominant	GG	185 (62.6)	45 (53.6)	1	
			GA	107 (35.6)	30 (35.7)	1.17 (0.69–1.96)	0.566
			AA	8 (2.6)	9 (10.7)	3.57 (1.30–9.80)	0.013*
		Dominant	GG	185 (62.6)	45 (53.6)	1	
			GA-AA	115 (38.3)	39 (46.4)	1.36 (0.83–2.22)	0.223
		Recessive	GG-GA	292 (9703)	75 (89.2)	1	
			AA	8 (2.6)	8 (2.6)	3.37 (1.25–9.06)	0.016*
		Log-additive				1.49 (0.99–2.22)	0.055
Hip lesions	rs17057846	Codominant	GG	81 (57.0)	40 (72.7)	1	
			GA	55 (38.7)	12 (21.8)	2.41 (1.14–5.09)	0.021*
			AA	6 (4.2)	3 (5.5)	0.88 (0.19–4.04)	0.872
		Dominant	GG	81 (57.0)	40 (72.7)	1	
			GA-AA	61 (43)	15 (27.3)	2.11 (1.05–4.26)	0.037*
		Recessive	GG-GA	136 (95.8)	52 (94.5)	1	
			AA	6 (4.2)	3 (5.5)	0.65 (0.15–2.91)	0.574
		Log-additive				1.63 (0.89–2.99)	0.111
Age (older than 42)	rs1582417	Codominant	GG	151 (41.9)	24 (27.3)	1	
			GA	158 (43.8)	45 (51.1)	1.74 (1.00–3.00)	0.048*
			AA	51 (14.2)	19 (21.6)	2.31 (1.17–4.58)	0.016*
		Dominant	GG	151 (41.9)	24 (27.3)	1	
			GA-AA	209 (58.1)	64 (72.7)	1.88 (1.12–3.14)	0.017*
		Recessive	GG-GA	309 (85.8)	69 (78.4)	1	
			AA	51 (14.2)	19 (21.6)	1.68 (0.93–3.03)	0.087
		Log-additive				1.54 (1.11–2.15)	0.011*
	rs7727115	Codominant	TT	164 (45.4)	53 (60.9)	1	
			TG	156 (43.2)	28 (32.2)	0.54 (0.33–0.90)	0.019*
			GG	41 (11.4)	6 (6.9)	0.45 (0.19–1.14)	0.092
		Dominant	TT	164 (45.4)	53 (60.9)	1	
			TG-GG	197 (54.6)	34 (39.1)	0.52 (0.32–0.85)	0.009*
		Recessive	TT-TG	320 (88.6)	81 (93.1)	1	
			GG	41 (11.4)	6 (6.9)	0.59 (0.24–1.44)	0.244
		Log-additive				0.61 (0.42–0.90)	0.012*

ONFH, osteonecrosis of the femoral head; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval. *p* values were calculated by logistic regression. * *p* < 0.05 indicates statistical significance.

Table 5. The association of genotypes in *MIR3142HG* genes with the clinical phenotypes

SNP	Genotype	TC, mmol/L	TG, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	ApoA1, mmol/L	ApoB, mmol/L
rs1582417	GG (n = 63)	4.46±0.95	2.04±1.71	1.08±0.27	2.54±0.79	1.24±0.21	0.98±0.78
	AG (n = 102)	4.52±0.91	1.74±1.35	1.05±0.26	2.69±0.76	1.23±0.25	0.93±0.24
	AA (n = 34)	4.54±0.91	1.75±1.22	1.09±0.30	2.67±0.71	1.26±0.25	0.98±0.42
	p value	0.897	0.416	0.726	0.485	0.868	0.736
rs2431689	GG (n = 151)	4.47±0.93	1.89±1.54	1.04±0.25	2.62±0.76	1.21±0.22	0.96±0.56
	GA (n = 44)	4.62±0.91	1.67±1.16	1.17±0.31	2.69±0.880	1.33±0.27	0.92±0.24
	AA (n = 3)	5.18±0.28	2.33±0.73	1.05±0.15	3.21±0.56	1.32±0.29	1.13±0.01
	p value	0.288	0.580	0.012*	0.388	0.010*	0.756
rs7727115	TT (n = 12)	4.79±0.95	2.81±2.54	1.01±0.25	2.66±0.87	1.24±0.24	0.95±0.22
	TG (n = 79)	4.50±0.95	1.76±1.38	1.06±0.26	2.67±0.78	1.22±0.24	0.90±0.24
	GG (n = 107)	4.47±0.90	1.78±1.33	1.08±0.28	2.61±0.75	1.26±0.23	0.99±0.65
	p value	0.542	0.056	0.699	0.838	0.472	0.515
rs17057846	GG (n = 123)	4.52±0.88	1.88±1.53	1.07±0.28	2.66±0.74	1.24±0.26	0.93±0.29
	GA (n = 67)	4.56±1.02	1.80±1.35	1.05±0.25	2.67±0.81	1.23±0.19	0.95±0.26
	AA (n = 9)	3.93±0.45	1.52±1.24	1.16±0.27	2.10±0.44	1.33±0.25	1.40±2.03
	p value	0.151	0.742	0.518	0.094	0.495	0.024*

SNP, single-nucleotide polymorphism. *p* values were calculated by logistic regression. * *p* < 0.05 indicates statistical significance.

Table 6. The haplotype frequencies of *MIR3142HG* polymorphisms and their association with the risk of steroid-induced ONFH

Haplotype				Freq	Without adjustment		With adjustment	
rs2431689	rs7727115	rs17057846			OR (95% CI)	<i>p</i> value ^a	OR (95% CI)	<i>p</i> value ^b
1	G	G	A	0.2157	1.10 (0.82–1.46)	0.531	1.03 (0.77–1.40)	0.806
2	G	T	G	0.2614	0.77 (0.59–1.00)	0.050	0.76 (0.58–1.00)	0.055
3	A	G	G	0.1269	0.82 (0.58–1.16)	0.267	0.81 (0.57–1.16)	0.244
4	G	G	G	0.3959	1.33 (1.04–1.70)	0.024*	1.40 (1.08–1.81)	0.010*

ONFH, osteonecrosis of the femoral head; OR, odds ratio; CI, confidence interval. *p* < 0.05 indicates statistical significance. ^a *p* values were calculated by unconditional logistic regression. ^b *p* values were calculated by unconditional logistic regression adjusted for age and gender.

inant model (OR = 2.11, 95% CI: 1.05–4.26, *p* = 0.037) (Table 4). In the age (older than 42), rs1582417 GA and AA genotype in the *MIR3142HG* gene are danger factors, increasing the risk of steroid-induced ONFH in the co-dominant model (OR = 1.74, 95% CI: 1.00–3.00, *p* = 0.048; OR = 2.31, 95% CI: 1.17–4.58, *p* = 0.016) and log-additive model (OR = 1.54, 95% CI: 1.11–2.15, *p* = 0.011). In addition, in the age (older than 42), rs7727115 TG genotype in the *MIR3142HG* gene are protect factors, reducing the risk of steroid-induced ONFH under the co-dominant model (OR = 0.54, 95% CI: 0.33–0.90, *p* = 0.019) and dominant model TG-GG (OR = 0.52, 95% CI: 0.32–0.85, *p* = 0.009) and log-additive model (OR = 0.61, 95% CI: 0.42–0.90, *p* = 0.012).

Considering the effect on lipid metabolism level in patients, we found that the HDL-C and ApoA1 levels of the AA genotype carriers in rs2431689 were higher than the GG genotype carriers, and the ApoB ratio of the GG genotype carriers in rs17057846 was significantly lower than that of the AA genes. For genotype carriers, TC level AA genotype is significantly lower than GG genotype carrier (Table 5).

In haplotype model analysis, one linkage disequilibrium block was detected in the *MIR3142HG* SNPs (rs2431689, rs7727115, rs17057846; Fig. 1; Table 6). The “GGG” sequence was associated with an increased risk of steroid-induced ONFH (OR = 1.33, 95% CI: 1.04–1.70, *p* = 0.021; adjusted OR = 1.40, 95% CI: 1.08–1.81, *p* = 0.010).

Fig. 1. LD analysis of the SNPs on *MIR3142HG*. Red squares display statistically significant associations between a pair of SNPs, as measured by D' ; darker shades of red indicate higher D' . LD, linkage disequilibrium; SNP, single-nucleotide polymorphism.

Discussion

Despite the introduction of many new treatment methods, and the rapid development of surgical techniques, the therapeutic effect of osteonecrosis has not improved significantly. Because many patients have reached the early stage of diagnosis, the final hip replacement is an inevitable choice. Therefore, researching the underlying mechanism of femoral head necrosis and development, and searching for more accurate biomarkers, is crucial for clinicians to formulate precise treatment strategies.

We assessed the relationship between the 4 SNPs in *MIR3142HG* and steroid-induced ONFH susceptibility in Han Chinese. The frequencies of 4 allelic SNPs of *MIR3142HG* genotype and haplotype in cases and control group were compared and further stratified. Among them, the rs1582417 polymorphism is associated with increased risk of steroid-induced ONFH, while the rs7727115 mutation is associated with reduced risk. For all we know, this is the first case-control study to examine the relationship between *MIR3142HG* gene polymorphism and the risk of steroid-induced ONFH. This research provides evidence for the biological significance of genetic variation in miRNA-coding genes, suggesting that it may affect the pathogenesis of steroid-induced ONFH.

MiRNA is highly conservative in evolution and its expression is tissue-specific. MiRNA has the following

characteristics: highly conserved; the order of time and space; and some miRNAs are tissue-specific. Studies have shown that miRNAs play a crucial role in different physiological and pathological processes via regulating gene expression post-transcriptionally; that is, it specifically binds to the untranslated region (3'-UTR) at the 3'-end of the mRNA of the target gene through base complementary pairing, inhibits the translation process of the mRNA, or directly mediates the degradation of the mRNA, so as to achieve the purpose of regulating the expression of the target gene [14–16]. Recently, more and more miRNAs have been studied in orthopedic diseases. Increasing evidence also indicates that miRNAs can directly play a role in the pathogenesis of many orthopedic diseases, regulating bone development and bone regeneration, such as disc degeneration, osteoarthritis, osteoporosis, and osteosarcoma [17–20]. In recent years, miRNAs have gradually become a research hotspot in the field of non-coding RNA, and some miRNAs have been proved to be closely related to the pathogenesis of steroid-induced ONFH [21, 22].

MiR-146a is abnormal in lung cancer, breast cancer, colorectal cancer, glioma, and other cancers [23–26]. MiR-146a may participate in ONFH and other physiological processes such as inflammation [27]. The study showed that *MIR3142HG* knockdown observably decreased the release of IL-8 and CCL2, and partially reduced the release of interleukin-6 in control fibroblasts, suggesting that the decreased expression of *MIR3142HG* might be the reason for the decreased inflammatory reaction of IPF fibroblasts stimulated by IL-1 [28]. It was found that *MIR3142HG* regulates the inflammatory response, and it is proved that the reduction of the inflammatory response of IPF fibroblasts is related to the decreased expression of *MIR3142HG*/miR-146a [28]. Yuan et al. [29] designed a study to compare miRNA expression profiles in 9 ONFH patients and 6 femoral neck fractures. It was confirmed that the expression of miR-146a was up-regulated in ONFH. So, we doped out that *MIR3142HG* polymorphism might affect the expression or function of miR-146a during the steroid-induced ONFH process. At present, the mechanism of *MIR3142HG* in steroid-induced ONFH has not been reported and remains to be studied.

We suggest that the induction of age-related steroid-induced ONFH may have relevance with rs1582417 and rs7727115 polymorphism in *MIR3142HG*. Further studies through gender stratification found that rs1582417 polymorphism increases steroid-induced ONFH susceptibility, suggesting that the polymorphism risk associa-

tion may be gender-related. We found that the HDL-C and ApoA1 levels of the AA genotype carriers in rs2431689 were higher than the GG genotype carriers, and the ApoB ratio of the GG genotype carriers in rs17057846 was well below that of the AA genes. For genotype carriers, TC level AA genotype is significantly lower than GG genotype carrier. These results further illustrate the correlation between rs2431689, rs17057846, HDL-C levels, ApoA1 levels, ApoB levels, metabolic disorders, and osteonecrosis. At present, few functional studies have been reported. Understanding the function of miRNAs is crucial, which will provide new ideas for future treatment.

Some important findings were revealed in our study, but in interpreting these results, some limitations of this study should be considered. First, the sample size of hormonal femoral head necrosis is too small and lacks representativeness, while the large sample study takes time and effort; second, the relationship between genetic polymorphisms of different regions and races and the incidence of hormonal femoral head necrosis is inconsistent, and further analysis is needed to reduce the variation within the interval and reduce the impact of the sampling error; again, the combination of large sample size etiology and SNP analysis has become the direction of future research on steroid-related femoral head necrosis, but the credibility of the experimental results needs to be further improved, such as application of statistical methods such as multivariate analysis of variance and avoidance of confounding factors in case-control intervals.

Conclusions

In a word, this is the first evidence that 2 polymorphisms (rs1582417 and rs7727115) in *MIR3142HG* might contribute to the susceptibility to steroid-induced ONFH in the Chinese Han population. However, it is not clear whether these changes in *MIR3142HG* are consistent with the onset of ONFH, or simply reflect the terminal stage of the disease.

References

- 1 Zhao J, Ma XL, Ma JX, Sun L, Lu B, Wang Y, et al. TET3 mediates alterations in the epigenetic marker 5hmC and Akt pathway in steroid-associated osteonecrosis. *J Bone Miner Res*. 2017;32:319–32.
- 2 Weinstein RS. Glucocorticoid-induced osteonecrosis. *Endocrine*. 2012;41:183–90.
- 3 Hirata T, Fujioka M, Takahashi KA, Asano T, Ishida M, Akioka K, et al. Low molecular

weight phenotype of Apo(a) is a risk factor of corticosteroid-induced osteonecrosis of the femoral head after renal transplant. *J Rheumatol*. 2007;34:516–22.

- 4 Hirata T, Fujioka M, Takahashi KA, Arai Y, Asano T, Ishida M, et al. ApoB C7623T polymorphism predicts risk for steroid-induced osteonecrosis of the femoral head after renal transplantation. *J Orthop Sci*. 2007;12:199–206.

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Statement of Ethics

This study was approved by the Hospital of Inner Mongolia Medical University Ethics Committee (Serial no.: YKD2016138). Written informed consent of all participants was collected.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Author Contributions

Jianzhong Wang and Huiqiang Wu conceived and designed the experiments, Huiqiang Wu, Tiantian Wang, Menghu Sun, Tingting Liu, and Feimeng An performed the experiments, and Tiantian Wang and Qiumei Dong analyzed the data. Jianzhong Wang and Tiantian Wang drafted the work or revised it critically for important content.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

- 5 Kuribayashi M, Fujioka M, Takahashi KA, Arai Y, Hirata T, Nakajima S, et al. Combination analysis of three polymorphisms for predicting the risk for steroid-induced osteonecrosis of the femoral head. *J Orthop Sci*. 2008; 13:297–303.
- 6 Du J, Jin T, Cao Y, Chen J, Guo Y, Sun M, et al. Association between genetic polymorphisms of MMP8 and the risk of steroid-induced osteonecrosis of the femoral head in the population of Northern China. *Medicine*. 2016;95:e4794.
- 7 Du J, Liu W, Jin T, Zhao Z, Bai R, Xue H, et al. A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. *Oncotarget*. 2016;7:68434–41.
- 8 Qi Y, Wang J, Sun M, Ma C, Jin T, Liu Y, et al. MMP-14 single-nucleotide polymorphisms are related to steroid-induced osteonecrosis of the femoral head in the population of Northern China. *Mol Genet Genomic Med*. 2019;7:e00519.
- 9 Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell*. 2012;148: 1172–87.
- 10 Ambros V. The functions of animal microRNAs. *Nature*. 2004;431:350–5.
- 11 Króliczewski J, Sobolewska A, Lejnowski D, Collawn JF, Bartoszewski R. MicroRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. *Gene*. 2018;640:66–72.
- 12 Preskill C, Weidhaas JB. SNPs in microRNA binding sites as prognostic and predictive cancer biomarkers. *Crit Rev Oncog*. 2013;18: 327–40.
- 13 Paterson MR, Kriegel AJ. MiR-146a/b: a family with shared seeds and different roots. *Physiol Genomics*. 2017;49:243–52.
- 14 Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. *Cell Prolif*. 2015;48:278–83.
- 15 Wang Y, Jia LS, Yuan W, Wu Z, Wang HB, Xu T, et al. Low miR-34a and miR-192 are associated with unfavorable prognosis in patients suffering from osteosarcoma. *Am J Transl Res*. 2015;7:111–9.
- 16 Lv C, Bai Z, Liu Z, Luo P, Zhang J. MicroRNA-495 suppresses human renal cell carcinoma malignancy by targeting SATB1. *Am J Transl Res*. 2015;7:1992–9.
- 17 Jing W, Jiang W. MicroRNA-93 regulates collagen loss by targeting MMP3 in human nucleus pulposus cells. *Cell Prolif*. 2015;48:284–92.
- 18 Jackson MT, Moradi B, Smith MM, Jackson CJ, Little CB. Activation of matrix metalloproteinases 2, 9, and 13 by activated protein C in human osteoarthritic cartilage chondrocytes. *Arthritis Rheumatol*. 2014;66:1525–36.
- 19 Li Z, Shen J, Chan MT, Wu WK. MicroRNA-379 suppresses osteosarcoma progression by targeting PDK1. *J Cell Mol Med*. 2017; 21:315–23.
- 20 He X, Zhang W, Liao L, Fu X, Yu Q, Jin Y. Identification and characterization of microRNAs by high through-put sequencing in mesenchymal stem cells and bone tissue from mice of age-related osteoporosis. *PLoS One*. 2013;8:e71895.
- 21 Hao C, Yang S, Xu W, Shen JK, Ye S, Liu X, et al. MiR-708 promotes steroid-induced osteonecrosis of femoral head, suppresses osteogenic differentiation by targeting SMAD3. *Sci Rep*. 2016;6:22599.
- 22 Gu C, Xu Y, Zhang S, Guan H, Song S, Wang X, et al. MiR-27a attenuates adipogenesis and promotes osteogenesis in steroid-induced rat BMSCs by targeting PPAR γ and GREM1. *Sci Rep*. 2016;6:38491.
- 23 Iacona JR, Lutz CS. MiR-146a-5p: expression, regulation, and functions in cancer. *Wiley Interdiscip Rev RNA*. 2019;10:e1533.
- 24 Iacona JR, Monteleone NJ, Lutz CS. MiR-146a suppresses 5-lipoxygenase activating protein (FLAP) expression and Leukotriene B4 production in lung cancer cells. *Oncotarget*. 2018;9:26751–69.
- 25 Si C, Yu Q, Yao Y. Effect of miR-146a-5p on proliferation and metastasis of triple-negative breast cancer via regulation of SOX5. *Exp Ther Med*. 2018;15:4515–21.
- 26 Bleau AM, Redrado M, Nistal-Villan E, Villalba M, Exposito F, Redin E, et al. MiR-146a targets c-met and abolishes colorectal cancer liver metastasis. *Cancer Lett*. 2018;414:257–67.
- 27 Guo X, Zhang M, Li Q, Zhao J, Wang B, Wang J, et al. Evaluation of genetic variants in MIR3142HG in susceptibility to and prognosis of glioma. *Am J Clin Oncol*. 2020;43:1–8.
- 28 Hadjicharalambous MR, Roux BT, Feghali-Bostwick CA, Clarke DL, Lindsay MA. Long non-coding RNAs are central regulators of the IL-1 β -induced inflammatory response in normal and idiopathic pulmonary lung fibroblasts. *Front Immunol*. 2018;9:2906.
- 29 Yuan HF, Von Roemeling C, Gao HD, Zhang J, Guo CA, Yan ZQ. Analysis of altered microRNA expression profile in the reparative interface of the femoral head with osteonecrosis. *Exp Mol Pathol*. 2015;98:158–63.