Osteoporosis International (2019) 30:871–877
https://doi.org/10.1007/s00198-019-04835-9

ORIGINAL ARTICLE

Genetic polymorphisms in *IL1B* predict susceptibility to steroid -induced osteonecrosis of the femoral head in Chinese Han population

Y. Yu[1] · Y. Zhang[2] · J. Wu[3] · Y. Sun[3] · Z. Xiong[3] · F. Niu[3] · L. Lei[3] · S. Du[3] · P. Chen[3] · Z. Yang[4]

Received: 5 June 2018 / Accepted: 2 January 2019 / Published online: 9 March 2019

Abstract

Summary The purpose of this research was to examine if the *IL1B* gene polymorphism has impact on the risk of steroid-induced ONFH in Chinese population. We found that *IL1B* rs1143630 decreased the SANFH's risk and *IL1B* rs2853550 increased the risk of steroid-induced ONFH. So, we guess that *IL1B* gene influences the genetic susceptibility of steroid-induced ONFH.

Introduction Genetic polymorphisms in *IL1B* gene could be related in the pathogenesis of osteonecrosis. Discusses on the relationship between the *IL1B* gene and steroid-induced osteonecrosis of the femoral head (steroid-induced ONFH) is still less in Chinese Han population. So, in this research, we want to examine whether the *IL1B* gene polymorphism has impact on the risk of steroid-induced ONFH in Chinese population.

Methods A total of 286 steroid-induced ONFH patients and 441 controls were recruited, and seven SNPs (rs2853550, rs1143643, rs3136558, rs1143630, rs1143627, rs16944, and rs1143623) in *IL1B* gene were selected; unconditional logistic regression analysis was used to research the influence on the risk of steroid-induced ONFH. Functional annotations of *IL1B* variants were performed by RegulomeDB and HaploReg.

Results rs1143630 (A>C) in the *IL1B* gene decreased the risk of steroid-induced ONFH in the allele model (OR = 0.69, 95%CI 0.51–0.93, $p = 0.014$). Further genetic model analyses found that *IL1B* rs2853550 AG genotype increased the risk of steroid-induced ONFH compared with the people who are carriers of the *IL1B* rs2853550 GG genotype (OR = 1.69, 95%CI 1.16–2.46, $p = 0.012$). In the dominant model, *IL1B* rs1143630 GG-GT genotype decreased the risk of steroid-induced ONFH (OR = 0.62, 95%CI 0.44–0.87, $p = 0.0051$). And further haplotype analysis was performed, while the result was not significant. Using RegulomeDB and HaploReg, rs2853550 is likely to affect TF binding, any motif and DNase peak.

Conclusions We guess that *IL1B* gene influences the genetic susceptibility of steroid-induced ONFH.

Keywords Steroid-induced ONFH · *IL1B* gene · Gene polymorphism · Case-control research

Introduction

Osteonecrosis of the femoral head (ONFH) is an intractable and complex orthopedic condition that is characterized by osteocyte apoptosis and deterioration and collapse of the bone structure of the femoral head, which ultimately lead to femoral head ischemia and death [1–3]. It is reported that the annual incidence of avascular necrosis of the femoral head (ANFH) in the USA in 1 to 2 million people, and China's annual incidence of 15 to 20 million people, occur in the 35 to 45-year-old crowd, such as untreated or improper treatment usually will progress to the femoral head collapse and secondary osteoarthritis [4]. Alcohol as well as steroid use is an important risk factor [5]. Hirota et al. [6] reported that a risk of osteonecrosis of the femoral head developing in occasional and regular drinkers becomes higher than in non-drinkers. It was reported that femoral head osteonecrosis was

Yan Yu and Yiming Zhang joint first authors

✉ Z. Yang
xgcgfd@126.com

[1] Department of Clinical Laboratory, HongHui Affiliated Hospital of Xi'an Jiaotong University College of Medicine, Xi'an 710054, Shaanxi, China

[2] Department of Orthopedics, Suizhou Hospital, Hubei University Of Medicine, Suizhou 441300, Hubei, China

[3] Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, School of Life Sciences, Northwest University, Xi'an 710069, Shaanxi, China

[4] Department of Osteonecrosis and Joint Reconstruction Ward, HongHui Affiliated Hospital of Xi'an Jiaotong University College of Medicine, #555 Youyi East Road, Xi'an 710054, Shaanxi, China

associated with fatty liver [7]. And with the hormone in the immune system diseases, spinal cord injury, and other diseases more and more widely used, the incidence of steroid-induced osteonecrosis of the femoral head (steroid-induced ONFH) showed an increasing trend year by year, the age of onset tends to younger, and patients with severe clinical symptoms, it has caused serious mental pressure and economic burden to individuals and society. Several studies have shown that genetic factors play an important role in the development of femoral head necrosis [8–10].

IL1β is a pro-inflammatory cytokine, encoded by the *IL1B* gene, belongs to the IL1 family (α, β monomer; its activity is usually reflected by the IL1β), and can regulate cell acute and chronic inflammatory response [11]. IL1β is mainly produced by blood mononuclear cells and tissue macrophages and can induce the expression of many inflammatory genes involved in the reaction, resulting in free radicals and causing a series of inflammatory reactions, such as induced tumor necrosis factor-a (TNF-α), leading to activation of the nuclear factor-κB (NF-κB) signal transduction pathway [12]. *IL1B* contains several polymorphisms including − 31 T/C (rs1143627) and − 511 T/C (rs16944) in the promoter region and + 3954C/T (rs1143634) in exon 5 [13].

Meulenbelt et al. [14] randomly selected 886 subjects to analyze the role of the interleukin-1β gene (*IL1B*) and the *IL1* receptor antagonist gene (*IL1RN*) in relation to the occurrence of radiographic osteoarthritis (ROA) in the hip, knee, and hand and disk degeneration of the spine. The findings suggest that the heterozygous and homozygous carriers of the rare *IL1B* allele − 511T increased the risk of osteoarthritis of the hip. Timms et al. reported that *IL1B* rs16944 was associated with ankylosing spondylitis (AS) in European ancestry [15]. Li et al. explored the association between *IL1A* and *IL1B* polymorphisms and ankylosing spondylitis and found that rs2853550 may reduce the risk of AS in Chinese population [16]. Studies on IL1B gene and femoral head necrosis have not been reported. Samara et al. [17] produced the comprehensive analysis of cytokine gene (*IL-1α*, *IL-1R*, *IL-1RA*, *IL-4Rα*, *IL-1β*, *IL-12*, *γ-IFN*, *TGF-β*, *TNF-a*, *IL-2*, *IL-4*, *IL-6* and *IL-10*) polymorphisms and femoral head osteonecrosis, and found that certain genotypes of the *IL-1α*, *TGF-β*, *IL-10*, and *TNF-a* genes could be related in the pathogenesis of osteonecrosis. So far, discusses on the relationship between the *IL1B* gene and steroid-induced ONFH is still less in Chinese Han population.

So, the purpose of this research was to examine whether the *IL1B* gene polymorphism has impact on the risk of steroid-induced ONFH in Chinese population. Therefore, we combined the literature reports and the minimum allele frequency more than 0.05 in the global population, selected seven loci (rs2853550, rs1143643, rs3136558, rs1143630, rs1143627, rs16944, and rs1143623) in IL1B gene, to research the influence on the risk of steroid-induced ONFH.

Materials and methods

Ethics statement

Our present study strictly followed the principles of the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of Zhengzhou Traditional Chinese Medicine Traumatology Hospital and HongHui Affiliated Hospital of Xi'an Jiaotong University College of Medicine. Informed consent forms were signed by all participants.

Study population

A total of 286 steroid-induced ONFH patients and 441 controls were selected from the Zhengzhou Traditional Chinese Medicine Traumatology Hospital and HongHui Affiliated Hospital of Xi'an Jiaotong University College of Medicine. ONFH was diagnosed based on evidence of osteonecrosis on anteroposterior and frog-view X-rays of both hips and/or magnetic resonance imaging [18]. Steroid-induced ONFH was defined by a history of a mean daily dose ≥ 16.6 mg or a high-dose steroid impulsion therapy for more than 1 week [19]. 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. 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.

SNP genotyping

Meulenbelt et al. [14] found that the heterozygous and homozygous carriers of the rare *IL1B* allele − 511T increased the risk of osteoarthritis of the hip. Timms et al. reported that *IL1B* rs16944 was associated with ankylosing spondylitis (AS) in European ancestry [15]. Li et al. explored the association between *IL-1A* and *IL-1B* polymorphisms and AS and found that rs2853550 may reduce the risk of AS in Chinese population [16]. Discusses on the relationship between the *IL1B* gene and steroid-induced ONFH is still less in Chinese Han population. Therefore, we combined the literature reports and the minimum allele frequency more than 0.05 in the global population, selected seven loci (rs2853550, rs1143643, rs3136558, rs1143630, rs1143627, rs16944, and rs1143623) in IL1B gene, to research the influence on the risk of steroid-induced ONFH. We used the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China) extracted from whole blood. DNA concentrations were

Table 1 The basic information of participants

	Case (286)	Control (441)	*p*
Gender			0.915
Female	113	176	
Male	173	265	
Age	41.83 ± 13.12	44.60 ± 11.55	0.006

$p < 0.05$ indicates statistical significance

measured using a NanoDrop 2000 (Gene Company Limited). We used Agena MassARRAY Assay Design 3.0 Software to design a Multiplexed SNP MassEXTEND assay [20]. Agena MassARRAY RS1000 (Agena Bioscience, San Diego, CA, USA) was used for genotyping, and the related data were managed using Agena Typer 4.0 Software [20, 21]. Laboratory personnel were blinded to the genotyping results of all samples.

Statistical analysis

Data analysis was performed using Microsoft Excel (Redmond, WA, USA) and SPSS 19.0 statistical package (SPSS, Chicago, IL, USA). All *p* values were two-sided, and $p < 0.05$ was indicated statistically significant. Each SNP frequency in the control subjects was assessed for departure from Hardy–Weinberg equilibrium (HWE) using an exact test. We calculated genotype frequencies of cases and controls using a χ^2 test [22]. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using unconditional logistic regression with adjustment for age and sex [23].

Four genetic models (genotype, dominant, recessive, and additive model) were performed using PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/), to characterize the potential association of each *IL1B* polymorphism with the risk of steroid-induced ONFH. We used the Power and Sample Size (PS) Calculation software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize) was used to calculate the power of the significant difference [24]. Finally, we used Haploview software package (version 4.2) to do haplotype analysis. Firstly, we make linkage disequilibrium analysis. The parameters D' and r^2 were used to measure the degree of linkage disequilibrium between the two SNPS loci. Using D' confidence interval method divided haplotype block haplotype block. $|D'| \leq 1$, the more close to 1, the higher the level of linkage disequilibrium between sites; $r^2 \leq 1$, the more close to 1, the higher the level of linkage disequilibrium between the loci. The ORs and CIs of haplotype were determined using unconditional logistic regression with adjustment for age and sex [25, 26].

The RegulomeDB [27] provides scores which refer to the data available for each individual SNP, with lower scores associated with a wider range of supporting data for functional importance. RegulomeDB was used to annotate these seven genetic variants (i.e., rs2853550, rs1143643, rs3136558, rs1143630, rs1143627, rs16944, rs1143623) in *IL1B* gene with known and predicted regulatory elements. HaploReg [28] is a tool for exploring annotations of the noncoding genome at variants on haplotype blocks, such as candidate regulatory SNPs at disease-associated loci. HaploReg v 4.1 analysis was done to predict the effect of risk allele on different regulatory motifs.

Results

In this research, we selected 286 steroid-induced ONFH, including 113 females and 173 males, and the mean age of case group was 41.83 ± 13.12 years. Also recruited were 441 healthy people, consist of 176 females and 265 males, and the mean age of the control group was 44.60 ± 11.55 years. There was a statistically significant difference in age ($p = 0.006$), and no statistical difference in the frequency distribution of gender ($p = 0.915$) (Table 1).

Table 2 The basic information of selected locus on the *IL1B* gene

SNP rs#	Chromosome	Position	Band	Allele		Gene(s)	Role	HWE-*p*	Allele model	
				A	B				OR (95%CI)	*p*
rs2853550	chr2	113587121	2q13	A	G	*IL1B*	Downstream	0.399	1.39 (0.99–1.95)	0.053
rs1143643	chr2	113588302	2q13	C	T	*IL1B*	Intron	0.181	1.03 (0.83–1.27)	0.782
rs3136558	chr2	113591275	2q13	G	A	*IL1B*	Intron	0.614	0.99 (0.80–1.23)	0.944
rs1143630	chr2	113591655	2q13	T	G	*IL1B*	Intron	1.000	0.69 (0.51–0.93)	0.014*
rs1143627	chr2	113594387	2q13	G	A	*IL1B*	Promoter	0.566	0.99 (0.80–1.22)	0.934
rs16944	chr2	113594867	2q13	A	G	*IL1B*	Promoter	0.568	1.00 (0.81–1.24)	0.981
rs1143623	chr2	113595829	2q13	G	C	*IL1B*	Promoter	0.552	0.81 (0.65–1.01)	0.065

ORs odds ratios, *CI* confidence interval

$p < 0.05$, statistical significance for HWE; *$p < 0.05$ indicates statistical significance

Table 3 The relationship between *IL1B* gene polymorphism and steroid-induced ONFH

SNP	Model	Genotype	Control	Case	Crude analysis		Adjustment by age and gender		Study power
					OR (95% CI)	*p* value	OR (95% CI)	*p* value	
rs2853550	Codominant	G/G	363 (82.7%)	216 (75.5%)	1	0.017	1	0.012*	
		A/G	71 (16.2%)	69 (24.1%)	1.63 (1.13–2.37)		1.69 (1.16–2.46)		56.4%
		A/A	5 (1.1%)	1 (0.4%)	0.34 (0.04–2.90)		0.36 (0.04–3.11)		
	Dominant	G/G	363 (82.7%)	216 (75.5%)	1	0.020	1	0.013*	
		A/G-A/A	76 (17.3%)	70 (24.5%)	1.55 (1.07–2.23)		1.60 (1.11–2.32)		46.5%
	Recessive	G/G-A/G	434 (98.9%)	285 (99.7%)	1	0.220	1	0.250	
		A/A	5 (1.1%)	1 (0.4%)	0.30 (0.04–2.62)		0.32 (0.04–2.79)		
	Log-additive	–	–	–	1.41 (1.00–1.99)	0.051	1.46 (1.03–2.06)	0.034*	30.9%
rs1143630	Codominant	G/G	299 (67.8%)	221 (77.3%)	1	0.016	1	0.016*	
		G/T	129 (29.2%)	57 (19.9%)	0.60 (0.42–0.85)		0.59 (0.42–0.85)		63.0%
		T/T	13 (3.0%)	8 (2.8%)	0.83 (0.34–2.04)		0.83 (0.34–2.04)		
	Dominant	G/G	299 (67.8%)	221 (77.3%)	1	0.005	1	0.005*	
		G/T-T/T	142 (32.2%)	65 (22.7%)	0.62 (0.44–0.87)		0.62 (0.44–0.87)		54.9%
	Recessive	G/G-G/T	428 (97.0%)	278 (97.2%)	1	0.910	1	0.900	
		T/T	13 (3.0%)	8 (2.8%)	0.95 (0.39–2.32)		0.95 (0.39–2.32)		
	Log-additive	–	–	–	0.69 (0.51–0.93)	0.014	0.69 (0.51–0.93)	0.014*	36.8%

*$p < 0.05$ indicates statistical significance

Table 2 shows that the basic information of seven SNPs, including chromosome, position, band, minor allele, and reference allele. Using an exact test found that all locus were met HWE. In the allele model, rs1143630 (A>C) in the *IL1B*

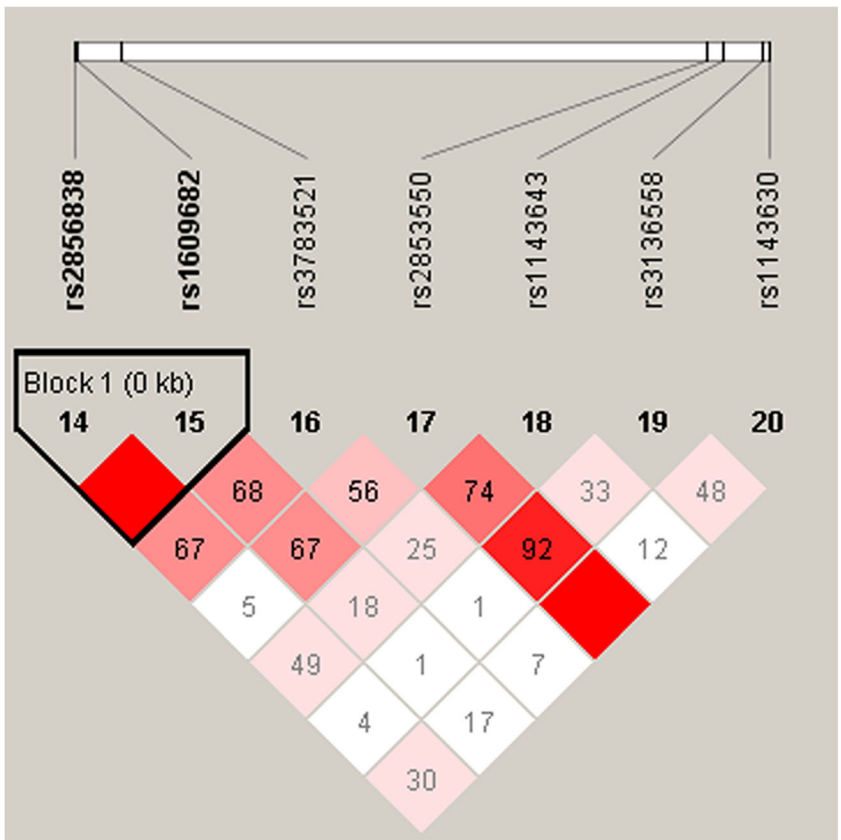

Fig. 1 Haplotype block map for all the SNPs of the *IL1B* gene

Table 4 The haplotype frequencies of *IL1B* gene polymorphisms and steroid-induced ONFH risk

rs1143627	rs16944	Freq	OR (95% CI)	*p* value	OR (95% CI)	*p* value
A	G	0.5144	1	–	1	–
G	A	0.4814	1.00 (0.81–1.24)	0.990	1.01 (0.81–1.25)	0.950

$p < 0.05$ indicates statistical significance

gene decreased the risk of steroid-induced ONFH (OR = 0.69, 95%CI 0.51–0.93, $p = 0.014$), on the occurrence of steroid-induced ONFH play a protective role.

In further genetic model analyses as shown Table 3, we found that the individuals with *IL1B* rs2853550 AG genotype increased the risk of steroid-induced ONFH compared with the people who are carriers of the *IL1B* rs2853550 GG genotype (OR = 1.63, 95%CI 1.13–2.37, $p = 0.017$). After correction of age and gender, *IL1B* rs2853550 AG genotype still increased the risk of steroid-induced ONFH (OR = 1.69, 95%CI 1.16–2.46, $p = 0.012$), with power value of 0.564. In the dominant model, *IL1B* rs1143630 GG-GT genotype decreased the risk of steroid-induced ONFH no matter crude analysis or modify analysis (crude analysis: OR = 0.62, 95%CI 0.44–0.87, $p = 0.005$; modify analysis: OR = 0.62, 95%CI 0.44–0.87, $p = 0.005$, power = 0.549).

The linkage disequilibrium degree between two SNP loci was measured by the parameter *D*' and r^2, and the haplotype block was divided by *D*' confidence interval method. The block consisted of rs1143624 and rs169944 (Fig. 1). And further haplotype analysis was performed, while the result was not significant (Table 4).

Using RegulomeDB, we identified that rs2853550 is likely to affect TF binding, any motif and DNase peak. As predicted by HaploReg v4.1, rs2853550 can bind to these proteins CEBPB, GABP, GATA, POL24H8, TAL1, and also lead to motifs changed (Table 5).

Discussion

In this case-control study, we assessed the potential effect of the *IL1B* gene polymorphisms on the susceptibility to steroid-induced ONFH in Chinese Han population. The individuals with *IL1B* rs2853550 AG genotype increased the risk of steroid-induced ONFH. And *IL1B* rs1143630 GG-GT genotype decreased the risk of steroid-induced ONFH.

Cytokines are cell-signaling molecules, which play an important regulatory role in cell-to-cell interactions, immune-regulation, hematopoiesis, and inflammation [29]. The family of interleukin-1 (*IL-1*) includes IL-1α, IL-1β, IL-1Ra, etc. IL-1alpha and IL-1beta are the major inducers of pro-inflammatory immune responses, which bind to many of the same cell surface receptors, leading to inflammatory cascade responses [30]. A study suggests that IL-1β can trigger inflammation and bone disorders [31]. In our research, we found that *IL1B* rs2853550 AG genotype increased the risk of steroid-induced ONFH. Previous studies did not find the rs2853550 affect the risk of osteonecrosis of the femoral head. In the research about AS, it is suggested that the minor allele "A" of rs2853550 may reduce the risk of AS [16]. RegulomeDB predicts discovery that rs2853550 is likely to affect TF binding, any motif and DNase peak. As predicted by HaploReg v4.1, rs2853550 can bind to these proteins CEBPB, GABP, GATA, POL24H8, TAL1, and also lead to motifs changed. So, we suspect that mutations in the rs2853550 site

Table 5 Functional annotation results using RegulomeDB and HaploReg

SNP rs#	Gene(s)	RegulomeDB	HaploReg	
			Proteins bound	Motifs changed
rs2853550	*IL1B*	3a	CEBPB, GABP, GATA, POL24H8, TAL1	Gm397,RP58
rs1143643	*IL1B*	6	–	Myb
rs3136558	*IL1B*	5	–	ERalpha-a, Mef2, RAR, RORalpha1, RXRA
rs1143630	*IL1B*	No data	–	Foxa, LXR, PPAR, TCF12
rs1143627	*IL1B*	1b	POL24H8, PU1, CEBPB, POL2, E2F6, MAX	Cdx2, Hoxa9, Hoxb13, Xoxb9, TATA
rs16944	*IL1B*	1f	–	Maf
rs1143623	*IL1B*	No data	–	CEBPB, EBF, Myc, TFIIA

1b: eQTL + TF binding + any motif + DNase Footprint + DNase peak; 1f: eQTL + TF binding/DNase peak; 3a: TF binding + any motif + DNase peak; 5: TF binding or DNase peak; 6: other

would affect the targeted binding of the IL1B gene to these proteins (CEBPB, GABP, GATA, POL24H8, TAL1), which in turn affected the occurrence of femoral head necrosis. And *IL1B* rs1143630 GG-GT genotype decreased the risk of steroid-induced ONFH, also lead to motifs changed by using RegulomeDB prediction. Rs1143630 is located in the intron region. Although it has no coding function, it can promote transcription initiation; participate in the modification, metabolism, transport, and storage of mRNA; and even affect translation. In the next step, we will use site-directed mutagenesis to construct mutant recombinants and in-depth analysis of whether this site affects the selective cleavage or translation of IL1B mRNA, thereby verifying whether this SNP is a pathogenic site of steroid-induced ONFH.

In our research, we did not find that these polymorphisms affected the risk of steroid-induced ONFH. Meulenbelt et al. [10] analyzed the role of the *IL1B* and the *IL1RN* in relation to the occurrence of ROA in the hip, knee, and hand and disk degeneration of the spine, and found that the heterozygous and homozygous carriers of the rare *IL1B* allele −511T increased the risk of OA of the hip. The −511 (rs16944) and 31 (rs1143627) are two important single nucleotide polymorphisms of *IL-1β* gene, *IL-1β*-31 T [32], and *IL-1β*-511 T [33] were associated with increased *IL-1β* expression, whose gene polymorphism could affect gene transcription and lead to functional changes. Small sample size may cause false negative results, so we continue to expand the sample size to analysis whether these two loci will affect the genetic susceptibility of steroid-induced ONFH.

Even so, we found that rs2853550 and rs1143630 were associated with the risk of steroid-induced ONFH no matter crude analysis or modify analysis. But the statistical effect is relatively low, which may be due to the lower minimum allele frequency. For this low-frequency mutation, we need to expand the sample to improve statistical effectiveness.

Conclusion

In summary, we guess that *IL1B* rs2853550 increased the risk of steroid-induced ONFH, and *IL1B* rs1143630 decreased the risk of steroid-induced ONFH. In future, we will expand our samples to verify our results. Afterwards, we will do some functional studies based on the reliability results to explore how *IL1B* gene affects the incidence of steroid-induced ONFH.

Acknowledgements We are grateful to all the patients and individuals in the study who made this work possible. We would also like to thank the clinicians and hospital staffs who contributed to data collection for this study.

Funding information This work was supported by the Shaanxi Science and Technology Development Plan Project (Grant Nos. 2013K12-17-03 and 2015SF025).

Compliance with ethical standards

Our present study strictly followed the principles of the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of Zhengzhou Traditional Chinese Medicine Traumatology Hospital and HongHui Affiliated Hospital of Xi'an Jiaotong University College of Medicine. Informed consent forms were signed by all participants.

Conflict of interest Yan Yu, Yiming Zhang, Jiamin Wu, Yao Sun, Zichao Xiong, Fanglin Niu, Lingyu Lei, Shuli Du, Peng Chen, and Zhi Yang declare that they have no conflict of interest.

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