Bone 101 (2017) 104–112

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

# Association of gene variants of transcription factors PPARγ, RUNX2, Osterix genes and COL2A1, IGFBP3 genes with the development of osteonecrosis of the femoral head in Chinese population

Yang Song [a,c], Zhenwu Du [a,b,c], Ming Ren [a,c], Qiwei Yang [b,c], Qingyu Wang [b,c], Gaoyang Chen [b,c], Haiyue Zhao [b,c], Zhaoyan Li [a,c], Jincheng Wang [a,c,*], Guizhen Zhang [a,b,c,*]

[a] Department of Orthopedics of Second Clinical College of Jilin University, Ziqiang Street 218, Changchun 130041,PR China
[b] Research Centre of Second Clinical College of Jilin University, Ziqiang Street 218, Changchun 130041,PR China
[c] The Engineering Research Centre of Molecular Diagnosis and Cell Treatment for Metabolic Bone Diseases of Jilin Province, Ziqiang Street 218, Changchun 130041,PR China

ARTICLE INFO

*Article history:*
Received 6 February 2017
Revised 28 April 2017
Accepted 1 May 2017
Available online 02 May 2017

*Keywords:*
ONFH
PPARγ
RUNX2
COL2A1
IGFBP3
Gene polymorphisms
Lipid metabolism disorder
Transcription factor

ABSTRACT

The molecular pathogenesis of osteonecrosis of the femoral head (ONFH) has been remained obscure so that its prevalence has been increasing in recent decades. Different transcription factors play critical roles in maintaining the balance between osteogenesis and adipogenesis. However, it has been unclear that the genes variants of the transcription factors exert the effects on the imbalance between steogenesis and adipogenesis during the development of ONFH. Here, we selected the 11SNPs from steogenesis, adipogenesis-specific transcription factors RUNX2, Osterix, and PPARγ genes, chondrogenesis or adipogenesis key factors COL2A1, IGFBP3 genes and analysed the genotypes, alleles, haplotypes and their association with the risk and clinical phenotypes of ONFH through Mass ARRAY® platformin in 200 ONFH patients and 177controls. The patients with ONFH (132 males, 68 females; age: 53.46 ± 11.48 yr) were consecutively enrolled at the Department of Orthopedics, the Second Clinical College of Jilin University, from March 2014 to June 2015 and were diagnosed and classified into 10 cases of stage II (5.6%), 54 cases of stage III (30.2%) and 115 cases (64.2%) of stage IV and alcohol-induced (71 cases (39.7%)), idiopathic (64 cases (34.0%)), and steroid-induced osteonecrosis (47 cases (26.3%)) subgroup, respectively. Our results showed that all models of logistical regression analysis, the co-dominants, dominants, and recessives of PPARγrs2920502, significantly associated with the increased risk of ONFH ($p = 0.004$, $p = 0.013$, $p = 0.016$), respectively. Both the minor homozygous CC genotype and the allele C of rs2920502 were evidently correlated with the enhanced risk of ONFH ($p = 0.005$, $p = 0.0005$),respectively. The recessives models of IGFBP3rs2132572 (G/A) as well as RUNX2 rs3763190(G/A) were statistically associated with the higher ONFH risk, $p = 0.030$, $p = 0.029$, respectively; the minor homozygous(AA) of IGFBP3rs2132572 (G/A) was also related to the increased risk of bilateral hips lesions, $p = 0.039$. Moreover, the ages on set of major homozygous(GG) and heterozygous(GT) of COL2A1rs2070739(G/A) were significantly younger than that of the minor homozygous(AA) of the SNP($p = 0.008$) while the A-T-G-A haplotype of COL2A1 gene revealed significant association with the decreased the risk of bilateral hip lesions, $p = 0.01$, OR:0.258. More important, the serum HDL-c level and the ratio of LDL-c/HDL-c in the ONFH group were significantly decreased and increased compared with those of the control group ($p = 0.02$, $p = 0.0001$), respectively. Particularly, the CC genotype of PPARγ rs2920502 was statistically correlated with the enhanced serum TG level, $p = 0.011$.These results suggest that the variants of PPARγ, RUNX2, COL2A1, and IGFBP3 genes closely associated with the development of ONFH.



## 1. Introduction

Osteonecrosis of the femoral head (ONFH) is a disabling and progressive complex disease caused by the interaction with the genetic and environmental factors [1–2].The animal experiments and clinical investigations have been showed that excess corticosteroids and long-term alcohol abuse are recognized as the environmental risk factors of ONFH, while multiple gene variants have also been proposed as the genetic risk factors of ONFH [3–5]. However, the molecular etiology and pathogenesis of ONFH have been remained obscure. ONFH prevalence has been increased in recent decades and 20,000 to 30,000 new patients in the United States [6] and 150,000 to 200,000 new cases in China are diagnosed with ONFH annually, respectively. Lipid metabolism disorder has been thought as a primary factor of ONFH pathogenesis because the

* Corresponding authors at: Department of Orthopedics of Second Clinical College of Jilin University, Ziqiang Street 218, Changchun 130041, PR China.
*E-mail addresses:* wangjinc@jlu.edu.cn (J. Wang), zgz@jlu.edu.cn (G. Zhang).

http://dx.doi.org/10.1016/j.bone.2017.05.002
8756-3282/

multiple investigations have well proved that the steroid- and alcohol-induced ONFH are closely associated with the lipid metabolism disorder, and moreover, excessive fat accumulation and replacement of myeloid tissue are often observed in the damaged bone marrow cavity concurrent with the increased serum lipid level [7]. Therefore, ONFH is thought as a stem cells disease to link the abnormal transdifferentiation between osteogenesis and adipogenesis of bone marrow mesenchymal stem cells (BMSCs), and the upregulated adipogenesis may exert a crucial effect on the fatty accumulation of ONFH [8,9].

Osteoblasts and adipocytes are derived from a common progenitor cell of BMSCs. The study results have showed that the induction factors of adipogenesis suppress osteogenesis, and, conversely, the induction factors of osteogenesis inhibit adipogenesis [10]. Different signaling pathways and transcription factors play critical roles not only in the maintaining the balance between the bone formation and resorption but also in the transdifferentiation between osteogenesis and adipogenesis of BMSCs. However, it has been unclear that the genes variants of the transcription factors exert the effects on the imbalance between steogenesis and adipogenesis during the development of ONFH. In order to optimize the selection of genes and SNPs of this study, our most important consideration is that the selected genes as well as SNPs need to be identified to maximise the potential associations. Therefore, Functionality of the genes was explored based on published literatures: ① PPARγ and IGFBP3, as an adipogenic transcription factor and central regulator of adipogenic differentiation, respectively, have been though to link the imbalance between adipogenesis and osteogenesis of ONFH [11–13]; ② ONFH is also thought as a stem cells disease to associate with the disorder of bone formation and resorption of BMSCs while osteogenesis-specific transcription factors, RUNX2 and Osterix, play critical roles in the maintaining the balance between the bone formation and resorption of BMSCs [14,15]; ③ the main pathological changes of ONFH involve in articular cartilage lesions, and as a master chondrocyte differentiation factor, COL2A1 controls the genetic program of differentiation of BMSCs into chondrocytes, which potentially correlated with articular cartilage lesions of ONFH [16,17]. Considering the possible effects of these genes on the imbalance between the bone formation and resorption as well as the abnormal transdifferentiation between adipogenesis and osteogenesis of BMSCs during the development of ONFH, we selected PPARγ, IGFBP3, RUNX2, Osterix, and COL2A1 as target genes.

In addition, our study was focused on the SNPs in promoter and coding region with the consideration of potential effects of the SNPs on the gene expression and gene function. Generally, polymorphisms in promoter regions potentially contribute to differential gene expression, presumably affecting the binding of transcription factors to DNA [18]. Accordingly, we selected the 2SNPs in promoter region of IGFBP3, RUNX2, COL2A1 genes, respectively and the 1SNP in promoter region of PPARγ and Osterix genes, respectively, as target SNPs. Moreover, in view of the possible effects of SNPs in exon region on protein expression, function or activity, we also selected the 1SNP in coding regions of COL2A1 and IGFBP3 gene, respectively. We analysed the SNPs genotypes, alleles, haplotypes and their association with the ONFH risk and the clinical phenotypes of ONFH in 200 ONFH patients and 177controls.

## 2. Materials and methods

### 2.1. Subjects

A total of 200 unrelated patients with ONFH (132 males, 68 females; age: 53.46 ± 11.48 yr) were consecutively enrolled at the Department of Orthopedics, the Second Clinical College of Jilin University, (Changchun, China) from March 2014 to June 2015 in the study. Patients with ONFH that was caused by direct trauma were excluded. The patients with ONFH concurrent with severe chronic diseases, such as cardiovascular diseases, congenital diseases, human immunodeficiency virus(HIV) infection, diabetes mellitus, renal dysfunction, and cancer were also excluded. ONFH were diagnosed by evidence of osteonecrosis using plain radiographs in Stages 2, 3, and 4 of the Ficat Classification system [19]. On the basic of the detailed inquiry of medical history and aetiological factors, ONFH patients were classified into one of the following subgroups: alcohol-induced (71 cases (39.7%)), idiopathic (64 cases (34.0%)), and steroid-induced osteonecrosis (47 cases (26.3%)). Steroid-induced osteonecrosis was defined by a history of taking prednisolone cumulative 2000 mg or an equivalent over 21 days. Alcohol-induced osteonecrosis was defined by the consumption of >900 ml of pure ethanol per week. The course of ONFH ranged from 0.5 months to 360 months, with an average of 71.75 months, and the clinical stages of ONFH consisted of 10 cases of stage II (5.6%), 54 cases of stage III (30.2%) and 115 cases (64.2%) of stage IV.

The unilateral and bilateral hips lesions were 76 cases (42.5%) and 103 cases (57.5%), respectively. There were 21 cases of ONFH patients who failed to undergo the clinical stages or aetiological classification duo to the defect of plain radiographs or unclear aetiological factors. Moreover, 177 unrelated health control subjects (112 males, 65 females; age: 50.73 ± 11.02 yr) who were age- and sex-matched for the ONFH group were consecutively enrolled at the Health Examination Centre of Second Clinical College of Jilin University, (Changchun, China) from October 2014 to December 2014. Health control subjects were defined in the following manner: they had no hip pain and their fasting blood glucose, triglyceride and total cholesterol levels in serum were in normal reference range, the abdominal ultrasound examination and chest X-ray radiography were normal, and they did not suffer from cardiocerebrovascular diseases. All of the 377 participants were Han Chinese from northeast China. The study was approved by the ethics committee of the Second Clinical College of Jilin University, Changchun, China, and conformed to the current ethical principles of the Declaration of Helsinki. All participants provided informed consent for their taking part in the study.

### 2.2. Genomic DNA extraction and SNP selection

Approximately 2 ml of venous blood was collected from all of the participants after a minimum of 10 h fasting. Genomic DNA was extracted from whole blood samples using the genomic DNA extraction kit (DP318,TianGen, BeiJing, China) following the manufacturer's protocols. DNA samples concentration and quality were detected spectrophotometrically at 260/280 nm and stored at −80 °C. The database: http://gvs.gs.washington.edu/GVS138/ and related literature were used to select SNPs of the genes by analysing their population distribution in different countries, nationalities and regions, particularly in data obtained from an Asian population. The search scope was from the upstream 2000 bp to downstream 1000 bp of RUNX2, Osterix, PPARγ, COL2A1, and IGFBP3 genes, respectively, including the promoter, 3-UTR, 5-UTR, intron, and exon region. The selection criteria of SNPs included in $r^2 > 0.8$ or $D' = 1$; Minority allele frequencies >0.05. List of 11SNPs of target genes is shown in Table 1.

**Table 1**
Basic information of SNPs in COL2A1, RUNX2, Osteris, PPARγ, and IGFBP3 genes.

| Gene | Chromosome | SNP ID | Allele | Minor Allele | Function |
|---|---|---|---|---|---|
| COL2A1 | 12q13.11 | rs1859444 | G/A | A | promoter |
| | | rs2070739 | G/A | A | missense |
| | | rs3803183 | T/A | A | missense |
| | | rs3809322 | G/A | A | promoter |
| RUNX2 | 6p21 | rs3763190 | G/A | A | promoter |
| | | rs7751427 | A/G | G | promoter |
| Osterix | 12q13.13 | rs4759113 | A/G | G | promoter |
| PPARγ | 3p25 | rs2920502 | G/C | C | promoter |
| IGFBP3 | 7p12.3 | rs2132572 | G/A | A | promoter |
| | | rs2854744 | A/C | C | promoter |
| | | rs2854746 | G/C | C | missense |