Original Article Association between the interaction of SMAD3 polymorphisms with body mass index and osteoarthritis susceptibility

Baolin Kang¹, Feng Zhao¹, Xin Zhang¹, Xiao Deng¹, Xijing He²

¹Department of Orthopaedics, Sengong Hospital of Shaanxi, Xi'an 710300, Shaanxi, China; ²Department of Orthopaedics, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, 157# West 5 Road, Xi'an 710004, Shaanxi, China

Received March 27, 2015; Accepted May 20, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: Purpose: This study aimed to investigate the relationship between the interaction of *SMAD3* polymorphisms (rs12102171 and rs2289263) with body mass index (BMI) and osteoarthritis (OA) susceptibility. Methods: This study involved 112 OA patients and 120 healthy people. The controls were frequency-matched with the cases by age and sex. Hardy-Weinberg equilibrium (HWE) was tested by χ^2 test in the control group. The rs12102171 and rs2289263 polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The relative risk of OA was represented by odds ratio (OR) with 95% confidence interval (CI) calculated by chi-squared test. Gene-environment interaction was analyzed by crossover analysis. Results: The TT genotype and T allele of *SMAD3* rs12102171 polymorphism were more frequent in case than control groups (*P*=0.04 in both of two polymorphisms), which increased the risk of OA (OR=3.39, 95% CI=1.03-11.11 and OR=1.64, 95% CI=1.03-2.59). GG genotype and G allele were also the risk factors for OA (OR=3.22, 95% CI=1.09-9.51 and OR=1.57, 95% CI=1.02-2.42). The BMI had interactions with genotype CC and CT+TT of rs12102171 and TT and TG+GG of rs2289263 (rs12102171: OR=2.15, *P*=0.02 and OR=3.99, *P*=1.00×10⁻³; rs2289263: OR=2.73, *P*=4.00×10⁻³ and OR=4.67, *P*=0). Conclusions: CC and CT+TT and TT and TG+GG genotypes of *SMAD3* rs12102171 and rs2289263 polymorphisms together with BMI may be susceptible factors to OA, and interactions there between can possibly confer risk to OA.

Keywords: SMAD3, polymorphism, body mass index, osteoarthritis

Introduction

Osteoarthritis (OA), also named osteoarthropathy or degenerative arthritis, is a degenerative joint disease that can gradually erode the joint function. Characterized by degenerative loss and abnormal repair of articular cartilage and subchondral bone reconstruction, the disease can be disabled and greatly affect the life qualities around the world [1]. The pathological changes of OA involve all structures of the joint, like the bone, cartilage and synovial tissue, among which the degeneration of the cartilage is the most obvious [2]. The occurrence of OA is a complex process involving many risk factors. such as age, sex, profession, race, obesity, heredity, trauma, inflammation, diet, hormones [3-5]. OA is especially common among middleto-old-aged population featuring the mild symptoms of accidental joint stiffness and intermittent pain, the severe symptoms of restricted range of joint activities and persistent severe pain, and the more serious symptoms of joint instability and limp. With the progression of aging population, the morbidity of OA is also rising year by year [6]. However, the exact etiology and pathogenesis of OA are still indistinct. Epidemiological studies exhibit that OA is a complicated disease induced by the combination of genetic factors and environmental factors [7, 8].

Sma and Mad homologue 3 (SMAD3), also known as mothers against decapentaplegic homolog 3, is a member of SMAD family and it is encoded by the *SMAD3* gene located on chromosome 15q21-22, including 9 exons and 8 introns. Because SMAD3 is the most important

SNP	Primer sequences	Length of amplified fragments		
rs12102171	Forward: 5'-AAAAGCACTGGGCAGAAGGT-3'	285 bp		
	Reverse: 5'-GAGACCTTCTGCCCAGTGCT-3'			
rs2289263	Forward: 5'-CTCTTCAGGGTCAGACTTTA-3'	249 bp		
	Reverse: 5'-ACTTTGTCCCCATACACCTT-3'			

 Table 1. Primer sequences

Table 2. Basic information of the study subjects

Table 2. Basic mormation of the study subjects								
Feature	Sex	Male (n, %)	Female (n, %)	Age $(\overline{x} \pm s)$	BMI	≤24.99	≥25	
Case		47 (41.96)	65 (58.04)	59.1±5.15		41 (36.61)	71 (63.39)	
Control		53 (44.17)	67 (55.83)	58.3±6.39		67 (55.83)	53 (44.17)	
Р	>0.05			>0.05	<0.01			

medium in transforming growth factor- β (TGF- β)/Smad signal transduction pathway and TGF- β plays an important role in the occurrence and development of OA, we predict that the abnormal expression of *SMAD3* gene may be one of the genetic factors affecting OA risk [9, 10].

Therefore, in present study, a case-control method enrolled 112 patients with OA and 120 healthy controls was conducted and rs12102171 and rs2289263 polymorphisms of *SMAD3* gene were selected to explore the correlation of interactions between the two single nucleotide polymorphisms (SNPs) and body mass index (BMI) with OA risk.

Materials and methods

Research objects

112 primary knee osteoarthritis (KOA) patients and 120 healthy people were enrolled in this case-control study. The case group included 47 males and 65 females diagnosed by clinical and imaging examinations at Joint Surgery Department of Tangdu hospital during January, 2013 and December, 2014, with a mean age of 59.1±5.15. The contemporaneous healthy persons frequency-matched with cases by sex and age from the physical examination center of the same hospital including 53 males and 67 females with an average age of 58.3±6.39 were as the controls. The diagnosis of OA was based on the established KOA diagnostic criteria [11]. The severity of OA was graded according to the grading system established Kellgren-Lawrence (K-L) and only patients with a score greater than 2 were included [12]. Patients whose OA was caused by other etiologies listed

below were excluded: (1) histories of knee joint trauma surgery, knee arthrosis infection, adult deformity of the knee and knee joint tumor; (2) inflammatory arthritis (rheumatism, rheumatoid disease, polyarthritis, and autoimmune diseases), traumatic arthritis, suppurative arthritis and bone dysplasia; (3) hypertension and diabetes. Medical examination reports of the controls showed that they did not have any symptoms or performances of arthritis or joint disease (pain, swelling, tenderness, and restricted movement), chronic diseases like cardiopulmonary disease, diabetes and high blood pressure, as well as tumor or autoimmune disease). In order to ensure the accuracy of the study, all study subjects belonged to the Han population of Xi'an and were unrelated each other by blood. Sample collection was carried out in accordance with ethics guidelines of National Human Genome Research Institute. This study was approved by Ethics Committee of Tangdu hospital hospital and received the informed consent of all participants.

Clinical data and sample collection

We recorded the clinical characteristics of all objects such as age, sex, BMI and manual labor status. Complete medical histories of the cases like histories of knee joint trauma, endocrine and metabolic disease, hormone taking, alcohol and tobacco taking were collected.

After obtaining the informed consent of all participants, we collected 5 ml morning fasting peripheral venous blood of every subject, then put them into ethylene diamine tetraacetic acid (EDTA) anticoagulation tubes. Genome DNA was extracted using the phenol-chloroform method and final preserved in a freezer at -20°C.

SMAD3 polymorphisms and osteoarthritis susceptibility

Genotype/		rs12102171					rs2289263				
allele	CC	СТ	TT	С	Т	TT	TG	GG	Т	G	
Case (n,%)	69 (61.61)	32 (28.57)	11 (9.82)	170 (75.89)	54 (24.11)	63 (56.25)	36 (32.14)	13 (11.61)	162 (72.32)	62 (27.68)	
Control (n, %)	85 (70.83)	31 (25.83)	4 (3.34)	201 (83.75)	39 (16.25)	78 (65.00)	37 (30.83)	5 (4.17)	193 (80.42)	47 (19.58)	
OR (95% CI)	1.00	1.27 (0.71-2.29)	3.39 (1.03-11.11)	1.00	1.64 (1.03-2.59)	1.00	1.21 (0.68-2.12)	3.22 (1.09-9.51)	1.00	1.57 (1.02-2.42)	
Р	-	0.42	0.04	-	0.04	-	0.52	0.03	-	0.04	

Table 3. Genotype distributions of rs12102171 and rs2289263 polymorphisms

rs12102171 genotype	CC	CC	CT+TT	CT+TT
BMI	≤24.99	≥25	≤24.99	≥25
Case	23	46	18	25
Control	44	41	23	12
OR (95% CI)	1.00	2.15 (1.11-4.14)	1.50 (0.68-3.32)	3.99 (1.70-9.36)
Р	-	0.02	0.32	1.00×10 ⁻³

Table 4. Interactions of SMAD3 rs12102171 polymorphism with BMI in the development of OA

Genotyping

We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to analyze the SMAD3 rs12102171 and rs2289263 polymorphisms. PCR primers was designed with Primer 5.0 program and synthesized by Shanghai GeneCore BioTechnologies Co., Ltd. The primer sequences were listed in
 Table 1. Each PCR reaction system had a total
 volume of 25 µl, which contained template DNA (1 µl), forward primer (1 µl), reverse primer (1 µI), PCR Master Mix (12.5 µI) and sterile water (9.5 µl). The PCR reaction conditions were presented as follows: 90°C for 5 min; 45 cycles of 90°C for 45 s, 65°C for 50 s, and 72°C for 30 s; and final 70°C for 1 min. The PCR products were digested by Xbal and Hindll restriction enzymes and genotyping results of the two SNPs were shown and analyzed with 2% agarose gel electrophoresis (AGE).

Statistical analysis

We used PASW Statistics 18.0 software to perform the statistical analysis. χ^2 test was applied to analyze the genotypes distribution of the two SNPs in cases and controls (there existed statistical significance in the differences only when *P*<0.05). The gender and BMI were also assessed by χ^2 test and the age was shown in the form of $\bar{x} \pm s$. The Hardy Weinberg equilibrium (HWE) of the controls was tested. Odds ratio (OR) with 95% confidence intervals (CI) calculated by chi-squared test were used to represent the association strength of the SNPs and OA risk. Crossover analysis was adopted for assessing gene-environment interactions.

Results

Clinical data comparison between two groups

Statistical analysis of the basic features in the 112 cases and 120 controls were presented in **Table 2.** No age and sex distribution difference

was observed between the OA patients and the healthy controls (P>0.05). The BMI of the case group was obviously higher than that of the control group (P<0.01).

HWE test

The genotype distributions of *SMAD3* rs121-02171 and rs2289263 polymorphisms in the control group were satisfied with HWE (P>0.05). Therefore, the representativeness of the sample subjects was remarkable.

Correlation analysis of SMAD3 polymorphisms and OA risk

The genotype distributions of *SMAD3* rs121-02171 and rs2289263 could be found in **Table 3.** Compared with the controls, the cases had apparently higher frequencies in genotype TT and allele T of rs12102171 compared with the control group (P<0.05) and they might be the risk factors for OA (OR=3.39, 95% Cl=1.03-11.11 and OR=1.64, 95% Cl=1.03-2.59). In the meanwhile, GG genotype and G allele of rs22-89263 polymorphism were associated with the increased risk of OA (OR=3.22, 95% Cl=1.09-9.51 and OR=1.57, 95% Cl=1.02-2.42).

Effects of the interactions between the two SNPs and BMI on OA

The influences of the interactions between SMAD3 rs12102171 polymorphism and BMI on OA were shown in **Table 4**. The risk of developing OA for CC genotype carriers with a BMI \geq 25.00 was 2.15 times as much as that the BMI \leq 24.99; and compared to CC genotype carriers with a BMI \leq 24.99, the risk of developing OA of CT+TT genotype carriers with a BMI \geq 25 was 3.99 times higher. The results suggested that there were interactions between genotype CC and CT+TT of rs12102171 and BMI, and the greater the BMIs of CC and CT+TT genotype carriers, the higher the risk of developing OA. The role of the interaction between

				•
rs2289263 genotype	TT	TT	TG+GG	TG+GG
BMI	≤24.99	≥25	≤24.99	≥25
Case	21	42	20	29
Control	45	33	29	13
OR (95% CI)	1.00	2.73 (1.37-5.44)	1.45 (0.67-3.13)	4.67 (2.03-10.78)
Р	-	4.00×10 ⁻³	0.35	0

Table 5. Interactions of SMAD3 rs2289263 polymorphism with BMI in the development of OA

AA and AC+CC genotypes of rs2289263 and BMI in the occurrence of OA can be found in **Table 5.** It was also demonstrated that the TT and TG+GG genotype carriers with higher BMIs would also have higher risk of developing OA.

Discussion

OA mainly has the following symptoms: progressive pain and dysfunction of the joint, destruction of the articular cartilage, sclerosis of subchondral bone and synovial hyperplasia. After the onset of the disease, the daily life of the patients will be seriously damaged because of the pain caused by it, and some cannot even stand up and walk. Being lack of related prevention and treatment knowledge and afraid of pain, some patients do not want to move about their knee joints during a long period of time; and as a result, their joints become stiff and they will become disabled at last. In addition to the pain, other common symptoms in the knee include joint swelling and squatting difficulties [13]. Furthermore, the high incidence and prevalence of OA can bring heavy burden to the patients and the society [14].

Matrix metalloproteinases (MMP) have been recently discovered to play critical roles in the process of cartilage and matrix transformation of OA [15, 16]. SMAD is the signal transduction molecule of TGF- β and can transduce the TGF- β signal from the cell membrane into the nucleus, thus mediating the various regulations of TGF-β on cells. SMAD3 is a receptor-activated SMAD, and studies have proved that mice by knockout SMAD3 gene will show symptoms of OA and have significantly higher MMP-13 content in serum than wild type mice [17]. Tardif et al. have found that TGF-ß can regulate content changes of MMP-13 through SMAD3 during the development process of OA [18]. More and more studies have confirmed the close relationship between SMAD and OA [19]. Similarly, the present study ascertained that the frequencies of genotype TT of *SMAD3* rs12102171 and GG of rs2289263 were remarkably higher in case group than in control group and there existed statistically significant differences, and we concluded that they might confer susceptibility to OA.

In the meanwhile, we should not ignore the role of environmental factors in the occurrence and development of OA. People who do heavy manual work are more likely to have OA [20-22]. Also, the incidence of the disease is very high among people who live in damp and cold environment year in year out. That is because low temperature can slow down the blood stream and even cause blood circulatory disorders in local parts of the body. Some scientists have found that OA risk may be related to estrogen deficiency [23]. However, some studies have reported that high estrogen content in the body can increase bone mass and the load on weight-bearing joints, thereby inducing pathological changes of OA [24, 25]. But some other publications have said that a certain level of estrogen in the body may inhibit the occurrence of OA [26, 27]. The mitochondrial DNA mutations in the aging process of human beings can directly affect the cell respiration and energy acquisition, which may be linked to degenerative diseases of the elders. Because women are sensitive to pathogenic factors of OA or have low tolerance to it. OA is more common in women, especially in menopausal women [28, 29]. In addition, serum leptin level will increased in perimenopausal women and it is positively related to BMI [30, 31].

Obesity is one of the major risk factors for KOA [32]. According to the standards recommended by Chinese Nutrition Society, a BMI value $\geq 24 \sim 27.9 \text{ kg/m}^2$ means overweight and a BMI $\geq 28 \text{ kg/m}^2$ stands for obesity. There exist gender differences in the influence of BMI on the onset of OA. Overweight can significantly increase the OA risk of women, but has no

remarkable influence in men. The same conclusion was obtained in the study of Shiozaki et al., which shows that overweight is one of the causes for OA. In the present study, we proved that there existed the interaction between BMI and rs12102171 (CC and CT+TT) and rs2289263 (TT and TG+GG) and it could possibly contribute to the occurrence of OA. Therefore, weight loss is of great significance for the preventing of KOA [33].

OA is a complex process influenced by mutual effects. This study suggested that both *SMAD3* rs12102171 and rs2289263 polymorphisms and BMI had a close correlation with the occurrence of OA. The occurrence of OA can be fundamentally prevented only when we take effective prevention measures and pay great attention to the high risk group. In the mean time, people should control their weight, avoid strenuous exercises or frequent mountain-climbing activities, try to keep warm and get away from humid environment. All in all, researches on the pathogenesis mechanism of OA are very significant for the early diagnosis, prevention and treatment of OA.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xijing He, Department of Orthopaedics, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, 157# West 5 Road, Xi'an 710004, Shaanxi, China. Email: xijingh@gmail.com

References

- [1] Forestier R, Francon A, Briole V, Genty C, Chevalier X and Richette P. Prevalence of generalized osteoarthritis in a population with knee osteoarthritis. Joint Bone Spine 2011; 78: 275-278.
- [2] Horton WE Jr, Bennion P and Yang L. Cellular, molecular, and matrix changes in cartilage during aging and osteoarthritis. J Musculoskelet Neuronal Interact 2006; 6: 379-381.
- [3] Bae JY, Park KS, Seon JK, Kwak DS, Jeon I and Song EK. Biomechanical analysis of the effects of medial meniscectomy on degenerative osteoarthritis. Med Biol Eng Comput 2012; 50: 53-60.
- [4] Felson DT, Zhang Y, Hannan MT, Naimark A, Weissman B, Aliabadi P and Levy D. Risk factors for incident radiographic knee osteoarthri-

tis in the elderly: the Framingham Study. Arthritis Rheum 1997; 40: 728-733.

- [5] Venn G, Nietfeld JJ, Duits AJ, Brennan FM, Arner E, Covington M, Billingham ME and Hardingham TE. Elevated synovial fluid levels of interleukin-6 and tumor necrosis factor associated with early experimental canine osteoarthritis. Arthritis Rheum 1993; 36: 819-826.
- Schuh A, Jezussek D, Fabijani R and Honle W.
 [Conservative Therapy of Knee Osteoarthritis].
 MMW Fortschr Med 2007; 149: 31-32.
- [7] Peach CA, Carr AJ and Loughlin J. Recent advances in the genetic investigation of osteoarthritis. Trends Mol Med 2005; 11: 186-191.
- [8] Arden N and Nevitt MC. Osteoarthritis: epidemiology. Best Pract Res Clin Rheumatol 2006; 20: 3-25.
- [9] Roberts AB, Russo A, Felici A and Flanders KC. Smad3: a key player in pathogenetic mechanisms dependent on TGF-beta. Ann N Y Acad Sci 2003; 995: 1-10.
- [10] Brown KA, Pietenpol JA and Moses HL. A tale of two proteins: differential roles and regulation of Smad2 and Smad3 in TGF-beta signaling. J Cell Biochem 2007; 101: 9-33.
- [11] Doherty M, Watt I and Dieppe P. Influence of primary generalised osteoarthritis on development of secondary osteoarthritis. Lancet 1983; 2: 8-11.
- [12] Shlopov BV, Lie WR, Mainardi CL, Cole AA, Chubinskaya S and Hasty KA. Osteoarthritic lesions: involvement of three different collagenases. Arthritis Rheum 1997; 40: 2065-2074.
- [13] van Weeren PR and de Grauw JC. Pain in osteoarthritis. Vet Clin North Am Equine Pract 2010; 26: 619-642.
- [14] Fautrel B, Hilliquin P, Rozenberg S, Allaert FA, Coste P, Leclerc A and Rossignol M. Impact of osteoarthritis: results of a nationwide survey of 10,000 patients consulting for OA. Joint Bone Spine 2005; 72: 235-240.
- [15] Nagase H and Woessner JF Jr. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491-21494.
- [16] Woessner JF Jr and Gunja-Smith Z. Role of metalloproteinases in human osteoarthritis. J Rheumatol Suppl 1991; 27: 99-101.
- [17] Yang X, Chen L, Xu X, Li C, Huang C and Deng CX. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol 2001; 153: 35-46.
- [18] Tardif G, Reboul P, Dupuis M, Geng C, Duval N, Pelletier JP and Martel-Pelletier J. Transforming growth factor-beta induced collagenase-3 production in human osteoarthritic chondrocytes is triggered by Smad proteins: cooperation between activator protein-1 and PEA-3 binding sites. J Rheumatol 2001; 28: 1631-1639.

- [19] Yuan W and Varga J. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. J Biol Chem 2001; 276: 38502-38510.
- [20] Bosomworth NJ. Exercise and knee osteoarthritis: benefit or hazard? Can Fam Physician 2009; 55: 871-878.
- [21] Minns Lowe CJ, Barker KL, Dewey M and Sackley CM. Effectiveness of physiotherapy exercise after knee arthroplasty for osteoarthritis: systematic review and meta-analysis of randomised controlled trials. BMJ 2007; 335: 812.
- [22] Jamtvedt G, Dahm KT, Holm I, Odegaard-Jensen J and Flottorp S. Choice of treatment modalities was not influenced by pain, severity or co-morbidity in patients with knee osteoarthritis. Physiother Res Int 2010; 15: 16-23.
- [23] Horowitz MC. Cytokines and estrogen in bone: anti-osteoporotic effects. Science 1993; 260: 626-627.
- [24] Wluka AE, Cicuttini FM and Spector TD. Menopause, oestrogens and arthritis. Maturitas 2000; 35: 183-199.
- [25] Parazzini F. Menopausal status, hormone replacement therapy use and risk of self-reported physician-diagnosed osteoarthritis in women attending menopause clinics in Italy. Maturitas 2003; 46: 207-212.
- [26] Richmond JC. Surgery for osteoarthritis of the knee. Rheum Dis Clin North Am 2008; 34: 815-825.

- [27] Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR and Boyce BF. Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. Nat Med 1996; 2: 1132-1136.
- [28] Samson DJ, Grant MD, Ratko TA, Bonnell CJ, Ziegler KM and Aronson N. Treatment of primary and secondary osteoarthritis of the knee. Evid Rep Technol Assess (Full Rep) 2007; 1-157.
- [29] Flugsrud GB, Nordsletten L, Reinholt FP, Risberg MA, Rydevik K and Uhlig T. [Osteoarthritis]. Tidsskr Nor Laegeforen 2010; 130: 2136-2140.
- [30] Michael JW, Schluter-Brust KU and Eysel P. The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. Dtsch Arztebl Int 2010; 107: 152-162.
- [31] Feeley BT, Gallo RA, Sherman S and Williams RJ. Management of osteoarthritis of the knee in the active patient. J Am Acad Orthop Surg 2010; 18: 406-416.
- [32] Manek NJ, Hart D, Spector TD and MacGregor AJ. The association of body mass index and osteoarthritis of the knee joint: an examination of genetic and environmental influences. Arthritis Rheum 2003; 48: 1024-1029.
- [33] O'Connor MI. Sex differences in osteoarthritis of the hip and knee. J Am Acad Orthop Surg 2007; 15 Suppl 1: S22-25.