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The genetic relationship of *SOX9* polymorphisms with osteoarthritis risk in Chinese population

A case-control study

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Abstract

This research aimed to reveal the relationship of SRY-type HMG box 9 (*SOX9*) gene polymorphisms with osteoarthritis (OA) risk in a Chinese population.

Polymerase chain reaction and direct sequencing were used for genotyping polymorphism in 152 OA patients and 139 controls. Firstly, the conformity of genotype distribution to Hardy–Weinberg equilibrium in the control group was checked. The differences in genotype and allele frequencies of our studied polymorphism were compared between the two groups using chi-square test. Odds ratio (OR) with 95% confidence interval (95%CI) was used to appraise the strength of the relationship between the polymorphism and OA occurrence. Cross-over analysis was conducted to reveal the interaction between polymorphisms in *SOX9*.

The AA genotype of the polymorphism rs1042667 was significantly correlated to the increased susceptibility to OA (OR = 2.075, 95%CI = 1.042–4.132). We also detected that the A allele of the polymorphism rs1042667 also obviously increased the occurrence of OA in our study (OR = 1.401, 95%CI = 1.009–1.945). Moreover, the G allele of the polymorphism rs12601701 and the A allele of the polymorphism rs1042667 could significantly elevate the risk of OA (OR = 2.075, 95%CI = 1.021–4.218).

SOX9 polymorphism rs1042667 may be a risk factor for OA in Chinese Han population. The interaction between the polymorphisms rs1042667 and rs12601701 also contribute to OA risk.

Abbreviations: 95%CI = 95% confidence interval, ASPN = Asporin, For. = forward, HWE = Hardy–Weinberg equilibrium, OA = osteoarthritis, OR = odds ratio, PCR = polymerase chain reaction, Rev. = reverse, SNP = single nucleotide polymorphism, SOX = SRY-type HMG box.

Keywords: interaction, osteoarthritis, polymorphisms, *SOX9*

1. Introduction

Osteoarthritis (OA) is the most common degenerative osteoarthropathy, and caused by articular cartilages and bones breakdown.[1,2] It is the leading cause of disability in the middle-aged and elderly people.[3] Basic symptoms of OA include joint pain, stiffness, and movement limitation.[4] In 2009, the number of OA patients was nearly 85 million worldwide, and reached 122 million in 2017 according to previous statistics.[5] OA brings about huge economic burden and heavy life stress on the patients and their family.[4,6] Unfortunately, the pathogenesis of OA is still not fully elucidated. Age, obesity, hormone, and joint injury are several known risk factors for OA occurrence.[7,8] A twin study showed that genetic factors play an important role in the disease pathology.[9] So, this complex multifactorial disease is affected by a large amount of genetic factors apart from other aspects.

Recently, *SOX9* is reported to be possibly correlated to OA occurrence.[10] *SOXs* (SRY-type HMG box) is a novel gene family encoding transcriptional factors which contain a conservative HMG motif domain and are involved in multiple early embryo development progresses including sex determination, bone tissues and nervous system development, and hemocytogenesis.[11] *SOX9* is an important member in SOX family and its coding gene is located on chromosome 22q13.[12] *SOX9* has been proved to regulate the rate of chondrocyte differentiation and hypertrophy, and the expression of proper matrix molecules in chondrocyte, including Col2A1, Col9A2, Col11A1, and proteoglycan.[13] Reportedly, *SOX9* can inhibit the transformation of proliferated chondrocytes into mast cells.[14] Meanwhile, it also plays an important role in chondrocytes hypertrophy, chondrovascularization, and myelopoiesis.[15] It is well known that articular cartilage degeneration and abnormal chondrocyte metabolism are the major etiology of OA.[16,17] However, the role of *SOX9* in OA occurrence is ambiguous.

Single nucleotide polymorphism (SNP) in genes has been regarded as common means of exploring genetic predisposition.

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Table 1

The information of PCR primer sequences.

SNP	Position	Primer sequence	Annealing temperature
Rs12601701	Promoter		54°C
	For.	5'CTAAGTACAGACGACCTGGCTAAA3'	
	Rev.	5'TCAGAAAGTGTAAGAACAGCATCG3'	
Rs1042667	3'UTR		60°C
	For.	5'CCTTCCATCCCGCAGACCCACAG3'	
	Rev.	5'ATCATCTCGGCCATCTTCGCCCT3'	

3'UTR = 3' untranslated region, For. = forward, Rev. = reverse, PCR = polymerase chain reaction, SNP = single nucleotide polymorphism.

Therefore, in the present study, we selected two common SNPs in *SOX9* to investigate their association with OA risk in a Chinese Han population, hoping to provide some clues for OA pathogenesis.

2. Materials and methods

2.1. Subjects

This case-control study selected its subjects of Chinese Han population from Inner Mongolia Autonomous Region People's Hospital, who had no consanguinity between each other. Our study also obtained the permission from the Research Ethics Committee of Inner Mongolia Autonomous Region People's Hospital, and every participant was informed about the study objective and process. Before this study, these subjects also signed written informed consents.

A total of 152 OA patients were enrolled in the case group, who were diagnosed on the basis of their clinical manifestations and radiographic results in the Department of Orthopaedics of Inner Mongolia Autonomous Region People's Hospital, according to the diagnosis criteria of American Rheumatism Association for OA.[18] Exclusion criteria were as follows: with incomplete clinical data; having the history of joint injury and/or any other joint diseases, such as rheumatoid arthritis, arthropathy due to goat, developmental dysplasia, post-traumatic skeletal dysplasia, previous joint infection or injury, etc. Finally, 58 males and 94 females conforming to the inclusion criteria were recruited, and their ages ranged between 39 and 82 years old. In the meanwhile, 139 healthy people were selected from the same hospital as the control group, who experienced physical examination without the history of joint injury. The control group was composed of 42 males and 97 females aged between 35 and 78 years old. The controls were matched with the cases in age and gender distribution.

2.2. Sample collection

Two milliliters of venous blood from every participant was collected in early morning and put into specific blood collection tube with anticoagulation EDTA-2Na. Then blood genomic DNA was extracted using TIANamp Blood DNA Kit (TIANGEN, Beijing China) according to the manufacturer's instruction and stored at −20°C for standby application.

2.3. Genotyping

Genotyping for *SOX9* polymorphisms rs12601701 and rs1042667 were conducted using the method of polymerase chain reaction (PCR) with sequencing. PCR primers were designed with Primer Premier 5.0 software based on *SOX9* sequence in GenBank database at NCBI website, and synthesized in Invitrogen (Shanghai). The primer sequences are shown in Table 1. A volume of 25 μl PCR system was used, and PCR procedures were as follows: 95°C pre-degeneration for 5 min, followed by 33 to 35 cycles of degeneration at 95°C for 45 s, annealing at specific temperatures (Table 1) for 30 s and extension at 72°C for 30 s, and final extension at 72°C for 7 min. The quality of PCR products was detected through 1.0% agarose gel electrophoresis.

The eligible PCR products were sent to Shanghai Sangon Biotech Co., Ltd for sequencing so as to detect corresponding genotypes in the cases and controls.

2.4. Statistical analysis

Genotype and allele frequencies of *SOX9* polymorphisms were gained via direct counting. Genotype distribution of our studied polymorphisms in the control group was checked for whether it accorded with Hardy–Weinberg equilibrium (HWE) using chi-square test. The differences in genotype and allele frequencies of the polymorphism were compared between the two groups via chi-square test, too. Odds ratio (OR) with 95% confidence interval (95%CI) was calculated to estimate the association intensity of *SOX9* polymorphisms with OA risk. The effect of the interaction between *SOX9* polymorphisms on OA occurrence was explored using cross-over analysis. All data processing in the study was completed adopting SPSS 18.0 software, and $P < .05$ meant the presence of statistically significant difference.

3. Results

3.1. HWE test

The genotype distribution of *SOX9* polymorphisms rs12601701 and rs1042667 was checked in the control group, and the results showed their consistency with HWE ($P = .631$ and .405, respectively). Therefore, our control group was a typical Mendelian population.

3.2. Comparing the genotype and allele frequency for SOX9 polymorphisms between the case and control groups

The genotype and allele frequencies of *SOX9* polymorphisms rs12601701 and rs1042667 were compared between the two groups to determine their association with OA susceptibility, and the results are listed in Table 2. Accordingly, the frequencies of the AA, AG, and GG genotypes of the polymorphism rs12601701 were 28.95%, 46.71%, and 24.34% in OA patients and 34.53%, 46.76%, and 18.71% in the controls, with no significant difference ($P = .516$ for AG and $P = .182$ for GG)

Table 2

The genotype and allele distribution of *SOX9* polymorphisms in the case and control groups.

Genotype/allele	Case, n=152 (%)	Control, n=139 (%)	*P*	OR (95%CI)	P_{HWE}
Rs12601701					0.631
AA	44 (28.95)	48 (34.53)	–	Reference	
AG	71 (46.71)	65 (46.76)	0.516	1.192 (0.702–2.024)	
GG	37 (24.34)	26 (18.71)	0.182	1.552 (0.813–2.996)	
A	159 (52.30)	161 (57.91)	–	Reference	
G	145 (47.70)	117 (42.09)	0.174	1.255 (0.904–1.741)	
Rs1042667					0.405
CC	38 (25.00)	46 (33.09)	–	Reference	
AC	78 (51.32)	72 (51.80)	0.321	1.311 (0.767–2.241)	
AA	36 (23.68)	21 (15.11)	0.037	2.075 (1.042–4.132)	
C	154 (50.66)	164 (58.99)	–	Reference	
A	150 (49.34)	114 (41.01)	0.044	1.401 (1.009–1.945)	

95%CI = 95% confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odds ratio.

between the two groups. Besides, similar result was also revealed for the A and G alleles ($P = .174$), so rs12601701 might be not an independent effector for OA. In contrast, the AA genotype of the polymorphism rs1042667 exhibited significantly different frequencies between the two groups (23.68% & 15.11%, $P = .037$), which indicated that the AA genotype was associated with elevated risk of OA occurrence (OR = 2.075, 95%CI = 1.042–4.132). Additionally, the A allele of rs1042667 also had significantly higher frequency in OA patients than in the controls (49.34% & 41.01%, $P = .044$), compared with the common allele C (50.66% & 58.99%), so A allele might be a risk factor for OA as well (OR = 1.401, 95%CI = 1.009–1.945).

3.3. The interaction effect between SOX9 polymorphisms rs12601701 and rs1042667 on the onset of OA

In this study, the effect of the interaction between *SOX9* polymorphisms rs12601701 and rs1042667 on OA occurrence was also analyzed (Table 3). The results demonstrated that individuals carrying genotypes with risk alleles of both rs12601701 and rs1042667 would face increased OA risk by 1.075 times, compared with those with the AA genotype of rs12601701 and the CC genotype in rs1042667 (OR = 2.075, 95%CI = 1.021–4.218). However, harboring only one risk allele of rs12601701 or rs1042667 did not show significant association with OA occurrence ($P > .05$).

4. Discussion

In the present study, we primarily investigated the relationship of *SOX9* polymorphisms rs12601701 and rs1042667 with OA susceptibility in a Chinese Han population. The results showed that carrying the AA genotype in rs1042667 increased OA risk by 2.075 times when compared with the CC genotype, and so did the A allele. Rs1042667 might be the risk factor of OA in Chinese population. However, we did not find any significant association for the polymorphism rs12601701 with the disease. To our knowledge, this study for the first time paid attention to this topic. In addition, the interaction effect between *SOX9* polymorphisms rs12601701 and rs1042667 on OA development was also analyzed in the study, and the results manifested that the combination of any genotypes having the G allele in rs12601701 with any genotypes containing the A allele in rs1042667 obviously increased the susceptibility to OA

OA is common in elderly people, and usually causes joint pain and stiffness, the loss of joint function, and even disability. It is important to explicitly explain the disease pathogenesis for early precaution and symptomatic treatment. According to previous reports, the occurrence of OA is affected by multiple factors.[19] Age has been proved to be an important risk factor for the disease and can maximize multiple adverse factors involved in OA.[20] Obesity and sex hormone are also reportedly associated with the onset of OA.[21,22] Moreover, several singling pathways have already been proposed to be involved in OA development, such as Notch pathway.[23] However, these aspects could not explain all OA cases.

Abnormal changes in articular cartilage and cartilage cell metabolism indicate OA development. In previous studies, many genes associated with cartilage cell metabolism have been put forward to be correlated to OA. *GDF5* rs143383 polymorphism significantly increased the risk of knee OA in the study by Tawonsawatruk.[24] Asporin (*ASPN*) D14 allele and 13301537 polymorphisms were exhibited to possess obvious association with OA risk.[25,26] Mototani reported that the TT genotype of the SNP in promoter region of the gene *CALM1* was significantly associated with OA risk through genome-wide scanning in Japanese population.[27]*SOX9* has also been discovered to participate in the metabolism of cartilage cells.

Table 3

The interaction association of *SOX9* rs12601701 and rs1042667 polymorphisms in OA risk.

rs12601701	rs1042667	Case, n (%)	Control, n (%)	*P*	OR (95%CI)
AA	CC	15 (9.87)	26 (18.71)	–	Reference
AA	AC+AA	29 (19.08)	22 (15.83)	0.053	2.285 (0.983–5.309)
AG+GG	CC	23 (15.13)	20 (14.39)	0.120	1.993 (0.832–4.774)
AG+GG	AC+AA	85 (55.92)	71 (20.14)	0.041	2.075 (1.021–4.218)

95%CI = 95% confidence interval, OA = osteoarthritis, OR = odds ratio.

In fetuses and adults, *SOX9* is expressed in various tissues, including brain, lung, liver, and skeletal muscle, and can recognize different target genes in the tissues. In cartilage development, *SOX9* can activate the transcription of *Col2a* which encodes the most abundant protein Col2 (type II collagen) in cartilage tissue.[28] In cartilage cells, *SOX9* forms protein complex with L-SOX5 and SOX6 to affect the enhancer sequence *Col*2a and *Col*112a, and thus enhances their transcription.[29] What's more, β-catenin is a major molecule in Wnt pathway and regulates the formation and development of bone and joint, the key mechanism of OA.[30] Decreased expression of *SOX9* combined with Col2 caused by β-catenin overexpression leads to phenotypic alternation of cartilage cells. Therefore, it is well-founded that *SOX9* may be associated with OA. However, the associations of SNPs in *SOX9* with OA susceptibility are rarely reported. The polymorphism rs12601701 is located in the promoter region of the gene *SOX9*, and significantly associated with the risk of osteonecrosis of the femoral head.[31] Another SNP rs1042667 is a mutation in 3'UTR of *SOX9* and was reported to contribute to the risk of gliomas.[32]

In conclusion, *SOX9* polymorphism rs1042667 shows significant association with the occurrence of OA in Chinese Han population, but not rs12601701. Furthermore, the interaction between the polymorphisms rs12601701 and rs1042667 is suggested to contribute to the risk of OA as well. Nevertheless, we have to recognize several limitations in our study. Sample size was relative small and study population only contained one Chinese nationality, Han. Moreover, environmental factors were also not taken into account. Therefore, further studies with larger sample sizes and multiple populations are needed to verify our findings and give more clearer illustration on OA mechanism in the future.

Author contributions

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