Clinical Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Genetic variation of *SMAD3* is associated with hip osteoarthritis in a Chinese Han population

Journal of International Medical Research
2018, Vol. 46(3) 1178–1186

DOI: 10.1177/0300060517745186
journals.sagepub.com/home/imr
SAGE

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Abstract

Objective: This study was performed to investigate the association between genetic variation in *SMAD3* and hip osteoarthritis (OA) in a Chinese Han population.

Methods: The frequency of two single nucleotide polymorphisms of *SMAD3*, rs1470002 and rs12901499, was examined in 500 patients with hip OA and 1080 healthy controls in a Chinese Han population. Further analysis was performed according to sex and age.

Results: We detected statistically significant differences in the allele frequency and genotype between the hip OA and healthy control groups. The frequency of the GA+GG and GA genotypes of rs12901499 and the G variant were much higher in patients with hip OA than in healthy controls. This association was also present when the participants were stratified by sex and age. However, there was no significant association between the risk of hip OA and the presence of rs1470002 GA, AA, or GA+AA genotypes, even after sex- and age-stratified analysis.

Conclusions: The *SMAD3* SNP rs12901499 GA genotype and G variant may increase the risk of hip OA in Chinese Han patients.

Keywords

Hip osteoarthritis, single nucleotide polymorphism, *SMAD3*, Chinese Han, case–control study, PCR

Date received: 4 April 2017; revised 15 October 2017; accepted: 8 November 2017

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Introduction

Osteoarthritis (OA) is characterized by the degeneration of articular cartilage.[1] It is a main cause of skeletal disability secondary to narrowed joint spaces and subchondral sclerosis, leading to pain and stiffness.[2] OA has multiple etiologies, including obesity, aging, and previous injury.[3–5] Genetic studies have shown that many candidate genes encoding proteins involved in vitamin D metabolism and collagen synthesis as well as genes encoding bone and cartilage growth factors are associated with OA.[6,7]

The gene encoding SMAD family member 3 (*SMAD3*) is located on chromosome 15q21–22.[8] SMAD3 is a key intracellular messenger in the transforming growth factor-β (TGFβ) signaling pathway, which has important anabolic effects on chondrocytes. TGFβ stimulates type II collagen and proteoglycan synthesis and downregulates the expression of cartilage-degrading enzymes. Moreover, TGFβ can counteract interleukin-1-induced suppression of proteoglycan synthesis.[9] The role of TGFβ in OA is likely derived from its effects in maintaining the stable phenotype of articular chondrocytes.[10] The TGFβ signaling pathway is initiated by phosphorylation of the intracellular mediators SMAD2 and SMAD3 (also known as MADH3) in response to the activation of TGF receptors. Once activated, SMAD3 translocates into the nucleus to interact with transcription factors and DNA to modulate target gene transcription.[11] SMAD3 is thus a downstream mediator of TGFβ signals.[12] A previous study showed that the single nucleotide polymorphism (SNP) rs12901499, which maps to intron 1 of *SMAD3*, was associated with both knee and hip OA in European populations.[13] Another study revealed that this SNP was related to both knee OA and hand OA in a Chinese population.[14]

In this study, we investigated whether rs12901499 and another *SMAD3* SNP, rs1470002 which was previously found not to be significantly associated with hip OA in European populations,[13] are associated with the pathogenesis of hip OA in a Chinese Han population and performed subgroup analyses according to age and sex.

Methods

Study population

This population-based case–control study involved Chinese Han patients with hip OA treated at the Department of Orthopedics, Tongde Hospital of Zhejiang Province, Hangzhou, China from May 2009 to May 2016. Hip OA was defined by a modified Croft grade of ≥ 2 for either hip.[15] The controls were participants who had not undergone total hip replacement and who had a Croft grade of ≤ 1 in both hips.[16] The exclusion criteria were inflammatory arthritis, post-traumatic skeletal dysplasia, and developmental dysplasia of hip joint disease.

All participants provided written informed consent. The study protocol was approved by the Ethics Committee of Tongde Hospital of Zhejiang Province, Hangzhou, China.

Genotyping

Two *SMAD3* SNPs were selected for this study: rs12901499 and rs1470002. Venous blood samples were acquired from all participants using 20 g/L of ethylenediaminetetraacetic acid anticoagulant. Genomic DNA was immediately extracted from a 250-μl sample of blood with the QIAamp DNA Blood Kit according to the manufacturer's instructions (Qiagen, Basel, Switzerland).[17] Samples were stored at –80°C until analysis. SNPs rs1470002 and rs12901499 in *SMAD3* were genotyped using the TaqMan SNP Genotyping Assay

(Thermo Fisher Scientific, Foster City, CA).[18] PCR was performed using the 9700 GeneAmp PCR system (Applied Biosystems, Foster City, CA) according to previously described methods. [19,20] ABI PRISM 9700 Sequence Detection System software version 2.0 (Applied Biosystems) was used to analyze the data. Approximately 5% of the samples were subjected to a second genotyping for confirmation of results.

Real-time PCR analysis

Total RNA was extracted from venous blood using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and real-time PCR analysis was performed. The relative expression levels of matrix metalloproteinase 13 (*MMP13*) and type II collagen (*COL2A1*) mRNA were measured, while 18S rRNA was used as an internal reference as described in our previous study.[21]

Statistical analyses

We used the χ^2 test to confirm the Hardy–Weinberg equilibrium of observed and expected genotype frequencies in healthy controls. We also used the Wilcoxon test to analyze the age, and the χ^2 test for all other variances to evaluate differences in the distributions of demographic characteristics for rs1470002 and rs12901499 genotypes in patients with hip OA and healthy controls. We estimated the associations between the *SMAD3* variants and risk of hip OA by determining the 95% confidence intervals and odds ratios using multivariate and univariate logistic regression model analyses after adjusting for age, sex, and body mass index (BMI). We performed two-sided tests using SPSS version 16.0 (SPSS Inc., Chicago, IL) to determine whether the differences were statistically significant. Statistical significance was defined as $P < 0.05$. The statistical power of the study, 0.9, was determined using Power and Sample Size Calculation software version 3.0 (Department of Biostatistics, Vanderbilt University, Nashville, TN) and previously described methods.[22]

Results

Baseline characteristics

This study included 1580 Chinese Han participants, of whom 500 were patients with hip OA and 1080 were controls. The demographic characteristics were matched between the controls and patients. There were no significant differences in baseline characteristics between the two groups (Table 1).

The genotype frequencies of rs1470002 and rs12901499 *SMAD3* SNPs in the healthy controls were consistent with the expected scores calculated from the Hardy–Weinberg principle (data not shown). Repeated genotyping results were 100% concordant between trials.

Association of SMAD3 polymorphisms with hip OA susceptibility

The allele distributions and genotype of *SMAD3* rs12901499 in patients with hip

Table 1. Demographic characteristics of Chinese Han patients with hip osteoarthritis and controls

Characteristic	Patients with hip osteoarthritis (n = 500)	Controls (n = 1080)
Age, years	65.9 ± 7.5	64.4 ± 8.9
Weight, kg	68.6 ± 8.6	67.2 ± 7.7
Height, cm	162.2 ± 7.1	161.6 ± 7.4
Body mass index, kg/cm^2	26.0 ± 3.9	25.7 ± 4.4

Data are presented as mean ± standard deviation.
No statistically significant between-group differences ($P \geq 0.05$) were observed using Wilcoxon's test for age and the χ^2 test for other parameters.

Table 2. Associations between *SMAD3* SNPs rs12901499 and rs1470002 and risk of hip OA in Chinese Han patients with hip OA and controls

SNP	Genotype/ allele	Patients with hip OA (n = 500)	Controls (n = 1080)	Statistical significance[a]	Adjusted odds ratio[b]	95% confidence intervals[b]
rs12901499	GG	290 (58.0)	450 (41.7)	$P < 0.001$	1.93	[1.20, 3.13]
	GA	200 (40.0)	610 (56.4)	$P < 0.001$	0.51	[0.32, 0.83]
	AA	10 (2.0)	20 (1.9)	NS	1.08	[0.19, 6.01]
	GA+AA	210 (42.0)	630 (58.3)	$P < 0.001$	0.52	[0.32, 0.84]
	A	220 (22.0)	650 (30.1)	$P < 0.001$	0.66	[0.44, 0.97]
	G	780 (78.0)	1510 (69.9)	$P < 0.001$	1.53	[1.03, 2.26]
rs1470002	CC	270 (54.0)	600 (55.5)	NS	0.94	[0.58, 1.51]
	CT	190 (38.0)	400 (37.0)	NS	1.04	[0.64, 1.70]
	TT	40 (8.0)	80 (7.7)	NS	1.09	[0.45, 2.63]
	CT+TT	230 (46.0)	480 (44.4)	NS	1.06	[0.66, 1.71]
	T	270 (27.0)	560 (25.9)	NS	1.06	[0.72, 1.54]
	C	730 (73.0)	1600 (74.1)	NS	1.12	[0.65, 1.38]

Data are presented as n (%) of patients. OA, osteoarthritis.

[a]Patients with knee OA versus controls; χ^2 test.

[b]Versus TT genotype or T allele for rs1470002, or [b]Versus AA genotype or A allele for rs12901499; estimated using multiple logistic regression analyses and adjusted for age, sex, and body mass index. NS, no statistically significant difference ($P \geq 0.05$).

OA and controls showed significant differences ($P < 0.001$) (Table 2). An increased susceptibility to hip OA was significantly associated with the GA or GG genotype of rs12901499 compared with the AA genotype after adjusting for age, sex, and BMI. Additionally, allele carriage (GA+AA genotype) was associated with a decreased susceptibility to hip OA compared with the GG genotype. Meanwhile, neither the CT nor TT genotype of *SMAD3* rs1470002 was significantly associated with susceptibility to hip OA (Table 2).

We also performed a stratification analysis to evaluate the detailed associations of *SMAD3* rs12901499 genetic variants with hip OA susceptibility in subgroups classified by age and sex. In the model stratified by age, the study participants were classified as "old" (>65 years) or "young" (≤65 years). The stratified analysis showed that both young and old patients with hip OA had significant differences in genotype frequencies compared with controls ($P < 0.001$) (Table 3). When the association between carriers of one allele (GA or AA genotype) and the susceptibility to hip OA was evaluated, young GA heterozygotes had a 0.44-fold decrease in hip OA susceptibility relative to GG homozygotes (Table 3). Similarly, in older patients with hip OA, GA heterozygotes had a 0.60-fold reduction in hip OA susceptibility compared with GG homozygotes (Table 3). Sex-stratified analysis showed that men and women exhibited significant differences in genotype frequencies among patients with hip OA compared with healthy controls ($P < 0.01$) (Table 3). Moreover, male and female GA heterozygotes had a 0.51- and 0.52-fold reduction in hip OA susceptibility, respectively, relative to GG homozygotes (Table 3).

However, logistic regression analysis of *SMAD3* rs1470002 variants revealed that CT and TT genotypes were not associated with hip OA susceptibility or significantly stratified by sex or age (Table 4).

We regarded an rs12901499 GG status as SNP-positive and an rs12901499 AA status as SNP-negative. SMAD signaling in the

Table 3. Logistic regression analysis of rs12901499 genotype frequencies and risk of hip OA in Chinese Han patients with hip OA and controls

	Patients with hip OA (n = 500)				Controls (n = 1080)				AA versus GG		GA versus GG	
	n	GG	GA	AA	n	GG	GA	AA	Adjusted OR[a]	95% CI[a]	Adjusted OR[a]	95% CI[a]
Age group	500	290	200	10	1080	450	610	20				
≤65 years	270	155 (57.4)	110 (40.7)	5 (1.9)	550	205 (37.3)	335 (60.9)	10 (1.8)	1.02	0.09–11.49	0.44	0.23–0.86
>65 years	230	135 (58.7)	90 (39.1)	5 (2.2)	530	245 (46.2)	275 (51.9)	10 (2.0)	1.16	0.39–3.42	0.60	0.44–0.82
Sex												
Male	240	135 (56.2)	100 (41.7)	5 (2.1)	500	200 (40.0)	290 (58.0)	10 (2.0)	1.04	0.09–11.79	0.52	0.26–1.04
Female	260	155 (59.6)	100 (38.5)	5 (1.9)	580	250 (43.1)	320 (55.2)	10 (1.7)	1.12	0.38–3.30	0.51	0.38–0.68

Data are presented as n (%) of patients.

OA, osteoarthritis; OR, odds ratio; CI, confidence interval.

[a]Adjusted for the other covariate presented in this table and for body mass index using a logistic regression model for each stratum.

Table 4. Logistic regression analysis of rs1470002 genotype frequencies and risk of hip OA in Chinese Han patients with hip OA and controls

	Patients with hip OA (n = 500)				Controls (n = 1080)				TT versus CC		CT versus CC	
	n	CC	CT	TT	N	CC	CT	TT	Adjusted OR[a]	95% CI[a]	Adjusted OR[a]	95% CI[a]
Age group	500	270	190	40	1080	600	400	80				
≤65 years	270	150 (55.6)	100 (37.0)	20 (7.4)	550	310 (56.3)	200 (36.4)	40 (7.2)	1.02	0.29–3.55	1.03	0.52–2.02
>65 years	230	120 (52.2)	90 (37.0)	20 (8.7)	530	290 (54.7)	200 (37.7)	40 (7.5)	1.17	0.33–4.09	0.97	0.47–1.98
Sex												
Male	240	135 (56.3)	85 (35.4)	20 (8.3)	500	275 (55.0)	185 (37.0)	40 (8.0)	1.05	0.30–3.66	0.93	0.46–1.91
Female	260	135 (55.8)	105 (36.5)	20 (7.7)	580	325 (56.0)	215 (37.1)	40 (6.9)	1.13	0.32–3.92	0.98	0.50–1.93

Data are presented as n (%) of patients.

OA, osteoarthritis; OR, odds ratio; CI, confidence interval.

[a]Adjusted for the other covariate presented in this table and for body mass index using a logistic regression model for each stratum.

OA was associated with catabolic events inducing the production of MMPs. The mRNA levels of *MMP13* and *COL2A1* were then tested in SNP-positive and -negative patients. The expression of *MMP13* was higher in SNP-positive than -negative patients (Figure 1), while *COL2A1* expression was lower in SNP-positive than -negative patients (Figure 2).

Discussion

This case–control study investigated associations between *SMAD3* rs1470002 and rs12901499 SNPs and the susceptibility to

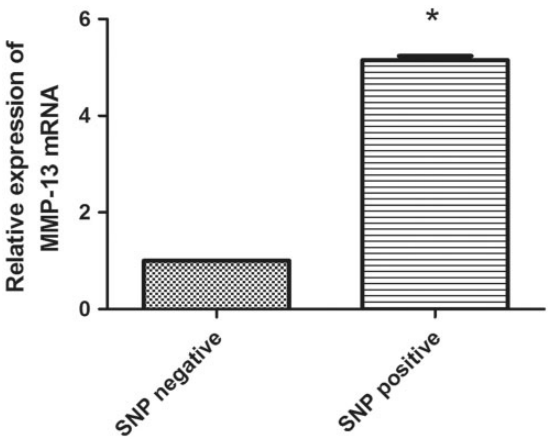

Figure 1. Relative expression levels of *MMP13* mRNA in SNP-negative and -positive patients were analyzed by real-time PCR. The bar represents the mean ± standard error of two independent experiments. *$P < 0.05$ compared with SNP-negative patients. *MMP13*, matrix metalloproteinase 13; SNP, single nucleotide polymorphism.

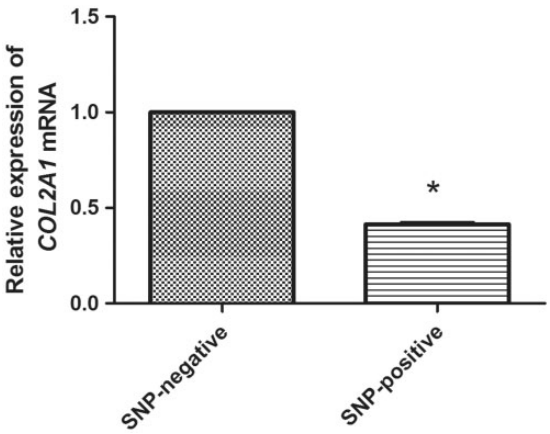

Figure 2. Relative expression levels of *COL2A1* mRNA in SNP-negative and -positive patients were analyzed by real-time PCR. The bar represents the mean ± standard error of two independent experiments. *$P < 0.05$ compared with SNP-negative patients. *COL2A1*, type II collagen; SNP, single nucleotide polymorphism.

hip OA in a Chinese Han population. The data demonstrated that the rs12901499 GA genotype and G variant may increase the risk of hip OA in Chinese Han patients. This risk was increased for female and older patients. This is the first study to associate the *SMAD3* SNP rs12901499 with hip OA in a Chinese Han population.

SMAD3 plays an important role in TGFβ signaling, which stimulates chondrocyte anabolism and maintains the phenotype of articular chondrocytes. Loss of SMAD3 enhances bone morphogenetic protein signaling in articular chondrocytes, resulting in OA-like changes. *SMAD3* has more than 10 polymorphic sites,[13] of which rs12901499leads to an intronic A-to-G transition.

A preliminary study indicated a feasible association between the rs12901499 SNP and adult hip and knee OA in a European population.[13] Another study demonstrated that rs12901499 polymorphisms could increase the risks of knee OA and hand OA in a Chinese population.[14,23,24] However, no previous studies have examined the association between SNP rs12901499 and risk of hip OA in a Chinese Han population. Consistent with earlier findings, this study supports the indication that SNP rs12901499 might be a risk factor for OA. Individuals with *SMAD3* rs12901499 GA or GG+GA genotypes carried a higher risk of hip OA than those with the AA genotype, indicating that the G allele of rs12901499 might be associated with the development of hip OA in the Chinese Han population.

Based on our stratification analysis, the association between hip OA susceptibility and rs12901499 GA heterozygosity was also positively stratified by age and sex compared with GG homozygosity. This result is consistent with the increased incidence of hip OA seen in females and patients older than 50 years.[25] Because OA is a multifactorial disease, both gene–environment and gene–gene interactions may be involved in disease development and pathology; thus one SNP is unlikely to be sufficient to predict the overall risk. Therefore, additional research is needed to reveal the role of other SNPs of related genes which participate in other pathways that may affect the etiology of OA.

We found that the rs1470002 SNP was not related to the risk of hip OA in this Chinese Han population, which is similar to the findings of a previous study showing no significant association between the rs1470002 SNP CT, TT, or CC genotype and hip OA in a European population.[13] Additionally, further stratification analysis with respect to sex and age revealed no significant associations between rs1470002 genotypes and hip OA susceptibility.

SMAD signaling in patients with OA is associated with catabolic events that induce MMP production. Type II collagen is the major component of the extracellular matrix degraded by MMP13. In the present study, the expression of *MMP13* was higher and that of *COL2A1* was lower in the *SMAD3* rs12901499-positive than -negative group among patients with hip OA, which suggests a possible mechanism underlying the association between rs12901499 and the risk of hip OA.

This study has several limitations. First, as a hospital-based study, inherent biases are likely to exist. However, our results were free from selection bias as indicated by the fact that the frequency of the *SMAD3* G allele in the controls was similar to the expected values from the haplotype map database,[26] and that rs1470002 and rs12901499 genotype distributions in the controls agreed with Hardy–Weinberg equilibrium. Second, this study had a modest sample size, and the results should therefore be further confirmed in larger studies. Third, we only investigated two *SMAD3* SNPs in this study, and other SNPs located

in other loci may be associated with the risk of hip OA. These SNPs should be investigated in future studies.

Conclusions

In conclusion, this was the first study to demonstrate that the *SMAD3* rs12901499 genotype distribution was different between hip OA patients and healthy controls in a Chinese Han population. Larger-scale studies and evaluations at a molecular level are needed to confirm the current results and to investigate the detailed roles of the rs12901499 polymorphism in the pathology of hip OA.

Acknowledgments

We thank John Martin for carefully reading the manuscript and Wei Wang for providing technical support.

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the Zhejiang Provincial Department of Science and Technology (no. 2015C37131), the Chinese Medicine Research Program of Zhejiang Province, China (no. 2015ZB028), and the Zhejiang Natural Science Foundation of China (LQ18H060001).

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