SYSTEMATIC REVIEW

THE ROLE OF RS12901499 POLYMORPHISM IN ASSOCIATION WITH SMAD3 GENE AND OSTEOARTHRITIS SUSCEPTIBILITY: SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Osteoarthritis is regarded as one of the most frequent disorders of musculoskeletal, which is characterized by the degeneration of articular cartilage and loss of cartilage of the joints. However, the relationship of OA susceptibility with rs12901499 polymorphism in SMAD3 is controversial. Although multiple studies have investigated the correlation of rs12901499A/G polymorphism in SMAD family member 3 (SMAD3) and osteoarthritis (OA) susceptibility, the results from previous studies remain controversial and unsolved. A meta-analysis utilizing fixed and random effects model was performed to clarify the association. **Methods:** Eligible studies were systematically searched from PubMed, Web of Science, Cochrane Library and EMBASE on April 17, 2019 for reporting the correlation of rs12901499 polymorphism and osteoarthritis susceptibility. Pooled Odds ratio of 95% confidence interval was performed to estimate the strength of relationship of rs12901499 polymorphism and osteoarthritis susceptibility. Publication bias was detected by Begg's test and STATA 11.0 software was used to evaluate statistical analysis. **Results:** Seven case-control papers involving eight studies from Caucasian and Asian populations were included. A significant increase in osteoarthritis susceptibility was found in recessive, homozygous and allele models. Stratified analysis on ethnicity suggested that the polymorphism with increased risk of OA only in Asians under allele model. Stratified analysis related to population-based studies indicated the increased risk of OA with polymorphism in recessive, homozygous, allele and dominant models. **Conclusion:** This meta-analysis demonstrated that there may be a weak association of rs12901499 polymorphism and OA susceptibility. Due to the limited size of sample and given ethnic groups, more studies need to validate the result in future.

Keywords: SMAD3; rs12901499; Osteoarthritis; Polymorphism; Meta-analysis

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INTRODUCTION

Osteoarthritis is regarded as one of the most frequent disorders of musculoskeletal, which is characterized by the degeneration of articular cartilage and loss of cartilage of the joints.[1,2] Osteoarthritis is a multifactorial disease involved by aging, sex, hormones, obesity, and previous injury.[3–5] Several human disorder processes, including Osteoarthritis showed an association with Transforming growth factor-beta (TGF-β) family signalling function pathways.[6] Growing evidence indicates that TGF-β makes a great contribution to the pathogenesis and progression of OA.[7] TGF-β contributes to anabolic effects on chondrocytes by way of the SMAD3 genes signalling, plays a significant role to maintain homeostasis of synovial joints.[7] The gene encoding SMAD3 is located on chromosome 15q21–22, which acts as a key intracellular messenger in the TGF-β signalling pathway.[8] Recently, increasing studies reported the correlation of SMAD3 with rs12901499 polymorphism and risk of OA, but the results are not consistent.[9–15] Previous literatures indicated the link of OA susceptibility with rs12901499 polymorphism in SMAD3.[10,11,14,16] On contrary, few studies could not affirm the association.[12,15] Small sample studies lack enough statistical data to evaluate the correlation of rs12901499 polymorphism with Osteoarthritis risk. Therefore, we investigated the uncertain correlation of rs12901499 polymorphism and OA susceptibility risk by a genetic meta-analysis.

MATERIAL AND METHODS

Eligible potential papers reporting the association of rs12901499 polymorphism and osteoarthritis susceptibility were systematically searched from Web of Science, Cochrane Library, PubMed, and EMBASE on April 17, 2019. Following search

terms were used without any language limitation; "osteoarthritis," OR "OA," AND "SMAD3," OR "MADH3," "SMAD family member 3," OR "polymorphism," OR "variant", OR "mutations," OR "Alleles," OR "single nucleotide polymorphism," OR "genotype," OR "SNP". Besides, studies from references were also searched manually to gain potential articles about this field. The results with disagreements were resolved by consensus.

Studies which met the following criteria were included: (1) original papers designed in a case-control mode; (2) studies assessing the correlation of rs12901499 polymorphism in SMAD3 and risk of osteoarthritis susceptibility; (3) availability of data corresponding to number of case-controls and number of case-controls related to A/A, A/G, and G/G genotypes; and (4) research population was human being. Studies were excluded if they were: (1) insufficient OR data calculated from raw studies, review articles, case reports, and comments; (2) studies without the raw data regarding the rs12901499 polymorphism; (3) animal studies. The latest studies were retained once the studies were duplicated.

Data extraction was performed according to a pre-defined data extraction form by two independent experienced researchers and disagreements were resolved by discussion among all the authors. The following article characteristics were recorded: first author, country of study performed, year of publication, ethnicity of subjects, total number of case-control groups, number of case-control related to A/A, A/G, and G/G genotypes, genotyping method, lesion locations, source of control, and the P value for Hardy-Weinberg equilibrium (HWE).

Two independent authors evaluated the quality of observational studies by one of the widely used rating systems 9-star Newcastle-Ottawa Scale (NOS).[17] The Newcastle-Ottawa Scale system involves 3 aspects: comparability, exposure and case-control selection. Each aspect consists of 2, 3, and 4 items respectively. Each item values one score, with a total of nine scores. The NOS scores of each single study equal or greater than 6 stars were defined as high quality.

STATA 11.0 software (Stata Corp, College Station, TX, USA) was performed for all pooled statistical analyses. The correlation strength of rs12901499 polymorphism in SMAD3 and risk of osteoarthritis susceptibility was assessed by calculating OR along with 95% CI. Pooled OR values were calculated by the Z-test. The Hardy-Weinberg equilibrium (HWE) for each single nucleotide polymorphism was measured by the $\chi 2$ test. The fixed effects model or the random-effects model were used to combine the summary OR values.[18] p-value < 0.05 was recognized as significantly different. Heterogeneity was tested among studies by Q-test and I2 statistic tests with $I^2<50\%$ indicating little difference. Sensitivity analysis was performed to evaluate the effect of each study on the pooled results by deleting each study to test the stability and consistency of results. Begg's funnel plot test was used to assess the publication bias, with p-value <0.05 to indicate a significant difference.

RESULTS

An initial search was made through electronic database which yielded a total of 67 references. Figure-1 show the study selection process which is displayed through a flow chart. Eventually, according to the inclusion criteria, seven original studies involving eight studies were identified for this study. Table-1 described the detailed characteristics of eight eligible studies. All included articles were designed in a case-control format. Among eight studies, 7 were recorded among Asians and 1 among Caucasians. The analysed single nucleotide polymorphism was within HWE in five studies[10–12,14], while another three studies were not consistent with the HWE[9,13,15]. The Newcastle-Ottawa Scale results showed the score of studies ranged from 7–8, which indicates that the methodological quality was reliable in eight included studies (Table-1). The frequency of each allele and genotype with Hardy-Weinberg equilibrium value is presented in table-2.

The meta-analysis to estimate the relationship between rs12901499 polymorphism and OA risk comprised 8 studies including 2403 cases and 3209 controls. Study results of five genetic models are presented in Table-3. Overall, when eight included studies were combined with random-effects model, a significant increase in OA susceptibility was found in the allele model (A vs. G: OR=1.32, 95%CI: 1.08-1.62, p=0.006), homozygous model (AA vs. GG: OR=1.46, 95% CI: 1.04–2.05; p=0.03), and recessive model (GG vs. AA+AG: OR=1.38, 95% CI: 1.01–1.88; p = 0.04), but not for the dominant and heterozygous model (AG+GG vs. AA : OR=1.04, 95%CI: 0.96-2.04; p=0.082), (AA vs. AG: OR= 1.29, 95%CI: 0.82–2.02; p=0.275) respectively. The forest plot of pooled OR of the association of rs12901499 and risk of OA susceptibility under homozygous model is presented in figure-2. To investigate the potential association among ethnicities, lesion

locations, source of control, and genotypic methods, a stratified analysis was further performed. In the subgroup analysis related to ethnicity, a significant increase in OA susceptibility was found only in Asian subgroup in the allele model (A vs. G: OR=1.34, 95% CI: 1.07–1.69, p = 0.012). Subgroup analysis based on lesion locations suggested that increased risk of knee OA in five models has no association with polymorphism rs12901499. Stratified analysis related to population-based studies indicated the association of polymorphism with increased risk of osteoarthritis in the allele (A vs. G: OR = 1.71, 95% CI: 1.43–2.05; p<0.001), homozygous (AA vs. GG: OR=2.04, 95% CI: 1.26–3.29; p=0.003), recessive (GG vs. AA+AG: OR=1.56, 95% CI: 1.05–2.30; p=0.027), and dominant models (AG+GG vs. AA: OR=2.49, 95% CI: 1.13–5.51; p=0.024). The detailed outcomes of subgroup analysis are shown in table-4.

The effect of each single study on the pooled results was evaluated by excluding individual study in five models. No significant quantitative changes were found among the value of pooled OR and 95% CI, which indicates the reliability of the pooled results. Figure-3 shows the sensitivity analysis of correlation of OA susceptibility with polymorphism rs12901499 in dominant model.

Publication bias was estimated by Begg's test. The correlation of rs12901499 polymorphism and OA susceptibility showed no significant publication bias (p=0.104, Figure-4).

Table-1: Characteristics of the studies included in the meta-analysis

Author	Year	Country	Ethnicity	Source of controls	Genotyping method	Lesion locations	Number (case/control)	HWE	NOS
Zhong	2018	China	Asian	PB	TaqMan	hip	500/1080	<0.001	8
Liva	2017	Greece	Caucasian	HB	PCR	spine	258/243	0.0022	8
Jiang	2013	China	Asian	PB	PCR–RFLP	Knee	102/217	0.1833	7
Jiang	2013	China	Asian	PB	PCR–RFLP	Hand	111/217	0.1879	7
Su	2015	China	Asian	HB	PCR–RFLP	Knee	518/468	0.5833	8
Xiao	2015	China	Asian	HB	PCR	Temporomandibular	114/126	0.1675	7
Zhang	2017	China	Asian	HB	PCR	Knee	350/400	0.5336	7
Sharma	2017	India	Asian	HB	PCR-RFLP	Knee	450/458	0.0089	8

HWE=Hardy–Weinberg equilibrium. NOS=Newcastle-Ottawa Scale. PB=population-based. HB=hospital-based. CR=polymerase chain reaction. RFLP=restriction fragment length polymorphism

Table-2: Polymorphisms genotype distribution and allele frequency in cases and controls

	Genotype (N)						Allele frequency (N)			
First author		Case			Control		Case		Control	
rs12901499 A>G	AA	AG	GG	AA	AG	GG	A	G	A	G
Zhong	10	200	290	20	610	450	220	780	650	1510
Liva	87	144	27	96	130	17	318	198	322	164
Jiang	22	68	12	114	83	23	112	92	311	129
Jiang	25	73	13	114	83	23	123	99	311	129
Su	142	274	129	116	228	124	558	532	460	476
Xiao	31	53	30	44	67	15	115	113	155	97
Zhang	82	173	91	81	202	111	337	355	364	424
Sharma	165	131	154	158	198	102	461	439	514	402

Table-3: Meta-analysis of the association between rs12901499 polymorphism and osteoarthritis susceptibility

	Association results			Heterogeneity	
SNP	OR (95% CI)	$p_{(Z-t)}$	$p_{(Q-t)}$	I^2(%)	Model
A vs. G	1.32 (1.08-1.62)	0.006	<0.001	83	random
AA vs. GG	1.46 (1.04-2.05)	0.03	0.003	67.3	random
AA vs. AG	1.29 (0.82-2.02)	0.275	<0.001	88.7	random
GG vs. AA+AG	1.38 (1.01-1.88)	0.04	<0.001	79.2	random
AG+GG vs. AA	1.04 (0.96-2.04)	0.082	<0.001	85.6	random

OR=odds ratios. SNP= single nucleotide polymorphism. $p_{(Z-t)}$ value for association test, $p_{(Q-t)}$ value forheterogeneity test

Table-4: Subgroup analysis of the association of rs12901499 polymorphism and osteoarthritis susceptibility under five models

Subgroup analysis	N	OR	95% CI	$p_{(Z-t)}$	I^2 (%)	$p_{(Q-t)}$	Model
A vs. G							
Asians	7	1.34	1.07-1.69	0.012	85.4	<0.001	random
Knee	4	1.31	0.83-1.93	0.181	89.9	<0.001	random
population-based	3	1.71	1.43-2.05	<0.001	26.7	0.256	fixed
hospital-based	5	1.11	0.93-1.32	0.262	68.3	0.013	random
PCR–RFLP	4	1.4	0.99-1.98	0.056	87.9	<0.001	random
PCR	3	1.17	0.86-1.59	0.318	74.5	0.002	random
HWE	5	1.35	0.96-1.90	0.085	88	<0.001	random
Not-HWE	3	1.33	1.14-1.56	<0.001	44.9	0.163	random
AA vs. GG							
Asians	7	1.44	0.99-2.09	0.059	70.5	0.002	random
Knee	4	1.32	0.75-2.30	0.334	76.2	0.006	random
population-based	3	2.04	1.26-3.29	0.003	6	0.345	fixed
hospital-based	5	1.27	0.86-1.87	0.237	72	0.006	random
PCR–RFLP	4	1.53	0.93-2.54	0.293	74.7	0.008	random
PCR	3	1.51	0.70-3.25	0.097	78.9	0.009	random
HWE	5	1.53	0.88-2.67	0.132	78.7	0.001	random
Not-HWE	3	1.47	1.11-1.94	0.006	0	0.826	fixed
AA vs. AG							
Asians	7	1.3	0.76-2.21	0.335	90.2	<0.001	random
Knee	4	1.89	0.85-4.16	0.116	93	<0.001	random
population-based	3	2.32	0.82-6.57	0.114	88.5	<0.001	random
hospital-based	5	0.91	0.72-1.16	0.451	52.6	0.071	random
PCR–RFLP	4	1.75	0.73-4.19	0.206	94.8	<0.001	random
PCR	3	1.03	0.81-1.31	0.802	0	0.371	fixed
HWE	5	1.7	0.89-3.26	0.11	90.7	<0.001	random
Not-HWE	3	0.82	0.50-1.33	0.42	72.7	0.026	random
GG vs. AA+AG							
Asians	7	1.37	0.97-1.92	0.071	82.1	<0.001	random
Knee	4	0.92	0.75-1.12	0.38	0	0.833	fixed
population-based	3	1.56	1.05-2.30	0.027	41.7	0.18	fixed
hospital-based	5	1.35	0.90-2.02	0.149	81.3	<0.001	random
PCR–RFLP	4	1.21	0.76-1.90	0.42	76.7	0.005	random
PCR	3	1.47	0.77-2.80	0.241	76.7	0.014	random
HWE	5	1.11	0.80-1.56	0.529	57.7	0.051	random
Not-HWE	3	1.87	1.58-2.21	<0.001	0	0.795	fixed
AG+GG vs. AA							
Asians	7	1.42	0.91-2.22	0.119	87.6	<0.001	random
Knee	4	1.78	0.83-3.81	0.136	93	<0.001	random
population-based	3	2.49	1.13-5.51	0.024	81.1	0.005	random
hospital-based	5	1	0.84-1.19	0.981	22.6	0.27	fixed
PCR–RFLP	4	1.81	0.89-3.66	0.101	93.1	<0.001	random
PCR	3	1.11	0.80-1.56	0.526	50.3	0.134	random
HWE	5	1.7	0.91-3.17	0.094	90.8	<0.001	random
Not-HWE	3	1.03	0.81-3.17	0.82	12	0.321	fixed

OR=odds ratios. CI=confidence intervals. PCR= polymerase chain reaction. RFLP=restriction fragment length polymorphism. N= the number of studies. $p_{(Z-t)}$ value for association test, $p_{(Q-t)}$ value forheterogeneity test. HWE=Hardy–Weinberg equilibrium

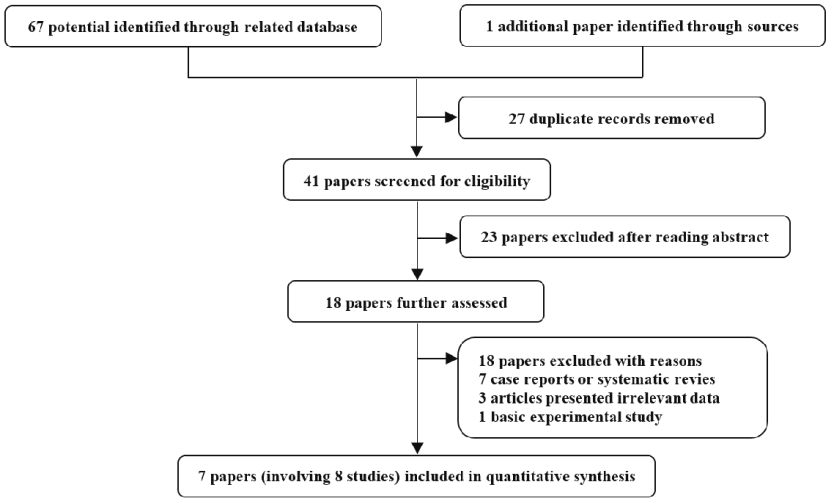

Figure-1: Flow diagram of study inclusion process

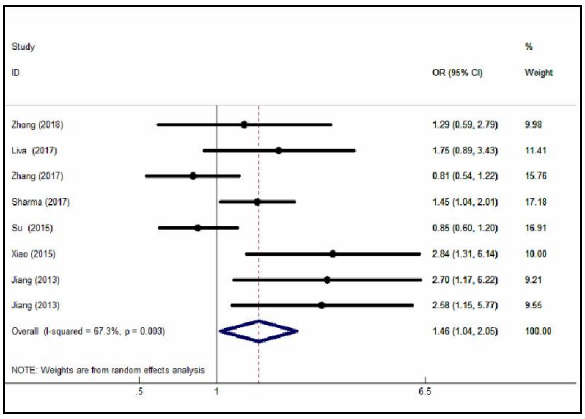

Figure-2: The forest plot of pooled OR of the association of rs12901499 and risk of OA susceptibility under homozygous model

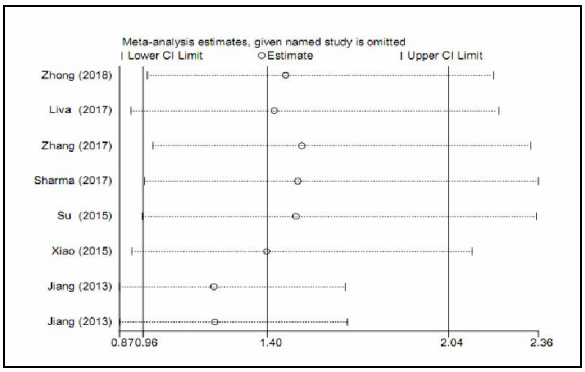

Figure-3: Sensitivity analysis of correlation of OA susceptibility with polymorphism rs12901499 in dominant model

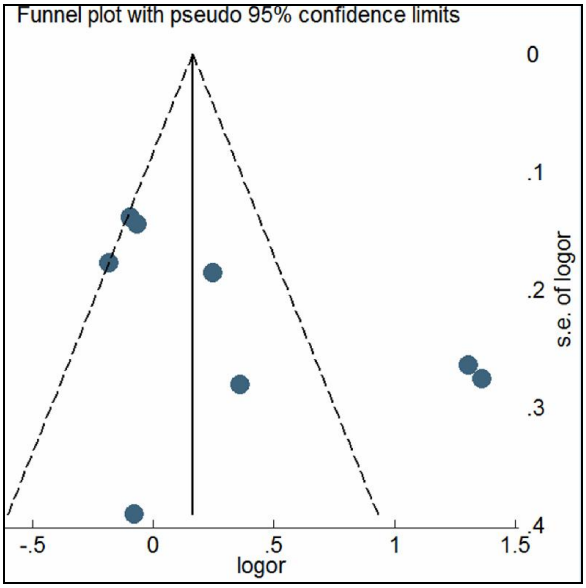

Figure-4: Begg's funnel plot was used to assess the publication bias

DISCUSSION

To our knowledge, no previous study has investigated the correlation of polymorphism rs12901499 and osteoarthritis susceptibility by meta-analysis. A total of 2403 OA patient with 3209 healthy control subjects were included in eight studies. The present study suggests a significant correlation of polymorphism rs12901499 and osteoarthritis susceptibility in allele, homozygous and recessive models. A significant increase in OA susceptibility was found only in Asian subgroup in the allele model. Subgroup analysis based on lesions location suggested no correlation of rs12901499 polymorphism and knee OA in five models. Stratified analysis on population-based studies showed that the polymorphism has correlation with increased risk of OA in the allele, homozygous, recessive and dominant models. Recently, several genetic studies investigated the correlation of rs12901499 polymorphism in SMAD3 and OA risk. A study was performed among European populations including 527 cases and 520 controls concluded that the risk of OA increased with polymorphism rs12901499.[16] Two studies from China also proved that the rs12901499 polymorphism in SMAD3 increased the risk of OA.[11,14] The study was focused on female Han Chinese revealed that rs12901499 polymorphism showed an obvious association with temporomandibular joint ORIGINAL ARTICLE.[11] Another study conducted in northeast Chinese population demonstrated that genetic variation in rs12901499 polymorphism increases the susceptibility of hand and knee OA.[14] Two recently published studies also revealed an association between knee and hip OA.[9,10] In another study conducted by Zhang *et al* showed a significant association which was observed only in the homozygous model.[10] However, another study performed in Taiwan which involved 518 knee OA cases and 468 controls could not conclude a significant correlation of rs12901499 polymorphism and osteoarthritis susceptibility.[12] The data to evaluate the different races among these studies was unavailable because all the participants were from Chinese Han populations. Above inconclusive conclusions of these researchers in varied studies might be resulting due to following factors such as difference in size of sample, genetic and clinical heterogeneity. However, association of spinal degenerative osteoarthritis susceptibility and rs12901499 polymorphism was not found in a study performed in Greece.[15] The conclusions of above-mentioned studies are contradictory. Small sample studies lack enough statistical power to demonstrate the relationship between rs12901499 polymorphism

and OA risks. Therefore, we used meta-analysis, a powerful statistical method, to pool the results of association in varied studies.

Although this study has presented a more comprehensive evaluation of correlation of rs12901499 polymorphism and osteoarthritis, there are some limitations which should be noted too. Firstly, analysis was limited because of only eight studies were included in our study. Secondly, no study had a satisfactory match on age, gender, body weight index and other confounding factors, which might be the reason of potential confounding bias of this study. Thirdly, we could not confirm the results from the level of molecular mechanism. We should not ignore the heterogeneity presented in this meta-analysis. Heterogeneity still existed after performing subgroups analysis by source of control and ethnicity suggesting that source of control and ethnicity could not fully account for the heterogeneity among studies. Therefore, large sample studies are still needed to provide strong evidence of correlation of rs12901499 polymorphism and osteoarthritis susceptibility

In conclusion, this study suggests that there may be a weak correlation of rs12901499 polymorphism and osteoarthritis susceptibility. Due to small size of sample and ethnic groups, more studies will be required in future to validate the result.

REFERENCES

1. Sun BH, Wu CW, Kalunian KC. New developments in osteoarthritis. Rheum Dis Clin North Am 2007;33(1):135–48.
2. Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum 1998;41(8):1343–55.
3. Blagojevic M, Jinks C, Jeffery A, Jordan KP. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage 2010;18(1):24–33.
4. Grotle M, Hagen KB, Natvig B, Dahl FA, Kvien TK. Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. BMC Musculoskelet Disord 2008;9:132.
5. Bijsterbosch J, Kloppenburg M, Reijnierse M, Rosendaal FR, Huizinga TW, Slagboom PE, et al. Association study of candidate genes for the progression of hand osteoarthritis. Osteoarthritis Cartilage 2013;21(4):565–9.
6. Serra R, Chang C. TGF-beta signaling in human skeletal and patterning disorders. Birth Defects Res C Embryo Today 2003;69(4):333–51.
7. van der Kraan PM, Blaney Davidson EN, Blom A, van den Berg WB. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. Osteoarthritis Cartilage 2009;17(12):1539–45.
8. Li Y, Schang G, Boehm U, Deng CX, Graff J, Bernard DJ. SMAD3 Regulates Follicle-stimulating Hormone Synthesis by Pituitary Gonadotrope Cells in Vivo. J Biol Chem 2017;292(6):2301–14.
9. Zhong F, Lu J, Wang Y, Song H. Genetic variation of SMAD3 is associated with hip osteoarthritis in a Chinese Han population. J Int Med Res 2018;46(3):1178–86.
10. Zhang L, Zhang L, Zhang H, Wang W, Zhao Y. Association between SMAD3 gene rs12901499 polymorphism and knee osteoarthritis in a Chinese population. J Clin Lab Anal 2018;32(5):e22383.
11. Xiao JL, Meng JH, Gan YH, Zhou CY, Ma XC. Association of GDF5, SMAD3 and RUNX2 polymorphisms with temporomandibular joint osteoarthritis in female Han Chinese. J Oral Rehabil 2015;42(7):529–36.
12. Su SL, Yang HY, Lee HS, Huang GS, Lee CH, Liu WS, et al. Gene-gene interactions between TGF-beta/Smad3 signalling pathway polymorphisms affect susceptibility to knee osteoarthritis. BMJ Open 2015;5(6):e007931.
13. Sharma AC, Srivastava RN, Srivastava SR, Parmar D, Singh A, Raj S. Association between Single Nucleotide Polymorphisms of SMAD3 and BMP5 with the Risk of Knee Osteoarthritis. J Clin Diagn Res 2017;11(6):GC1–4.
14. Liying J, Yuchun T, Youcheng W, Yingchen W, Chunyu J, Yanling Y, *et al*. A SMAD3 gene polymorphism is related with osteoarthritis in a Northeast Chinese population. Rheumatol Int 2013;33(7):1763–8.
15. Liva E, Panagiotou I, Palikyras S, Parpa E, Tsilika E, Paschou P, *et al*. Candidate gene investigation of spinal degenerative osteoarthritis in Greek population. Spine J 2017;17(12):1881–8.
16. Valdes AM, Spector TD, Tamm A, Kisand K, Doherty SA, Dennison EM, *et al*. Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. Arthritis Rheum 2010;62(8):2347–52.
17. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25(9):603–5.
18. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7(3):177–88.

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