

Research Paper

# Rs143383 in the Growth Differentiation Factor 5 (*GDF5*) Gene Significantly Associated with Osteoarthritis (OA)-A Comprehensive Meta-analysis

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## Abstract

Family, twin, adoption studies show osteoarthritis (OA) has a substantial genetic component. Several studies have shown an association between OA and Growth Differentiation Factor 5 (*GDF5*), some others have not. Thus, the status of the OA-*GDF5* association is uncertain. This meta-analysis was applied to case-control studies of the association between OA and *GDF5* to assess the joint evidence for the association, the influence of individual studies, and evidence for publication bias. Relevant studies were identified from the following electronic databases: MEDLINE and current contents before Feb. 2012.

For the case-control studies, the authors found 1) support for the association between OA and *GDF5*. The rs143383 polymorphism was significantly associated with OA [fixed: OR and 95%CI: 1.193 (1.139-1.249),  $p < 0.001$ ; random: OR and 95%CI: 1.204 (1.135-1.276),  $p < 0.001$ ], 2) no evidence that this association was accounted for by any one study, and 3) no evidence for publication bias. Although the effect size of the association between OA and *GDF5* is small, there is suggestive evidence for an association. Further studies are needed to clarify what variant of *GDF5* (or some nearby gene) accounts for this association.

Key words: *GDF5*; Osteoarthritis; polymorphism; meta-analysis.

## Introduction

Primary osteoarthritis (OA) is an idiopathic phenomenon, occurring in previously intact joints, with no apparent initiating factor such as joint injury or developmental abnormalities. It is characterized by the progressive failure of the extracellular cartilage matrix leading to articular cartilage destruction.[1-3] It is widely believed that OA develops from an im-

balance between anabolic and catabolic processes or homeostasis of cartilage metabolism.[4-5] Although the detailed aetiology is not currently fully understood, it has been suggested that OA resulted from the combination of aging, hormonal, environmental, and genetic factors.[6-7] It is well known that genetic factors contribute to the susceptibility for OA. Several

studies have demonstrated that polymorphisms in many genes might be related to the pathogenesis of OA.[8-18]

Growth differentiation factor 5 (*GDF5*), also known as cartilage-derived morphogenetic protein 1 or bone morphogenetic protein (BMP) 14, is a member of TGF-beta superfamily.[19] A number of studies have demonstrated that *GDF5* plays important roles in musculoskeletal processes, affecting endochondral ossification, synovial joint formation, tendon maintenance, and bone formation.[20-21] Defects of this gene was shown to be correlated to abnormal joint development or skeletal disorders in humans and mice.[22-25] Moreover, it has been reported that the polymorphism in *GDF5* gene is related with low expression of the *GDF5* protein in knee joint.[26] In addition, *GDF5* deficient mice exhibited biomechanical abnormalities in the tendon, which may be associated with altered type I collagen and skeletal abnormalities, one hypothesis of the mechanism behind that was *GDF5* might modulate the rate of endochondral bone growth by affecting the duration of the hypertrophic phase in growth plate chondrocytes.[27]

Several studies have shown that the association of rs143383 polymorphism in the promoter region might be a risk factor of OA,[26,28-33] but other groups did not confirm these results.[34] To deal with the ambiguities raised by inconsistent results among molecular genetic studies, Rice suggested that the statistical method of meta-analysis could be used to reconcile conflicting findings.[35] This method is used to examine whether the aggregate evidence across all available studies provides evidence of statistical significance. Thus, to examine the putative association between OA and the *GDF5*, we applied this comprehensive meta-analysis to all available case-control association studies.

## Materials and methods

**Search strategy.** To assess the total evidence of

association between *GDF5* gene and OA, we performed this meta-analysis of published studies. We considered all studies that examined the association of the rs143383 polymorphisms with OA. Sources included PUBMED and EMBASE (search last updated in Feb. 2012). The search strategy was based on combinations of the terms “*GDF5*” “growth and differentiation factor 5” “*BMP14*” “1104T/C” “rs143383” and “OA”, and “osteoarthritis.” Reference lists in retrieved articles were also screened.

**Inclusion and exclusion criteria.** All the studies included satisfied all the following criteria: they (1) were association studies between the rs143383 polymorphisms in the *GDF5* gene and OA; (2) used disease-free people as controls; (3) provided genotypes or alleles distribution in both case and control groups; (4) were independent studies and the subject groups investigated in each studies did not overlap with other’s; (5) were published in peer-reviewed journals and were indexed by PubMed or cited by articles indexed by PubMed. Authors were contacted where clarification was required.

The phenotype definitions were accepted basing on clinical criteria and radiographic criteria. 1) For the clinical criteria, the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria was used [36-38] but also accepted other definitions that may have been preferred by local investigators, if information on ACR criteria was not available. 2) For the radiographic criteria, we used the Kellgren/Lawrence (K/L) classification system (grades 0-4, with 0 representing normal findings and 4 representing severe OA) [39], which is the most widely used scale for identifying and grading OA.[28] A cutoff of K/L grade 2 was used to classify OA, unless the data had been generated with another cutoff and the definition could not be revisited. The phenotype details which were used in every study are shown in Table 1.

**Table 1.** Characteristics of the studies included.

Table 1. Characteristics of the studies included													Mean age		BMI(kg/m2)					
Study(Reference no.)	Year	Country	Polymorphisms	Case	Control	Genotype method	Frequencies of Case			Frequencies of Control			Case(Numbers)		Control(Numbers)		Case	Control	Case	Control
							TT	TC	CC	TT	TC	CC	Females	Males	Females	Males				
Southam-1 (29)	2007	UK	rs143383	1669	822	PCR-RFLP	672	796	201	324	372	126	1006	663	368	454	65.1	69.1	/	/
Southam-2 (29)	2007	Spain	rs143383	818	1196	Taqman	302	398	118	439	563	194	617	119	667	529	/	>55.2	/	/
Miyamoto Y-1 (26)	2007	Japan	rs143383	718	861	Sequence	444	243	31	473	330	58	644	74	405	456	59.7	56.1	23.2	23.5
Miyamoto Y-2 (26)	2007	China	rs143383	313	485	Sequence	197	97	19	244	193	48	205	108	316	169	58.8	56.8	24.9	23.6
Tsezou (34)	2007	Greece	rs143383	251	268	Sequence	95	126	30	99	125	44	205	46	169	99	67.9	65.2	29.5	25
Chapman K (30)	2008	Netherlands	rs143383	363	724	/	179	112	72	359	215	150	/	/	/	/	55.3	55.1	/	/
Evangelou-1 (28)	2009	UK	rs143383	608	822	Taqman	287	231	90	341	453	28	334	274	452	370	61.7	/	/	/
Evangelou-2 (28)	2009	Holland	rs143383	745	1614	Taqman	296	282	167	789	612	213	745	0	1614	0	76.7	/	/	/
Evangelou-3 (28)	2009	Iceland	rs143383	1071	1169	Taqman	535	379	157	552	442	175	725	346	748	421	74.8	/	/	/
Valdes AM-4 (31)	2009	UK	rs143383	259	509	allele-specific PCR	126	98	35	181	244	84	259	0	509	0	66.8	63	26.8	26.2
Valdes AM-5 (31)	2009	UK	rs143383	1003	647	allele-specific PCR	337	313	85	238	329	79	631	372	369	278	67.3	66.9	28.3	26.5
Tawonsawatruk (32)	2011	Thailand	rs143383	90	103	PCR-RFLP	38	41	11	33	47	23	79	93	11	10	68.46	59.25	26.66	24.57
Valdes AM-1 (33)	2011	UK	rs143383	867	758	allele-specific PCR	377	397	93	290	315	153	425	442	371	387	66.5	/	/	29.3
Valdes AM-2 (33)	2011	Holland	rs143383	745	1614	allele-specific PCR	412	211	122	864	413	337	417	328	904	710	56.0	/	/	27.7
Valdes AM-3 (33)	2011	Estonia	rs143383	65	427	allele-specific PCR	33	21	11	230	166	31	45	20	295	132	47.1	/	/	28.1

Nonfamilial case-control studies were eligible if the researchers had determined the distribution of genotypes for the polymorphism in OA cases and disease-free controls. We excluded studies with family-based designs in which the analysis was based on linkage considerations.

Only studies published in English were included. Studies presenting non-original data were excluded, such as reviews, editorials, opinion papers, or letters to the editor. Studies using non-human subjects or specimens were excluded. Studies in which rheumatoid, inflammatory, or other forms of arthritis were incorporated in the OA datasets were excluded. Studies with no extractable, numerical data were excluded. Only those articles which had some measure of diagnostic performance were included. Any duplicates which came up in the preliminary search were excluded.

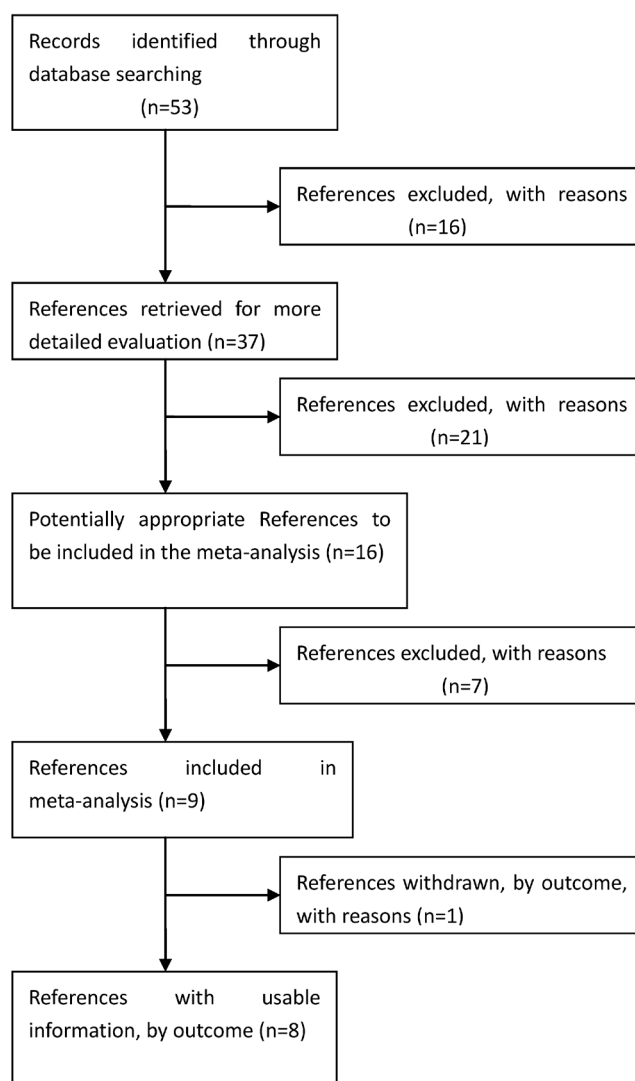
**Data extraction.** The following data was extracted from all identified studies, by two independent investigators: 1) first author, 2) journal, 3) year of publication, 4) study design, 5) ethnicity of the study population, 6) gender, 7) clinical characteristics, 8) genotyping method, 9) the number of cases and controls or OR and 95%CI (odds ratio and 95 percent confidence interval), 10) country in which the study was conducted and confirmation of diagnosis. Inconsistencies were resolved in consensus.

**Statistical analysis.** We used a comprehensive meta-analysis to analyze the odds ratios by using the method of Carlin.[40] To determine whether the results of the meta-analysis were unduly influenced by any one study, we recomputed the meta-analysis statistic after deleting each study one at a time. We assessed publication bias by using the method of Egger et al.[41] This method is based on the fact that the precision of the odds ratio increases with larger study groups. The method regresses the standard normal deviate of the odds ratio (the odds ratio divided by its standard error) against the precision of the odds ratio (the inverse of its standard error). In the absence of bias, Egger et al. showed that the regression of the standard normal deviate on precision of the odds ratio should run through the origin (i.e., small study groups with low precision have large standard errors and therefore small standard normal deviates; large study groups have higher precision, smaller standard errors, and large standard normal deviates). The publication bias statistic of Egger et al. is the intercept of the regression, which will be significantly greater than zero in the presence of publication bias.

The effect size was represented by an odds ratio (OR) with 95% confidence interval (CI). Sensitivity analysis was conducted by removing each study and

analyzing the others to ensure no single study was totally responsible for overall results. The significance level was set at 0.05, and all P values were two-tailed. Between-study heterogeneity was tested using Cochran's Q statistic, which is considered significant at  $P < 0.10$  [42]. The extent of inconsistency across studies was quantified with the  $I^2$  statistic [43]. The  $I^2$  ranges between 0 and 100%. For operational purposes, values of 0-24%, 25-50%, 50-75%, and >75% are considered low, moderate, large, and very large, respectively [28,44]. When there was very large or large (>50%) between-study heterogeneity, we used a simulation algorithm to evaluate how many studies had to be removed for the  $I^2$  to reach <25% [45].

The comprehensive meta-analysis was performed using Comprehensive Meta-Analysis software (Version 2.2.046, BIOSTAT, Englewood, NJ, USA).



**Figure 1.** Process used to select published studies for a systematic review and meta-analysis of the relation between GDF5 and OA, 2007–Feb 2012. MeSH, Medical Subject Headings.

## Results

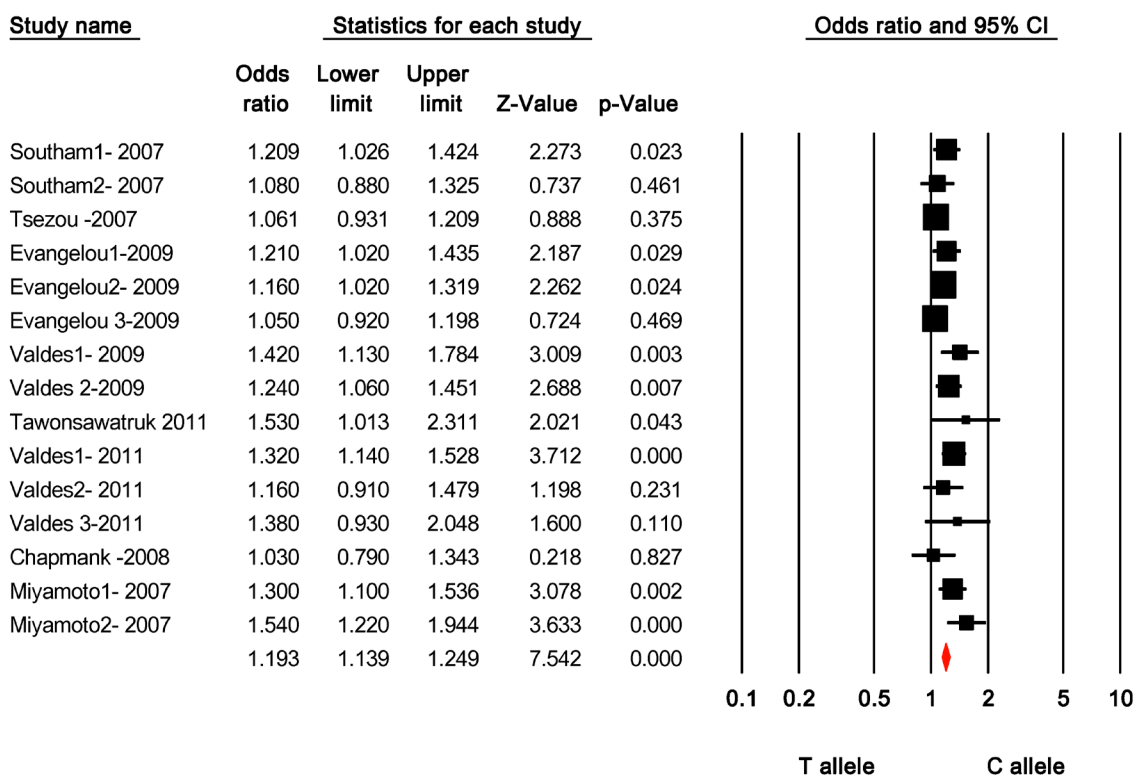
**Eligible studies.** The combined search yielded at least 53 references. After discarding overlapping references and those that clearly did not meet the criteria, 37 references were retained. These references were then filtered to ensure conformity with the inclusion criteria. 21 references were discarded for insufficient and equivocal data (although we tried unsuccessfully to obtain further information from the authors), 7 references were excluded because they were not about the researches of SNPs, and 1 reference was excluded because its data were about lumbar disc degeneration. In total, 15 studies (8 references) met our inclusion criteria, altogether consisting of a total of 7881 cases and 12019 controls.[26, 28-34] The main characteristics of these studies are described in Table 1.

**Quantitative data synthesis.** The eligible studies for analysis included a total of 7881 cases with OA

and 12019 controls (Table 1). The meta-analysis of all the studies about rs143383 polymorphism was significantly associated with OA [fixed: odds ratio and 95 percent confidence interval (CI): 1.193 (1.139-1.249),  $p < 0.001$ ; random: odds ratio and 95 percent confidence interval (CI): 1.204 (1.135-1.276),  $p < 0.001$ ] (Fig. 2 and Fig. 3).

**Publication bias and heterogeneity.** The sensitivity analysis showed that when any one of the studies was removed, the heterogeneity of the population was not changed deeply. In other words, when one study was removed, the result also showed significant (Fig. 4). This indicated that no heterogeneity existed in the population. There was no evidence that the magnitude of the overall odds ratio estimates changed in the same direction over time. And the Egger's funnel plots of publication bias analysis for the rs143383 polymorphism was shown in Fig. 5.

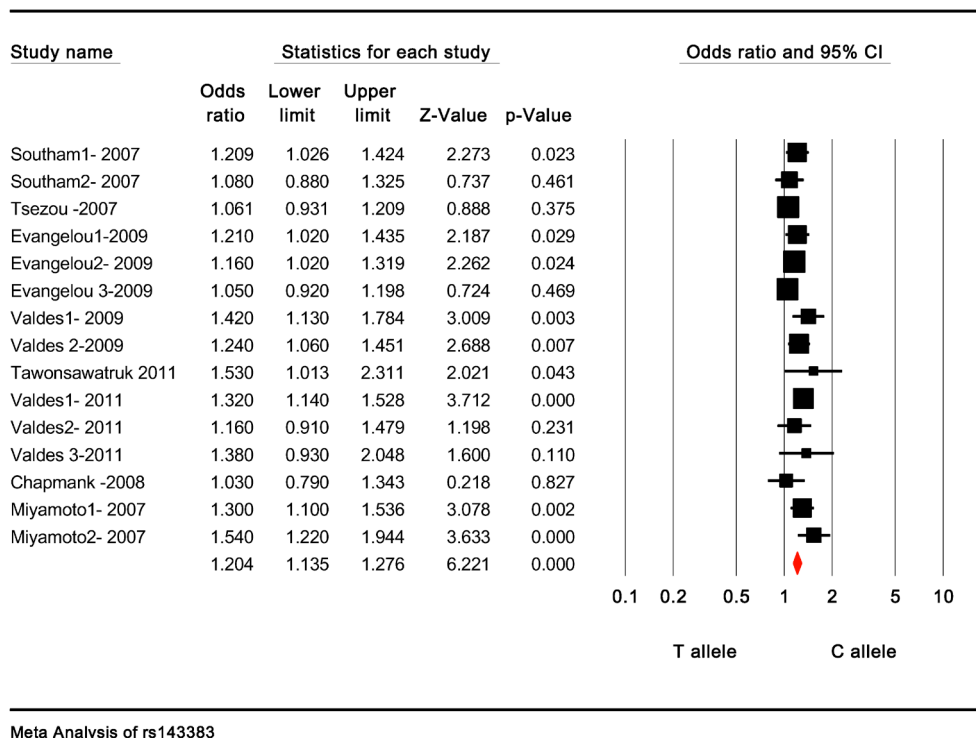
## Meta Analysis of rs143383



Meta Analysis of rs143383

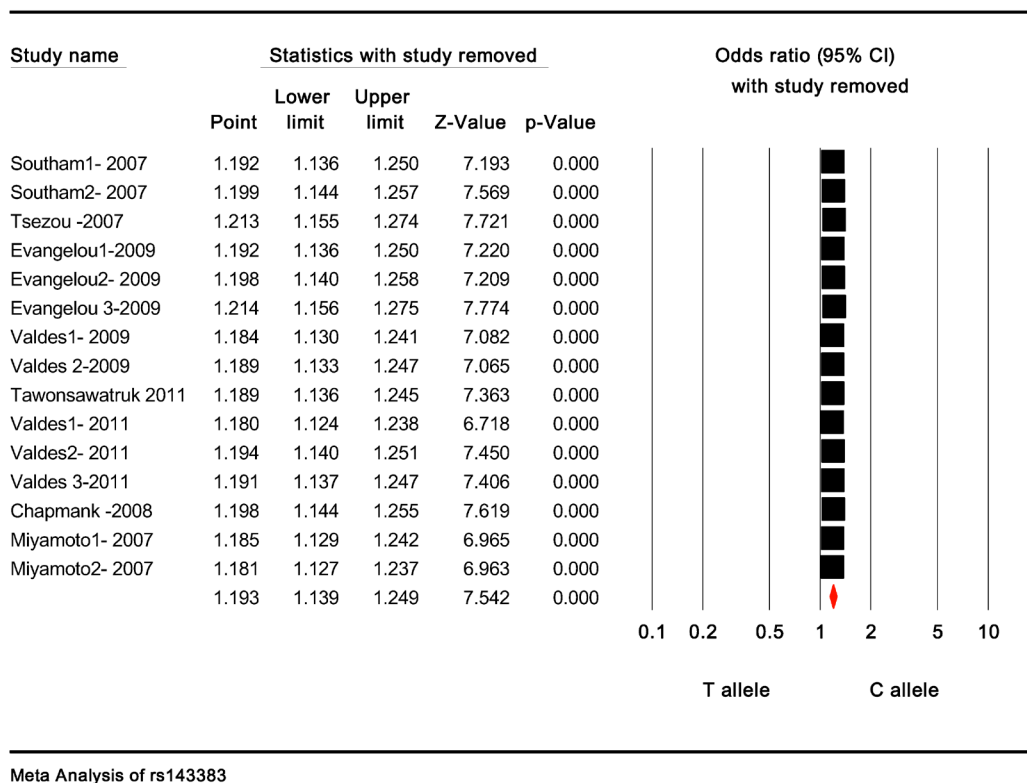
**Figure 2. Meta-analysis of association studies of the rs143383 polymorphism and OA (fixed model).** The overall OR is shown. The OR of each study is marked with a black square. The overall OR is indicated by diamond.

### Meta Analysis of rs143383

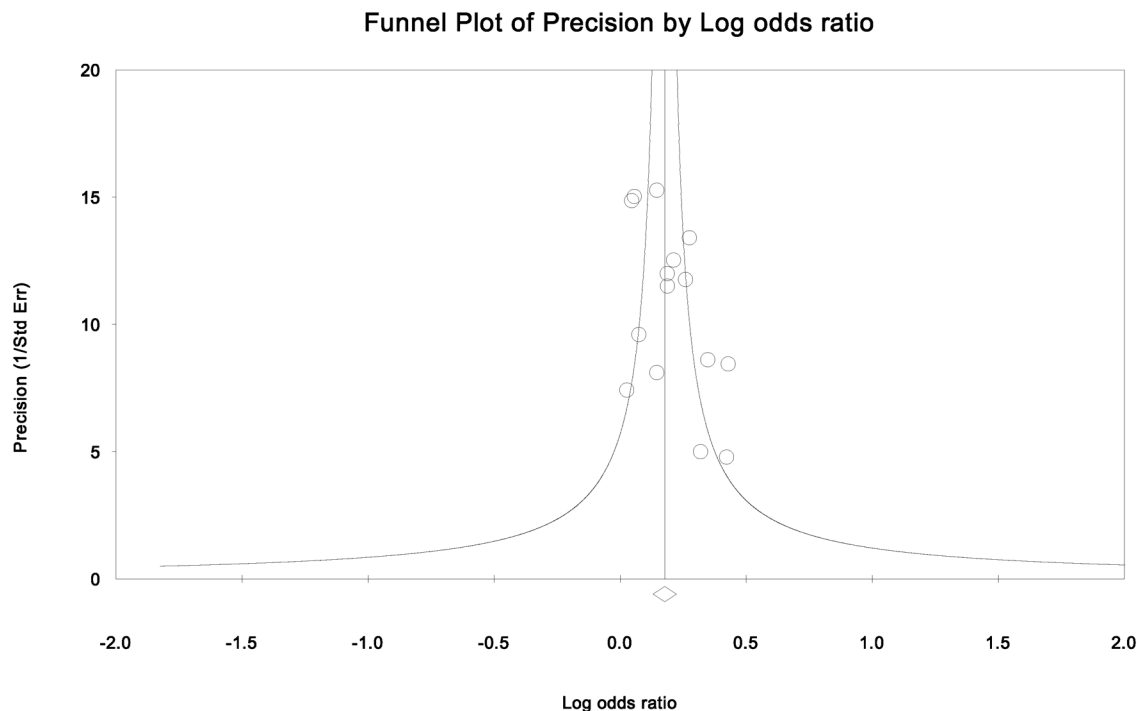


**Figure 3. Meta-analysis of association studies of the rs143383 polymorphism and OA (random model).**The overall OR is shown. The OR of each study is marked with a black square. The overall OR is indicated by diamond.

### Meta Analysis of rs143383



**Figure 4. The sensitivity analysis of rs143383.**When any one of the studies was removed, the heterogeneity of the population was not changed.



**Figure 5. Egger's funnel plots of publication bias analysis for the rs143383 polymorphism.** The larger the deviation from the funnel curve of each study, the more pronounced the asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies.

## Discussion

The pathogenesis of the development and progression of OA is far from being clear at present. Several studies indicated that variants of the *GDF5* gene may contribute to the disease, but the results of genetic association studies were confusing because of the difficulty in replicating significant associations. Different characteristics among studies such as ethnicities, definition of case and control, introduced heterogeneity and made the results of association studies hard to be interpreted. A meta-analysis aiming at finding out the origin of heterogeneity and assessing overall effects of these variants on OA was performed. This comprehensive meta-analysis included data from 8 references including 15 studies with approximately 19900 OA cases and controls. It revealed highly significant evidence of association between the rs143383 polymorphism of *GDF5* gene and OA.

*GDF5* is an extracellular signaling molecule that participates in bone and cartilage morphogenesis as well as in joint formation.[46] Mutations in human *GDF5* gene results in a broad spectrum of skeletal disorders.[47] Based on this functional knowledge, Miyamoto et al. genotyped a number of common *GDF5* polymorphisms and demonstrated that rs143383, a T to C transition located in the 5' untrans-

lated region (5'UTR) of the gene, was significantly associated with OA.[26] Further studies have revealed that rs143383 is functional, the OA-associated T-allele mediating reduced *GDF5* transcription relative to the C-allele in all of the joint tissues.[47-48] Moreover, the A-allele of -41C/A polymorphism is able to compensate for the reduced expression mediated by the T-allele of rs143383.[49] In some other studies, rs143383 has also been shown to be associate with other phenotypes such as variation in normal height, Achilles tendon pathology, fracture risk and congenital dysplasia of the hip.[50-54] As Loughlin described, this highlights the tendency of a common genotype to influence multiple phenotypes (pleiotropy), and indicates that developmental factors may play an important role on conditions that manifest in the mature individual.[55]

Functional study has demonstrated that T allele of rs143383 was associated with the decrease of *GDF5* molecule expression and might increase susceptibility to OA.[26] The differential binding of deformed epidermal autoregulatory factor 1 (DEAF-1) could modulate the expression of *GDF5* via this polymorphism.[48] These evidences indicated that the functional polymorphism of *GDF5* gene plays critical roles in the etiology of OA. Mouse models have provided a basis for better understanding of the role of *GDF5* in skeletogenesis and joint maintenance.[22-24] The

brachypodism (bp) mice which carry a functional null allele of *GDF5* caused by a frame-shift mutation, exhibited abnormal skeletal and bone development.[56-57] However, *Gdf5*<sup>Bp-J/+</sup> mice appeared phenotypically normal, but does show an increased propensity of developing an OA phenotype when challenged.[58] This model suggested that decreased *GDF5* levels in mice contribute to osteoarthritis development, and it is supportive of the genetic data indicating the association between rs143383 polymorphism of *GDF5* and human osteoarthritis.

In the present study, we assessed all available literature. This effort to take a comprehensive and even-handed approach to the literature inclusion may have strengthened the robustness of the findings while it avoided publication bias and minimized heterogeneity.[59] Compared with previous study [30], the current meta-analysis pooled larger sample sizes, analyzed them both combined and separately, generated even more significant results with systematic design types and analysis approaches and included tests of heterogeneity, as well as sensitivity analyses. The current results showed that there is a potential association between the rs143383 allele of *GDF5* and OA. For greater insight into OA's genetic component, more work is required to confirm the role of other genes that may have a small effect, and to identify new genetic risk factors. The large samples required will necessitate multi-site projects and meta-analyses on the basis of national and international collaboration.

In summary, this meta-analysis supports significant association of marker in the *GDF5* gene with OA. It remains unclear why the frequency of the associated alleles varies across studies. Identification of functional variants will probably require biological as well as additional genetic assays.

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## Contributions

J. Liu and H.X. Zhang designed the study and drafted the manuscript. J. Liu, W. Cai, C. He, L.F. Deng and H.X. Zhang performed the statistical analysis. All authors critically revised the manuscript and gave final approval of the article for submission.

## Competing Interests

The authors have declared that no competing interests exist.

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