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 imbalance 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 were 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: (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 study did not overlap with others; (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.

| Study/Reference no | Year | Country | Polymorphism | Case | Control | Genotype method | Frequencies of Case | Frequencies of Control | Case Numbers | Control Numbers | Mean age | BM/(kg/m²) |
|--------------------|------|---------|--------------|------|---------|----------------|---------------------|----------------------|--------------|----------------|----------|-------------|
| Southam-1(26)      | 2007 | UK      | rs143383     | 1899 | 822     | PC-RFLP       | 177 192 314         | 324 372 126          | 1006         | 683 384 548 | 65.1     | 69.1       |
| Southam-2(26)      | 2007 | Spain   | rs143383     | 818  | 1156    |                | 302 398 118         | 439 563 194          | 617 119      | 667 529      | /        | /          |
| Miyamoto Y-1(25)   | 2007 | Japan   | rs143383     | 719  | 861     | Sequence       | 444 243 31         | 473 320 55          | 664 74       | 405 456      | 50.7     | 56.1       |
| Miyamoto Y-2(25)   | 2007 | China   | rs143383     | 313  | 485     | Sequence       | 197 19 244          | 193 48           | 205 108     | 316 169      | 58.8     | 58.3       |
| Tazoe (34)         | 2007 | Greece  | rs143383     | 251  | 268     | Sequence       | 95 126 30           | 95 125 44          | 205 46       | 169 99       | 67.9     | 55.2       |
| Chapman-K (28)     | 2008 | Netherland | rs143383 | 363  | 724     | /               | 179 112 71         | 369 216 150       | /           | /           | /        | /          |
| Evangelou-1(26)    | 2008 | UK      | rs143383     | 608  | 822     | TEJAN          | 287 231 90          | 341 453 28         | 334 274     | 452 370      | 61.7     | /          |
| Evangelou-2(26)    | 2009 | Holand  | rs143383     | 745  | 1614    | TEJAN          | 250 282 167         | 789 512 213        | 745 0        | 164 0        | 76.7     | /          |
| Evangelou-3(26)    | 2009 | Ireland | rs143383     | 1671 | 1185    | TEJAN          | 555 379 157         | 562 175 705       | 723 568     | 748 432      | 74.9     | /          |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 250  | 509     | allele-specific | 126 98 33           | 191 244 64        | 259 0        | 509 0        | 66.8     | 63       |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 1003 | 647     | allele-specific | 337 313 85          | 238 329 79        | 631 372     | 369 278      | 67.3     | 66.9       |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 90  | 193     | PC-RFLP       | 38 41 11         | 42 47 22          | 79 32        | 52 10        | 66.4     | 59.5      |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 867  | 738     | allele-specific | 377 397 93          | 296 315 153       | 425 442     | 371 367      | 66.5     | 29.3      |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 745  | 1614    | allele-specific | 412 211 122         | 864 413 337        | 417 328      | 904 710      | 56.0     | /          |
| Vladić A(44)       | 2009 | Croatia | rs143383     | 85  | 427     | allele-specific | 33 21 11           | 230 166 31        | 45 22        | 295 132      | 47.1     | 26.1      |

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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 I² statistic [43]. The I² 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 I² 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

| Study name          | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|---------------------|------------|-------------|-------------|---------|---------|
| Southam1-2007       | 1.209      | 1.026       | 1.424       | 2.273   | 0.023   |
| Southam2-2007       | 1.080      | 0.880       | 1.325       | 0.737   | 0.461   |
| Tsezou -2007        | 1.061      | 0.931       | 1.209       | 0.888   | 0.375   |
| Evangelou1-2009     | 1.210      | 1.020       | 1.435       | 2.187   | 0.029   |
| Evangelou2-2009     | 1.160      | 1.020       | 1.319       | 2.262   | 0.024   |
| Evangelou3-2009     | 1.050      | 0.920       | 1.198       | 0.724   | 0.469   |
| Valdes1-2009        | 1.420      | 1.130       | 1.784       | 3.009   | 0.003   |
| Valdes2-2009        | 1.240      | 1.060       | 1.451       | 2.688   | 0.007   |
| Tawonsawatruk 2011  | 1.530      | 1.013       | 2.111       | 2.021   | 0.043   |
| Valdes1-2011        | 1.320      | 1.140       | 1.528       | 3.712   | 0.000   |
| Valdes2-2011        | 1.160      | 0.910       | 1.479       | 1.198   | 0.231   |
| Valdes 3-2011       | 1.380      | 0.930       | 2.048       | 1.600   | 0.110   |
| Chapmannk -2008     | 1.030      | 0.790       | 1.343       | 0.218   | 0.827   |
| Miyamoto1-2007      | 1.300      | 1.100       | 1.536       | 3.078   | 0.002   |
| Miyamoto2-2007      | 1.540      | 1.220       | 1.944       | 3.633   | 0.000   |
| 1.193               | 1.139      | 1.249       | 7.542       | 0.000   |

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.
**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.

**Figure 4. The sensitivity analysis of rs143383.** When any one of the studies was removed, the heterogeneity of the population was not changed.
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' untranslated 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, Gdf5Bp-1/+ 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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