

Large-Scale Analysis of Association Between *GDF5* and *FRZB* Variants and Osteoarthritis of the Hip, Knee, and Hand

Evangelos Evangelou,¹ Kay Chapman,² Ingrid Meulenbelt,³ Fotini B. Karassa,¹ John Loughlin,⁴ Andrew Carr,² Michael Doherty,⁵ Sally Doherty,⁵ Juan J. Gómez-Reino,⁶ Antonio Gonzalez,⁶ Bjarni V. Halldorsson,⁷ Valdimar B. Hauksson,⁸ Albert Hofman,⁹ Deborah J. Hart,¹⁰ Shiro Ikegawa,¹¹ Thorvaldur Ingvarsson,¹² Qing Jiang,¹³ Ingileif Jonsdottir,¹⁴ Helgi Jonsson,¹⁵ Hanneke J. M. Kerkhof,⁹ Margreet Kloppenburg,³ Nancy E. Lane,¹⁶ Jia Li,¹⁷ Rik J. Lories,¹⁸ Joyce B. J. van Meurs,⁹ Annu Näkki,¹⁹ Michael C. Nevitt,²⁰ Julio Rodriguez-Lopez,⁶ Dongquan Shi,¹³ P. Eline Slagboom,³ Kari Stefansson,¹⁴ Aspasia Tsezou,²¹ Gillian A. Wallis,²² Christopher M. Watson,²² Tim D. Spector,¹⁰ Andre G. Uitterlinden,⁹ Ana M. Valdes,¹⁰ and John P. A. Ioannidis²³

Objective. *GDF5* and *FRZB* have been proposed as genetic loci conferring susceptibility to osteoarthritis (OA); however, the results of several studies investigat-

ing the association of OA with the rs143383 polymorphism of the *GDF5* gene or the rs7775 and rs288326 polymorphisms of the *FRZB* gene have been conflicting or inconclusive. To examine these associations, we performed a large-scale meta-analysis of individual-level data.

Methods. Fourteen teams contributed data on polymorphisms and knee, hip, and hand OA. For rs143383, the total number of cases and controls, respectively, was 5,789 and 7,850 for hip OA, 5,085 and

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¹Evangelos Evangelou, PhD, Fotini B. Karassa, MD: University of Ioannina School of Medicine, Ioannina, Greece; ²Kay Chapman, MSc, PhD, Andrew Carr, MD: University of Oxford, Oxford, UK; ³Ingrid Meulenbelt, PhD, Margreet Kloppenburg, MD, PhD, P. Eline Slagboom, PhD: Leiden University Medical Center, Leiden, The Netherlands; ⁴John Loughlin, PhD: Newcastle University, Newcastle upon Tyne, UK; ⁵Michael Doherty, MD, FRCP, Sally Doherty, RGN: University of Nottingham and City Hospital Nottingham, Nottingham, UK; ⁶Juan J. Gómez-Reino, MD, PhD, Antonio Gonzalez, MD, PhD, Julio Rodriguez-Lopez, PhD: Hospital Clinico Universitario de Santiago, Santiago de Compostela, Spain; ⁷Bjarni V. Halldorsson, PhD: Reykjavik University and deCODE Genetics, Reykjavik, Iceland; ⁸Valdimar B. Hauksson, BSc: deCODE Genetics, Reykjavik, Iceland; ⁹Albert Hofman, MD, PhD, Hanneke J. M. Kerkhof, MSc, Joyce B. J. van Meurs, PhD, Andre G. Uitterlinden, PhD: Erasmus Medical Center, Rotterdam, The Netherlands; ¹⁰Deborah J. Hart, PhD, Tim D. Spector, MD, Ana M. Valdes, PhD: St. Thomas' Hospital and Kings College London, London, UK; ¹¹Shiro Ikegawa, MD, PhD: Center for Genomic Medicine, RIKEN, Tokyo, Japan; ¹²Thorvaldur Ingvarsson, MD, PhD: University of Akureyri, Akureyri, and University of Iceland, Reykjavik, Iceland; ¹³Qing Jiang, MD, PhD, Dongquan Shi, MD: Drum Tower Hospital and Medical School of Nanjing University,

Nanjing, Jiangsu, China; ¹⁴Ingileif Jonsdottir, PhD, Kari Stefansson, MD, PhD: University of Iceland, Reykjavik, and deCODE Genetics, Reykjavik, Iceland; ¹⁵Helgi Jonsson, MD, PhD: University of Iceland, Reykjavik, and Landspítali University Hospital, Reykjavik, Iceland; ¹⁶Nancy E. Lane, MD: University of California at Davis Medical Center, Sacramento, California; ¹⁷Jia Li, PhD: Roche Molecular Systems, Pleasanton, California; ¹⁸Rik J. Lories, MD, PhD: Katholieke Universiteit Leuven, Leuven, Belgium; ¹⁹Annu Näkki, BSc: National Public Health Institute, ORTON Orthopedic Hospital, and ORTON Invalid Foundation, Helsinki, Finland; ²⁰Michael C. Nevitt, PhD: University of California, San Francisco; ²¹Aspasia Tsezou, PhD: University of Thessaly Medical School, Larissa, Greece; ²²Gillian A. Wallis, PhD, Christopher M. Watson, BSc (Hons): University of Manchester, Manchester, UK; ²³John P. A. Ioannidis, MD: University of Ioannina Medical School of Medicine and Foundation for Research and Development—Hellas, Ioannina, Greece, and Tufts Medical Center, Tufts University School of Medicine, Boston, Massachusetts.

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Address correspondence and reprint requests to Professor John P. A. Ioannidis, MD, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina 45110, Greece. E-mail: jioannid@cc.uoi.gr.

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8,135 for knee OA, and 4,040 and 4,792 for hand OA. For rs7775, the respective sample sizes were 4,352 and 10,843 for hip OA, 3,545 and 6,085 for knee OA, and 4,010 and 5,151 for hand OA, and for rs288326, they were 4,346 and 8,034 for hip OA, 3,595 and 6,106 for knee OA, and 3,982 and 5,152 for hand OA. For each individual study, sex-specific odds ratios (ORs) were calculated for each OA phenotype that had been investigated. The ORs for each phenotype were synthesized using both fixed-effects and random-effects models for allele-based effects, and also for haplotype effects for *FRZB*.

Results. A significant random-effects summary OR for knee OA was demonstrated for rs143383 (1.15 [95% confidence interval 1.09–1.22]) ($P = 9.4 \times 10^{-7}$), with no significant between-study heterogeneity. Estimates of effect sizes for hip and hand OA were similar, but a large between-study heterogeneity was observed, and statistical significance was borderline (for OA of the hip [$P = 0.016$]) or absent (for OA of the hand [$P = 0.19$]). Analyses for *FRZB* polymorphisms and haplotypes did not reveal any statistically significant signals, except for a borderline association of rs288326 with hip OA ($P = 0.019$).

Conclusion. Evidence of an association between the *GDF5* rs143383 polymorphism and OA is substantially strong, but the genetic effects are consistent across different populations only for knee OA. Findings of this collaborative analysis do not support the notion that *FRZB* rs7775 or rs288326 has any sizable genetic effect on OA phenotypes.

Primary osteoarthritis (OA) is the most prevalent articular disorder and accounts for substantial morbidity and disability, particularly among the elderly, with a considerable health care burden in the developed countries. Its etiology is multifactorial. Many common genetic variants have been proposed as being associated with the risk of OA, albeit with inconsistent results among studies. Among them, 2 genes that have received extensive attention in the recent literature are the growth differentiation factor 5 gene (*GDF5*) and the Frizzled-related protein gene (*FRZB*). *GDF5* has a role in the development and maintenance of bone and cartilage (1–5). *FRZB* is a key participant in the Wnt signaling pathway, which has been considered to be important in OA since it can influence chondrocyte differentiation and cartilage function (6–8).

With regard to *GDF5*, Miyamoto et al reported a strong association between the single-nucleotide polymorphism (SNP) rs143383 and hip and knee OA in

Asian populations (9): the per-risk allele (T) summary odds ratio (OR) was 1.79 ($P = 1.8 \times 10^{-13}$) in 2 Japanese cohorts evaluated for associations with hip OA, and the ORs obtained in a Japanese and a Chinese cohort evaluated for associations with knee OA were 1.30 ($P = 0.0021$) and 1.54 ($P = 2.8 \times 10^{-4}$), respectively. Several studies of Caucasian populations have yielded conflicting results (10,11). Findings of an earlier meta-analysis (12) suggested that rs143383 is associated with a 1.21-fold increase in the risk of knee OA per risk allele copy, but significance levels were modest by current standards ($P = 0.0004$ in the per-allele model). A modest association was also observed for all cases combined (any joint involved [$P = 0.006$]); however, a large heterogeneity was observed ($I^2 = 85\%$).

Several studies have also investigated the relationship between OA and 2 polymorphisms in *FRZB*, rs7775 and rs288326 (7,13,14). Much like the findings with *GDF5*, studies have yielded conflicting results (13–17). A preliminary meta-analysis of published data showed no clear evidence of association between the rs7775 SNP and hip or knee OA (18). The original proposed association was stronger at the haplotype level (7), but published data did not enable evaluation of haplotype effects.

The inconclusive and conflicting results leave uncertainty with regard to the effects of these polymorphisms. Single studies are hampered by small sample size and lack of power (19), inconsistent definitions of OA phenotypes, difficulty in assessing haplotypes, lack of standardization, and potentially selective reporting in the published literature (20). Meta-analyses based on consortia may help overcome some of these limitations. Herein we report the results of a collaborative meta-analysis of individual-level data, from 14 teams, on the relationship of the *GDF5* rs143383, *FRZB* rs7775, and *FRZB* rs288326 polymorphisms with OA phenotypes. We synthesized standardized data on hip, knee, and hand OA according to a common meta-analysis protocol.

METHODS

Study population. Groups from the Translational Research in Europe Applied Research in Osteoarthritis (TREAT-OA) consortium and other groups that were known to have genotyped the *GDF5* and *FRZB* SNPs in their OA cohorts were invited to participate in the present study. Fourteen teams had generated such data (Table 1), 9 of which had already published these data in the literature (7,10–13,17,18); 1 team had published only some of the data (14), and 4 (Nottingham, Twins UK, Kujala, and deCODE) had not published any of the data. Thirteen teams studied populations

Table 1. Characteristics of the study populations included in the meta-analysis, and definition of phenotypes*

Study or PI name	Country of origin	Study design	Female subjects, %	Age, mean \pm SD years	Hip OA definition		Knee OA definition		Hand OA definition	
					THR	Radiographic	TKR	Radiographic	Clinical	Radiographic
Loughlin (7)	UK	Case-control	53	NA	+		+			
Chapman (10)	UK	Case-control	57	71.2 \pm 7.8 (cases)	+		+			
Twins UK	UK	Twin study	100	54.3 \pm 7.8		Croft grade \geq 1		K/L grade \geq 2		K/L grade $>$ 2 in $>$ 3 joints
Chingford (10)	UK	Prospective cohort	100	53.6 \pm 6.0						
Tsezou (11)	Greece	Case-control	72	67.6 \pm 8.4						
Ikegawa	Japan	Case-control	70	57.8 \pm 16.9						
GARP (12)	The Netherlands	Family-based case-control study	100	60.4 \pm 7.5						K/L grade \geq 2 in $>$ 3 joints
Gonzalez (10,17)	Spain	Case-control	66	66.9 \pm 8.3	+					ACR
deCODE	Iceland	Prospective cohort	64	74.8 \pm 11.9	+					†
Rotterdam (18)	The Netherlands	Prospective cohort	59	69.4 \pm 9.1	+					K/L grade \geq 2 in 2 of 3 joint groups
Kujala	Finland	Family-based study (only index cases were used)	Hand OA 82, knee OA 76, controls 76	Hand OA 62 \pm 9, knee OA 67 \pm 8, controls 58 \pm 2						K/L grade \geq 2 in bilateral DIP joints
Nottingham	UK	Case-control	<i>GDF5</i> 60.4, <i>FRZB</i> 56.9	NA						ACR
SOF (14)	US	Prospective cohort	100	78.5 \pm 4.6						
Lories (16)	Belgium	Case-control	100	76.7 \pm 7.0	+					

* PI = principal investigator; OA = osteoarthritis; THR = total hip replacement; TKR = total knee replacement; NA = data not available; K/L = Kellgren/Lawrence; GARP = Genetics, Osteoarthritis, and Progression study; ACR = American College of Rheumatology; DIP = distal interphalangeal; SOF = Study of Osteoporotic Fractures; JSN = joint space narrowing.

† Clinical and/or radiographic.

of Caucasian descent (from Europe or the US), and 1 team studied a Japanese population (Table 1). Teams/studies are referred to below by the same designations that appear in Table 1, i.e., name of study or principal investigator.

Outcomes assessed. Three outcomes, i.e., hip OA, knee OA, and hand OA, were addressed separately. We accepted phenotype definitions based on joint replacement, radiographic criteria, and clinical criteria. For radiographic criteria, we preferred the Kellgren/Lawrence (K/L) classification system (21), which is the most widely used scale for identifying and grading OA (grades 0–4, with 0 representing normal findings and 4 representing severe OA). 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. For clinical criteria, we preferred the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria (22–24) but also accepted other definitions that may have been preferred by local investigators, if information on ACR criteria was not available. The phenotype definitions used in each study are shown in Table 1.

Participants with >1 affected joint were considered cases for each of the analyses pertaining to OA phenotypes for which they had affected joints. Participants without any known affected joint were included as controls. If information on controls was available for only 1 joint and information on the other joints was missing, the participant was counted as a control only for the specific joint. Thus, for example, if an individual was considered as a control for hip OA and there was no recorded information on knee and hand OA, he or she was counted as a control only in the analysis of hip OA.

We also performed an analysis of associations with any OA regardless of site, but this was considered, from the outset of our study, a secondary exploratory analysis given the very large diversity in phenotype definitions. For this analysis, cases were subjects who had OA in at least 1 joint (hip, knee, or hand), and controls were those who had no OA documented in the joints assessed in each study, as per each study's ascertainment procedures.

Genotyping procedures. Genotypes were determined by TaqMan analysis in the Rotterdam, Chingford, and Gonzalez studies, by other polymerase chain reaction methods in the Loughlin, Chapman, and Tsezou studies and the Study of Osteoporotic Fractures (SOF), by mass spectrometry in the Genetics, Osteoarthritis, and Progression (GARP) study and the Kujala study, and by pyrosequencing in the Lories study. The genotypes in the deCODE study were determined with the Centaurus platform. Twins UK study data were generated as part of ongoing genome-wide association studies using the Illumina platform. Invader assay, TaqMan, DNA fragment analysis, or direct sequencing were used in the Ikegama study. All teams performed random genotyping for quality controls, and the results were concordant.

Statistical analysis. Participants were stratified according to study and sex. Hardy-Weinberg equilibrium (HWE) was assessed in the control group in studies of unrelated cases and controls and in the whole cohort in population-based studies, by exact chi-square test. Deviation from HWE was considered nominally statistically significant at the $P < 0.05$ level.

For each phenotype of interest, allele-based natural logarithms of ORs and their respective standard errors were estimated across all strata. Specifically, an effect estimate

(natural logarithm of the OR) and its standard error were estimated within each study separately for each sex. ORs correspond to the risk conferred by each copy of the proposed risk allele of each SNP; i.e., allele T for 143383, allele G for rs7775, and allele T for rs288326. In the deCODE study, the ORs were calculated as implemented in the NEMO software package (25). In studies that used familial designs (GARP, Twins UK) or had potential relatedness among the participants due to the structure of the population (deCODE), adjusted standard errors were computed to take into account the relatedness among the participants.

Between-study heterogeneity was tested using Cochran's Q statistic, which is considered significant at $P < 0.10$. The extent of inconsistency across studies was quantified with the I^2 statistic (26). We also computed the 95% confidence intervals (95% CIs) for I^2 (27). The I^2 ranges between 0 and 100%, and for operational purposes, values of 0–24%, 25–50%, 50–75%, and >75% are considered low, moderate, large, and very large, respectively (28). 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% (29).

The natural logarithms of the OR estimates were synthesized using fixed-effects models (30) and random-effects models (31). Fixed-effects models assume that the true genetic effect of the risk allele is constant among groups and the observed differences are due to chance. In random-effects models the risk allele effects for the individual studies are assumed to vary around some overall average effect, and they take into account the between-study heterogeneity (32). In the absence of heterogeneity, fixed and random effects coincide.

As a main analysis, we synthesized separately the ORs for hip, knee, and hand OA. We also synthesized information on all OA cases. Sex-specific subgroup analyses were also undertaken. Heterogeneity between the sex-specific summary effects was calculated using Cochran's Q statistic. We also performed sensitivity analyses excluding studies in which there was nominally significant deviation from HWE. Furthermore, we undertook sensitivity analyses with only Caucasian-descent populations included.

For *FRZB*, we also performed an analysis of haplotypes. Haplotype reconstruction of rs7775 and rs288326 polymorphisms was performed using the population genotypic data separately for cases and controls, and separately for each sex stratum in each study. The possible haplotypes are CC, CT, GT, and GC (the first allele corresponds to the rs7775 and the second one to the rs288326 polymorphism). Haplotypes were inferred performing 1,000 iterations for 5 times with different seed for the random number generator. The frequency estimates were then checked to ensure consistency of the estimates across each run.

Nominal statistical significance is attributed using the traditional $P < 0.05$ level. However, this may be too lenient a criterion for claiming that an association is credible. Therefore, we also performed a calculation of the Bayes factors and credibility estimates for the observed associations. For each nominally statistically significant summary effect, we estimated the corresponding Bayes factor under a spike and smear prior, with average genetic effect, under the alternative corresponding to an OR of 1.2 (33,34). We also calculated the credibility of the association based on these Bayes factors under various assumptions for prior credibility (10% [strong prior based on

Table 2. Fixed-effects and random-effects summary odds ratios and 95% confidence intervals for osteoarthritis risk with *GDF5* rs143383 in various analyses*

Analysis (no. of studies included)	Fixed-effects model		Random-effects model		I ² , % (95% CI)
	OR (95% CI)	P	OR (95% CI)	P	
Hip OA					
Male subjects (7)	1.10 (1.01–1.22)	0.028	1.18 (0.98–1.42)	0.074	69 (32–86)
Female subjects (8)	1.17 (1.09–1.26)	1.1×10^{-5}	1.14 (0.97–1.36)	0.12	80 (61–90)
All (8)	1.15 (1.09–1.22)	1.4×10^{-6}	1.16 (1.03–1.31)	0.016	75 (58–85)
Excluding Japanese subjects (7)	1.07 (1.01–1.14)	0.034	1.07 (1.01–1.14)	0.034	0 (0–57)
Excluding studies without HWE (7)	1.16 (1.10–1.23)	5.9×10^{-7}	1.19 (1.04–1.35)	0.009	76 (58–86)
Excluding Japanese subjects and studies without HWE (6)	1.07 (1.01–1.15)	0.026	1.07 (1.01–1.15)	0.026	0 (0–60)
Knee OA					
Male subjects (9)	1.13 (1.03–1.25)	0.011	1.13 (1.03–1.25)	0.011	0 (0–65)
Female subjects (10)	1.16 (1.08–1.24)	2.5×10^{-5}	1.16 (1.09–1.22)	2.5×10^{-5}	7 (0–65)
All (10)	1.15 (1.09–1.22)	9.4×10^{-7}	1.15 (1.09–1.22)	9.4×10^{-7}	0 (0–49)
Excluding Japanese subjects (9)	1.13 (1.06–1.20)	9×10^{-5}	1.13 (1.06–1.20)	9×10^{-5}	0 (0–51)
Excluding studies without HWE (9)	1.16 (1.09–1.23)	6.9×10^{-7}	1.16 (1.09–1.23)	6.9×10^{-7}	0 (0–51)
Excluding Japanese subjects and studies without HWE (8)	1.13 (1.07–1.22)	7.3×10^{-5}	1.13 (1.07–1.22)	7.3×10^{-5}	0 (0–54)
Hand OA					
Male subjects (5)	1.04 (0.91–1.18)	0.59	1.03 (0.88–1.22)	0.69	21 (0–66)
Female subjects (6)	1.16 (1.06–1.27)	0.001	1.11 (0.94–1.31)	0.22	65 (17–86)
All (6)	1.12 (1.04–1.01)	0.003	1.08 (0.96–1.22)	0.19	53 (8–76)
Excluding studies without HWE (5)	1.12 (1.04–1.21)	0.003	1.09 (0.96–1.22)	0.20	56 (8–79)

* OR = odds ratio; 95% CI = 95% confidence interval; OA = osteoarthritis; HWE = Hardy-Weinberg equilibrium.

biologic, functional, or other evidence], 0.1% [modest prior], or 0.0001% [agnostic prior]). Moreover, the nominally significant associations were graded according to the Venice criteria, which take into account the amount of data, replication consistency, and protection from bias (35). These indexes generate a composite assessment of strong, moderate, or weak credibility. Specifically, grades of A, B, or C are assigned for each of the above-mentioned criteria. An association with an assigned grade of C in any of the composites is considered as having weak credibility, while an association with an assigned grade of A in all 3 criteria is considered to have strong credibility.

All statistical analyses were performed with Stata 10 (StataCorp, College Station, TX). Haplotype reconstruction was performed using Phase 2.1 (36,37).

RESULTS

Data. Fourteen teams, 13 studying populations of Caucasian descent and 1 studying a Japanese population, contributed data to the collaborative analysis. As far as the authors are aware, all study teams that participate in TREAT-OA and that have genotype data available on the *GDF5* and *FRZB* polymorphisms were included in this collaborative analysis. Eleven teams provided data on the *GDF5* rs143383 SNP, and 10 teams contributed data on the *FRZB* rs7775 and rs288326 SNPs. Summary descriptions of the data on the different OA phenotypes (according to affected joints) are provided in Supplementary Table 1, available in the online

version of this article at <http://www3.interscience.wiley.com/journal/76509746/home>.

***GDF5* rs143383.** Eleven teams contributed cases and controls for study of the association of *GDF5* rs143383 with OA in 1 or more affected joints (Supplementary Table 1). The allele contrast under analysis was T allele versus C allele. In 1 study (Gonzalez), genotype distributions were not consistent with HWE, in both sex-specific groups ($P = 0.025$ and $P = 0.03$ for men and women, respectively).

Hip OA. Eight teams contributed data for the analysis of *GDF5* rs143383 and hip OA. There were data on 5,789 cases and 7,850 controls. The per-allele summary OR by random-effects analysis was nominally statistically significant (OR 1.16, 95% CI 1.03–1.31) (Table 2); however, very large heterogeneity was observed when all data were included in the analysis ($I^2 = 75\%$) (Figure 1A). When applying a simulation algorithm for sensitivity analysis of the heterogeneity, we found that the source of the variability was the Japanese study. Indeed, the I^2 became 0% when only Caucasian subjects were considered, and the summary OR was 1.07 (95% CI 1.01–1.14) ($P = 0.034$). The summary effect remained nominally significant ($P = 0.026$) when studies with deviation from HWE and non-Caucasians were both removed from the analysis (OR 1.07 [95% CI

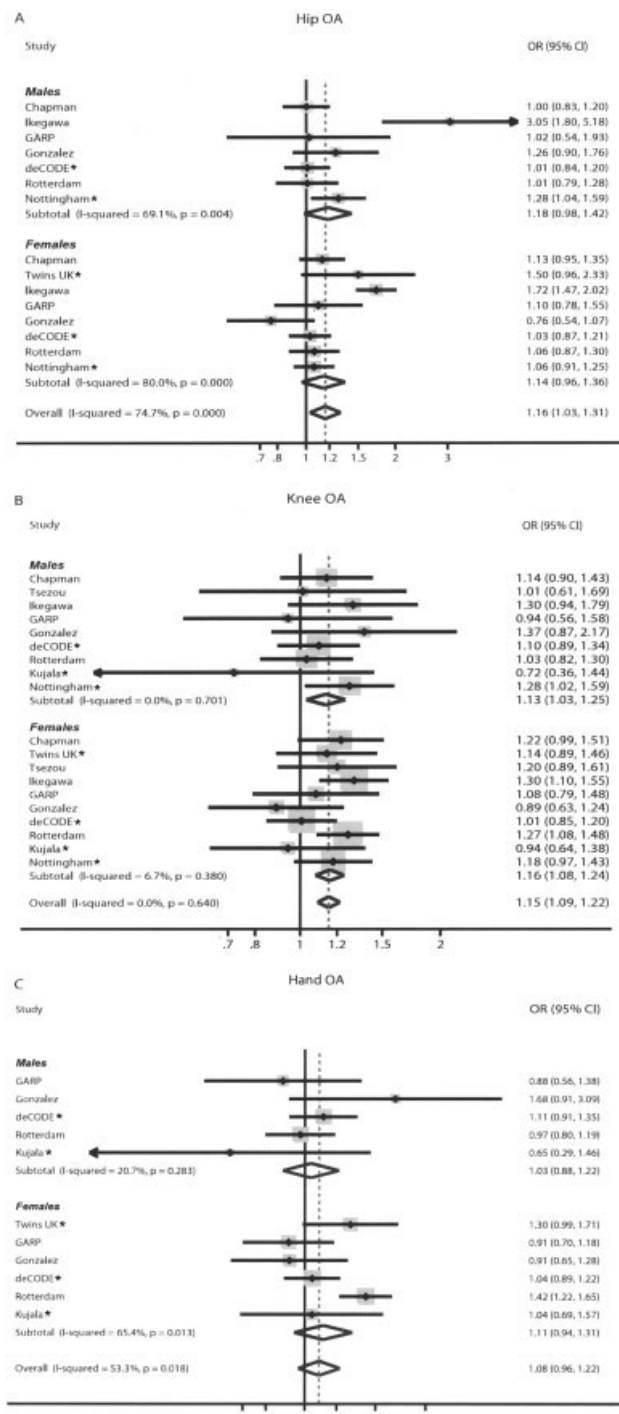


Figure 1. Forest plot of study-specific estimates and random-effects summary odds ratio (OR) estimates and 95% confidence intervals (95% CIs) for the association between the rs143383 polymorphism of the *GDF5* gene and hip osteoarthritis (OA) (A), knee OA (B), and hand OA (C). Diamonds represent the point estimate (center of each diamond) and the 95% CIs (horizontal tips of each diamond). Dashed lines represent the summary OR estimate. Sizes of the shaded boxes represent the weight of each study. Asterisks indicate that the data are entirely new or expanded compared with what was included in a previous meta-analysis (12). GARP = Genetics, Osteoarthritis, and Progression study.

1.01–1.15)). The summary ORs were not significantly different between men and women ($P = 0.35$).

Knee OA. Ten teams had available data for the analysis of *GDF5* rs143383 and knee OA, contributing a total sample size of 5,085 cases and 8,135 controls. The random-effects summary OR was 1.15 (95% CI 1.09–1.22) (Table 2). No significant heterogeneity was detected ($I^2 = 0\%$) (Figure 1B). Sensitivity analysis with only Caucasian participants and excluding studies with deviation from HWE yielded almost identical results (OR 1.13 [95% CI 1.07–1.22]) (Table 2). The sex-specific effect estimates were 1.16 and 1.13 for women and men, respectively; no statistically significant heterogeneity between the 2 estimates was observed ($P = 0.70$).

Hand OA. Six teams contributed data for the analysis of *GDF5* rs143383 and hand OA, with 4,040 cases and 4,792 controls. A non-statistically significant summary OR (1.08 [95% CI 0.96–1.22]) with large heterogeneity was observed (Figure 1C). One group from 1 study (the female subjects in the Rotterdam study, in which a very large effect had been found) would have to be removed in order for any substantial reduction in heterogeneity to be achieved ($I^2 = 1\%$). The results were unaltered when sensitivity analysis excluding studies without HWE was performed (Table 2). The estimates did not differ beyond chance between men and women ($P = 0.17$).

All OA. Overall, the summary OR for association between *GDF5* rs143383 and OA as assessed with random-effects models was not nominally statistically significant (OR 1.12 [95% CI 0.99–1.31]). Very large heterogeneity was observed ($I^2 = 80\%$, 95% CI 68–88%).

***FRZB* rs7775 and rs288326.** Ten teams provided data for the study of *FRZB* rs7775 and rs288326 in OA. All subjects were of Caucasian origin. In 1 study (Chingford) there was a nominally significant deviation from HWE in rs288326 ($P = 0.003$ and $P = 0.0001$ for knee OA controls and hip OA controls, respectively). That study included only female subjects. The allele contrasts under study were G versus C for rs7775, and T versus C for rs288326.

Hip OA. Overall 4,352 cases and 10,843 controls for rs7775 and 4,346 cases and 8,034 controls for rs288326 were available for the analyses of associations with hip OA. The summary effect was nominally statistically significant for rs288326. Specifically, the summary ORs assessed with random-effects models were 1.06 (95% CI 0.93–1.22) and 1.12 (95% CI 1.02–1.23) for rs7775 and rs288326, respectively (Table 3). There was moderate heterogeneity observed for rs7775 ($I^2 = 30\%$) and no significant heterogeneity for rs288326 ($I^2 = 0\%$)

Table 3. Fixed-effects and random-effects summary odds ratios and 95% confidence intervals for osteoarthritis risk with *FRZB* rs7775 and rs288326 polymorphisms in various analyses*

Analysis (no. of studies included)	Fixed-effects model		Random-effects model		I ² , % (95% CI)
	OR (95% CI)	P	OR (95% CI)	P	
Hip OA					
rs7775					
Male subjects (5)	1.01 (0.84–1.21)	0.95	1.00 (0.81–1.23)	0.98	14 (0–82)
Female subjects (9)	1.08 (0.95–1.24)	0.22	1.11 (0.92–1.34)	0.29	41 (0–73)
All (9)	1.06 (0.95–1.18)	0.30	1.06 (0.93–1.22)	0.39	30 (0–63)
rs288326					
Male subjects (5)	1.02 (0.87–1.20)	0.77	1.03 (0.84–1.27)	0.77	33 (0–75)
Female subjects (9)	1.17 (1.04–1.31)	0.007	1.17 (1.04–1.31)	0.007	0 (0–65)
All (9)	1.12 (1.02–1.23)	0.019	1.12 (1.02–1.23)	0.019	0 (0–55)
Excluding studies without HWE (8)	1.12 (1.02–1.23)	0.017	1.12 (1.02–1.23)	0.017	0 (0–57)
Knee OA					
rs7775					
Male subjects (6)	1.13 (0.92–1.39)	0.26	1.13 (0.92–1.39)	0.26	0 (0–75)
Female subjects (8)	1.03 (0.90–1.18)	0.67	1.03 (0.90–1.18)	0.67	0 (0–68)
All (8)	1.06 (0.95–1.19)	0.32	1.06 (0.95–1.19)	0.32	0 (0–55)
rs288326					
Male subjects (6)	0.91 (0.75–1.10)	0.33	0.91 (0.73–1.13)	0.39	12 (0–78)
Female subjects (8)	1.13 (1.00–1.28)	0.06	1.13 (1.00–1.28)	0.06	0 (0–68)
All (8)	1.06 (0.95–1.18)	0.28	1.06 (0.94–1.19)	0.34	15 (0–53)
Excluding studies without HWE (7)	1.05 (0.94–1.18)	0.40	1.05 (0.92–1.19)	0.48	20 (0–58)
Hand OA					
rs7775					
Male subjects (4)	1.01 (0.80–1.26)	0.96	1.01 (0.80–1.26)	0.96	0 (0–85)
Female subjects (5)	1.02 (0.88–1.19)	0.77	1.05 (0.86–1.28)	0.14	38 (0–77)
All (5)	1.02 (0.90–1.15)	0.79	1.02 (0.90–1.15)	0.79	0 (0–65)
rs288326					
Male subjects (4)	1.08 (0.89–1.32)	0.42	1.08 (0.88–1.33)	0.45	4 (0–79)
Female subjects (5)	1.08 (0.94–1.24)	0.27	1.08 (0.94–1.24)	0.27	0 (0–85)
All (5)	1.08 (0.97–1.21)	0.17	1.08 (0.97–1.21)	0.17	0 (0–65)

* See Table 2 for definitions.

(Figures 2A and 3A). Sensitivity analysis excluding the 1 study with HWE deviation did not alter the calculated summary effect size for rs288326. Effects did not differ significantly between men and women ($P = 0.52$ and $P = 0.19$ for rs7775 and rs288326, respectively), but of note, the association with rs288326 reached nominal significance for women (Table 3).

Knee OA. Eight teams contributed 3,545 cases and 6,085 controls for rs7775 and 3,595 cases and 6,106 controls for rs288326, for the analyses of associations with knee OA. For both polymorphisms under study, the summary ORs computed with random-effects models were not statistically significant and were very close to the null (summary OR 1.06 [95% CI 0.95–1.19] and OR 1.06 [95% CI 0.94–1.19]) for rs7775 and rs288326, respectively) (Table 3). No statistically significant between-study heterogeneity was seen, with $I^2 = 0\%$ and $I^2 = 15\%$ for rs7775 and rs288326, respectively (Figures 2B and 3B). There was no significant difference between men and women for rs7775 ($P = 0.48$) or rs288326 ($P = 0.064$ [nonsignificant trend toward stronger effect in women]) (Table 3).

Hand OA. Overall, 4,010 cases and 5,151 controls for rs7775 and 3,982 cases and 5,152 controls for rs288326 were available from 5 teams, for the analyses of

associations with hand OA. For both SNPs the summary ORs were not statistically significant and close to the null, with effect sizes of 1.02 and 1.08 for rs7775 and rs288326, respectively (Table 3). No significant heterogeneity was observed for either polymorphism (Figures 2C and 3C). There was no significant difference between men and women for rs7775 ($P = 0.90$) or rs288326 ($P = 0.17$).

All OA. The summary OR was not statistically significant for rs7775 or rs288326 when all OA cases were considered; by random-effects analysis, the summary ORs were 1.04 (95% CI 0.93–1.13) and 1.09 (95% CI 0.98–1.21), respectively. There was no between-study heterogeneity for either SNP ($I^2 = 80\%$ [95% CI 0–58% and 0–42% for rs7775 and rs288326, respectively]).

Analysis of *FRZB* haplotypes. No evidence of association was found when *FRZB* haplotypes were considered. By random-effects analysis, in hip OA the summary ORs were 1.08 (95% CI 0.92–1.26) ($P = 0.34$) and 1.10 (95% CI 0.96–1.27) ($P = 0.18$) for the CT and GC haplotypes, respectively, with the CC haplotype as the reference. For knee OA, the summary ORs were 1.02 (95% CI 0.91–1.13) ($P = 0.79$) and 1.07 (95% CI 0.95–1.21) ($P = 0.28$) for the CT and GC haplotypes, respectively, with the CC haplotype as the reference. For

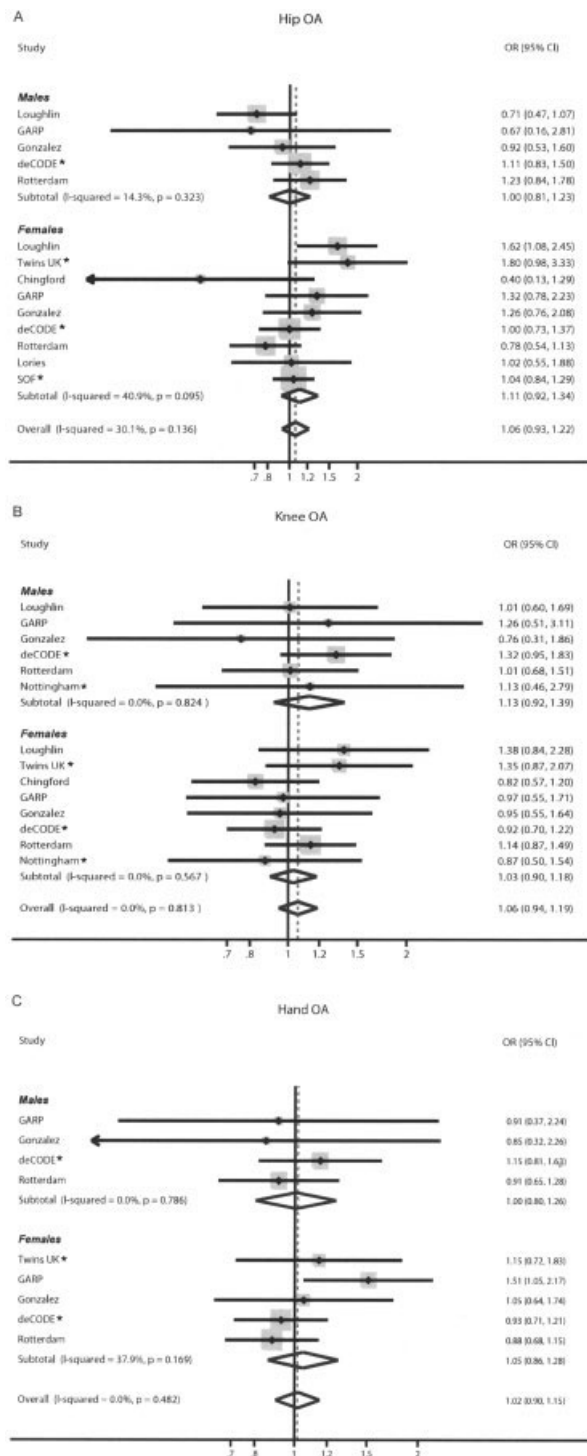


Figure 2. Forest plot of study-specific estimates and random-effects summary OR estimates and 95% CIs for the association between the rs7775 polymorphism of the *FRZB* gene and hip OA (A), knee OA (B), and hand OA (C). Diamonds represent the point estimate (center of each diamond) and the 95% CIs (horizontal tips of each diamond). Dashed lines represent the summary OR estimate. Sizes of the shaded boxes represent the weight of each study. Asterisks indicate that the data are entirely new or expanded compared with what was included in a previous meta-analysis (18). SOF = Study of Osteoporotic Fractures (see Figure 1 for other definitions).

the same comparisons, the summary ORs were 1.07 (95% CI 0.95–1.20) ($P = 0.28$) and 1.02 (95% CI 0.89–1.17) ($P = 0.76$) for hand OA.

The analysis of the GA haplotype that contains the rare alleles also showed no statistically significant effects, but data were sparse compared with data on the other haplotypes. Specifically, for hip OA the summary ORs were 0.96 (95% CI 0.41–2.24), 1.01 (95% CI 0.49–2.08), and 0.94 (95% CI 0.42–2.15) compared with the CT, CC, and GC haplotypes, respectively. The computed summary ORs for the same analysis for knee OA were 1.33 (95% CI 0.70–2.53), 1.31 (95% CI 0.68–2.54), and 1.26 (95% CI 0.60–2.62), respectively. For hand OA the computed results were 1.62 (95% CI 0.62–4.25), 1.63 (95% CI 0.65–4.10), and 1.63 (95% CI 0.62–4.27), respectively.

We also analyzed the haplotype consisting of the rare alleles versus all of the other 3 haplotypes combined. The summary OR using random-effects calculations was 0.99 (95% CI 0.48–2.07), 1.31 (95% CI 0.67–2.53), and 1.63 (95% CI 0.65–4.12) for hip, knee, and hand OA, respectively. When only women were considered, the summary OR was 1.70 (95% CI 0.82–3.50), 2.15 (95% CI 0.97–4.78), and 2.33 (95% CI 0.90–5.99) for hip, knee, and hand OA, respectively.

Bayes factors and credibility of significant signals. The estimated $-\log_{10}$ Bayes factor for the association between hip OA and *GDF5* rs143383 was 0.60, and the credibility for the association to be true was 28%, 0.4%, and 0.0004% for the strong, modest, and agnostic priors, respectively. For knee OA and *GDF5* rs143383, the estimated $-\log_{10}$ Bayes factor was 3.5, with the credibility for the association to be true being 99.7%, 76%, and 0.31% for the strong, modest, and agnostic priors, respectively. For the observed association between hip OA and *FRZB* rs288326, the estimated $-\log_{10}$ Bayes factor was 0.46 and the credibility for the association to be true was 22%, 0.29%, and 0.0003% for these 3 priors, respectively.

All 3 associations with nominal statistical significance are based on large-scale evidence ($n > 1,000$ minor alleles in cases and controls combined; grade A per the Venice criteria for amount of evidence). The between-study heterogeneity was large for hip OA and *GDF5* rs143383 (grade C per the Venice criteria), whereas no heterogeneity was observed for the knee OA and *GDF5* rs143383 association or for the hip OA and *FRZB* rs288326 association (grade A per the Venice criteria for replication consistency). Excluding data from the first study on each polymorphism and OA and studies with HWE deviation would result in loss of the nominal significance for the hip OA and rs288326 association (OR 1.11, $P = 0.052$; grade C for protection from bias per the Venice criteria). Therefore, only the association

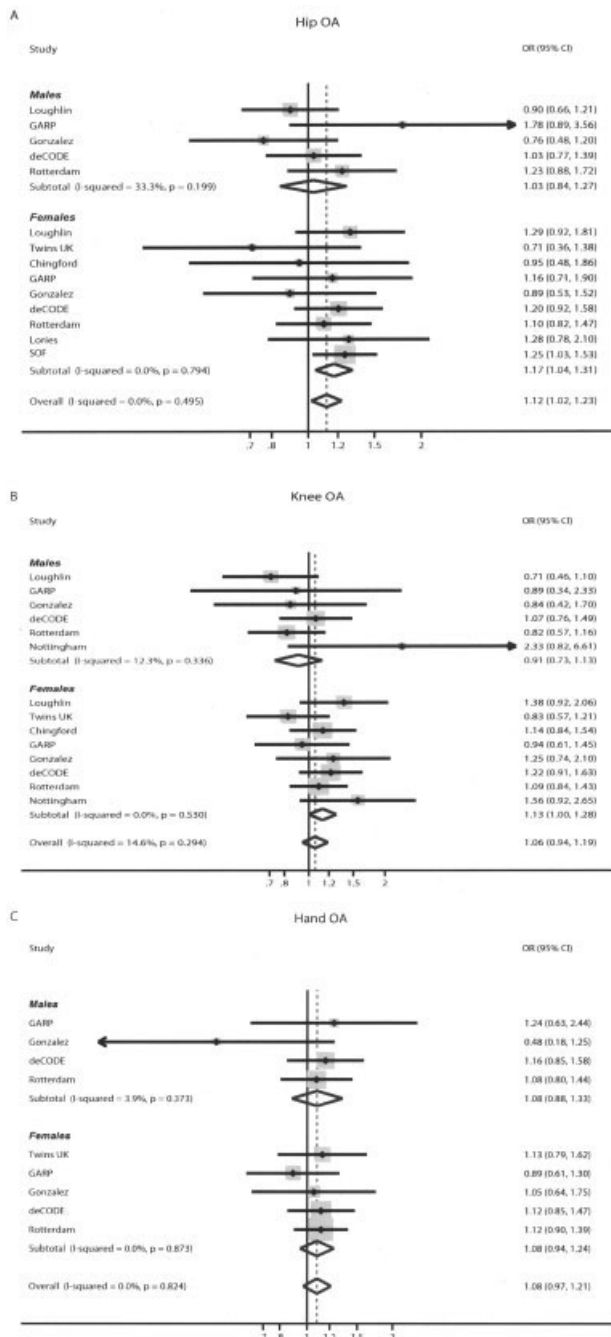


Figure 3. Forest plot of study-specific estimates and random-effects summary OR estimates and 95% CIs for the association between the rs288326 polymorphism of the *FRZB* gene and hip OA (A), knee OA (B), and hand OA (C). This polymorphism was not evaluated in a previous meta-analysis (18). Diamonds represent the point estimate (center of each diamond) and the 95% CIs (horizontal tips of each diamond). Dashed lines represent the summary OR estimate. Sizes of the shaded boxes represent the weight of each study. SOF = Study of Osteoporotic Fractures (see Figure 1 for other definitions).

of rs143383 would be graded as having strong credibility according to the Venice criteria.

DISCUSSION

In the present study, which is, to our knowledge, by far the largest study of genetics in OA undertaken to date, we found strong support for the notion that the *GDF5* rs143383 polymorphism is a determinant of risk of OA. The evidence is stronger and most consistent for knee OA, but the magnitude of the effect seems considerably smaller than originally proposed. Our meta-analyses used new or expanded data on 4 large studies compared with a previous meta-analysis of *GDF5* (12) and new or expanded data on 4 large studies compared with a previous meta-analysis of *FRZB* (18), and the amount of available data is now more than doubled for all phenotypes and polymorphisms. One of the new data sets (deCODE) is the largest single study conducted on these polymorphisms. Also, in the previous meta-analysis of *FRZB*, only 1 variant was addressed, haplotype analyses were not performed, and data on hand OA were not included. Our results yield far more robust statistical support for the association of *GDF5* rs143383 with OA, for which previous statistical support was more tenuous, even though the absolute magnitude of the effect (odds ratio) demonstrated in our meta-analysis is smaller than previously thought. For *FRZB* rs7775 and rs288326, the addition of more data demonstrates more conclusively that there is no overall effect, and excludes modest overall effects (that would be pertinent to both women and men) that could not have been excluded based on results of the previous meta-analysis.

Even for the strongest association signal we observed, that between *GDF5* rs143383 and knee OA, the effect size was very small, with an OR of only 1.15 overall (1.13 excluding the Japanese study and studies with deviation from HWE). This is much smaller than the effect of *GDF5* originally proposed in the primary publication that addressed populations of Asian descent (9). Diversity between populations of different racial descents may reflect different linkage disequilibrium patterns and may even indicate that this marker is not necessarily the true or only culprit. Nevertheless, Miyamoto et al found functional evidence for a role of the rs143383 polymorphism (9), and based on the findings of some previous studies, a functional role of *GDF5* in the pathogenesis of OA is plausible. In humans, *GDF5* is present in adult articular cartilage and also stimulates proteoglycan synthesis in articular cartilage explants (38). In addition, mutations in *GDF5* have been implicated in disorders of skeletal development, such as various forms of chondrodysplasia, synphalangism, and type C brachydactyly (9,39). Findings in a mouse study by Masuya et al further support the notion that *GDF5*

has a critical role in joint formation and OA development (40).

The more prominent effect sizes reported in earlier studies may have been inflated (41), and the large number of samples now analyzed provides an estimate of genetic effect in knee OA that is probably more realistic. Despite the very large sample size we accrued, our findings are not conclusive with regard to an association between *GDF5* rs143383 and OA of other joints. Small effects with ORs in the range of 1.1–1.15 are certainly plausible for these sites as well, but the evidence remains weak. These results highlight the need to perform extremely large studies with very careful definitions and measurements in order to understand the effects of common genetic variants on OA outcomes (42), and are consistent with experience regarding the magnitude of genetic effects that is currently obtained with high-throughput platform testing for various diseases (43). According to data in the HuGE Navigator (44) as of December 2, 2008, there are at least 95 genes that have been proposed to be associated with OA. However, the studies performed have almost always been too small for the results to be conclusive, or even considerably suggestive, for validation of such modest effects.

The ORs for *FRZB* gene polymorphisms rs7775 and rs288326 were very close to unity. In the first study from which a role of these polymorphisms was proposed, Loughlin et al (7) observed that the risk allele for rs7775 was responsible for a 1.5-fold increased risk of total hip replacement. Min and colleagues (13) also found a strong association of rs7775 with generalized OA. We observed only a nominally statistically significant association of rs288326 with hip OA; however, the potential association of rs288326 with hip OA in women should not be disregarded. One may argue that the anatomy predisposing to hip OA may differ in men and women, and Wnt signaling may affect anatomy. A possible sex-specific association may require further documentation, acknowledging that robust documentation of significantly different effects between men and women would require extremely large sample sizes (45).

Nevertheless, the overall epidemiologic credibility of this association is currently weak, despite the supporting biology. Effects did not differ significantly across studies, and the largest estimate of between-study inconsistency in the *FRZB* data was observed for rs7775 and hip OA, but it was still not large ($I^2 = 30\%$). It should be acknowledged that the Loughlin study and the SOF investigated subjects with severe radiographic hip OA, defined by findings equivalent to a K/L grade of ≥ 3 or total hip replacement, with these cases of severe hip OA compared with controls with no radiographic hip OA (K/L grade of 0 [SOF] or no signs or symptoms of

arthritis or joint disease [Loughlin study]). The phenotype definitions of radiographic hip OA and the severity of the disease varied across other studies included in the present meta-analysis. Additional studies with greater standardization of the definition of severe OA in cases and more rigorous ascertainment of lack of even mild disease in controls may be useful.

Very large collaborative studies would also be needed for better understanding of the heterogeneity of genetic effects across different populations and settings. For example, no significant heterogeneity was observed for the association of *GDF5* rs143383 with knee OA; however, the results must be interpreted with caution since the upper confidence interval of I^2 was 49%. A large degree of heterogeneity was observed in many of the other *GDF5* analyses. Part of this heterogeneity was attenuated when the only study of *GDF5* in an Asian population was excluded from the analysis.

In addition, similar to the above-described situation with regard to studies of *FRZB*, different criteria were used for definition of cases in different studies of *GDF5*, and this phenotypic heterogeneity may have led to heterogeneity in the magnitude of the genetic effects. Most of the participating study teams defined OA radiographically, using the K/L classification criteria. The K/L classification provides a composite estimate of osteophytes and joint space narrowing, and the lack of focus on joint space narrowing has been considered to be a flaw of that system (46) and might be the reason many different descriptions/definitions have been used for determination of K/L grade (47). Another important issue is that differences in radiographic assessment protocols across centers might have also introduced heterogeneity. In particular, differences in the position of the knee in which the radiographs were obtained yield different K/L grades (46). All of these issues underscore the fact that further consensus is needed in order to achieve standardization of phenotype definitions in future studies of OA.

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AUTHOR CONTRIBUTIONS

Dr. Ioannidis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Loughlin, Ingvarsson, Jonsson, Kloppenburg, Lane, Näkki, Nevitt, Spector, Valdes, Ioannidis.

Acquisition of data. Chapman, Meulenbelt, Loughlin, Carr, M. Doherty, S. Doherty, Gómez-Reino, Gonzalez, Hauksson, Hofman, Hart, Ikegawa, Jiang, Jonsdottir, Jonsson, Kerkhof, Lane, Li, Lories, van Meurs Näkki, Nevitt, Rodriguez-Lopez, Shi, Slagboom, Stefansson, Tsezou, Wallis, Watson, Uitterlinden, Valdes.

Analysis and interpretation of data. Evangelou, Meulenbelt, Karassa, Gómez-Reino, Gonzalez, Hauksson, Jonsdottir, Jonsson, Kloppenburg, Li, van Meurs, Näkki, Rodriguez-Lopez, Slagboom, Ioannidis.

Manuscript preparation. Evangelou, Meulenbelt, Karassa, Loughlin, Hauksson, Ingvarsson, Jonsson, Kerkhof, Kloppenburg, Lane, Li, Lories, Näkki, Slagboom, Valdes, Ioannidis.

Statistical analysis. Evangelou, Meulenbelt, Halldorsson, Hauksson, Lane, Näkki, Ioannidis.

Sample collection. Ingvarsson.

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