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# A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5′ UTR of *GDF5* with osteoarthritis susceptibility

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**We have performed a meta-analysis combining data for more than 11 000 individuals. It provides compelling evidence for a positive association between a functional single-nucleotide polymorphism (SNP) in the 5′-UTR of *GDF5* (+104T/C; rs143383) and osteoarthritis (OA) in European and Asian populations. This SNP has recently been reported to be associated with OA in Japanese and Han Chinese populations. Attempts to replicate this association in European samples have been inconclusive, as no association was found in the case–control cohorts from the UK, Spain and Greece when studied individually. However, the pooled data of UK and Spain found an association of the T-allele with an odds ratio (OR) of 1.10. Although the European studies had adequate power to replicate the original findings from the Japanese cohort (OR = 1.79), these results suggest that the role of the *GDF5* polymorphism may not be as strong in Europeans. To clarify whether the European studies were hampered by insufficient power, we combined new data from the UK and the Netherlands with the three published studies of Europe and Asia. The results provide strong evidence of a positive association of the *GDF5* SNP with knee OA for Europeans as well as for Asians. The combined association for both ethnic groups is highly significant for the allele frequency model ($P = 0.0004$, OR = 1.21) and the dominant model ($P < 0.0001$, OR = 1.48). These findings represent the first highly significant evidence for a risk factor for the development of OA which affects two highly diverse ethnic groups.**

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## INTRODUCTION

The role of growth and differentiation factor 5 (GDF5) in the development and maintenance of bone and cartilage has been recognized for some time (1–5). Also known as cartilage-derived morphogenetic protein 1, it is a member of the transforming growth factor-β superfamily and is closely related to the subfamily of bone morphogenetic proteins. Mutations in *GDF5* have been implicated in several disorders of skeletal development, for example various forms of chondrodysplasia, synphalangism and type C brachydactyly. Thus, its function in the aetiology of osteoarthritis (OA), a progressive degeneration of articular cartilage, appears highly plausible.

OA is a common multifactorial disease affecting ∼5% of the population mostly above 45 years of age. As such it has a large economic impact. Early changes most likely take place in the articular cartilage, which result in bone remodelling, new bone and (over a period of time) loss of articular cartilage which appears radiographically as joint space narrowing. A later severe end-stage of the disease is usually treated by surgery involving total joint replacement of the hip and/or knee (THR and TKR). A number of studies in recent years have reported finding susceptibility genes for the disease (6–10), although replication of many of these findings has been mixed (11). Possible reasons proposed for the noted inconsistencies in results include ethnic differences between cohorts, the heterogeneity of OA and perhaps the inadequate statistical power of some of the studies. These inconsistencies can be overcome by a meta-analysis which provides a quantitative approach for the combination of different independent studies, thereby maximizing the overall power.

It was to resolve some of these difficulties that a meta-analysis was conducted between an aspartic acid (D)-repeat polymorphism in the gene-encoding asporin (ASPN) and OA (12). The initial association was found in a Japanese cohort between knee OA and the D14 allele of the gene ($P = 0.0013$, OR = 2.49). This result was replicated in two independent samples of knee OA and hip OA, with functional analyses providing strong evidence for the causality of the asporin variant (7). Although three European studies from the UK, Greece and Spain failed to replicate the findings (13–15), a Chinese case–control study utilizing a cohort of radiographically defined knee OA did find significant association with the D14 allele ($P = 0.0013$, OR = 2.04) (16). A meta-analysis of all the data revealed significant heterogeneity between ethnic groups of Europeans and Asians (12). The study detected a strong association between the D14 allele and knee OA in Asian populations ($P = 0.0000013$, summary OR = 1.95) that was not replicated in the European samples. Thus, it was concluded that there are ethnic differences in the effect of the ASPN D14 allele on OA susceptibility.

Recently, an association with hip and knee OA of the single-nucleotide polymorphism (SNP) in the 5′-UTR of *GDF5* (+104T/C; rs143383) was reported in Japanese and Chinese cohorts (17). An elevated frequency of the T-allele was found in two independent Japanese hip cohorts with a combined OR = 1.79 ($P = 1.8 \times 10^{-13}$). This finding was replicated in two knee OA cohorts from Japan ($P = 0.0021$; OR = 1.30) and China ($P = 0.00028$; OR = 1.54). Furthermore, *in vitro* cell transfection studies employing luciferase reporter assays revealed that the T-allele mediated a moderate but significant reduction in the activity of the *GDF5* promoter in both chondrogenic and non-chondrogenic cell lines. Two European studies have attempted to reproduce these results (18,19). The first study employed two large independent populations from the UK ($n = 2491$) and Spain ($n = 2014$) with knee and hip OA as defined by having THR and/or TKR. No association was detected in the Spanish cohort either between allele or genotype frequencies. The UK population alone did reveal moderate association when carriers of the T-allele were combined (TT + TC) and compared with the CC genotypes (implying that the allele has a dominant effect on OA susceptibility). However, a trend towards an elevated T-allele in OA cases for both cohorts was observed. The data were pooled and this larger cohort of 4505 individuals revealed association at the allele level ($P = 0.03$), the genotype level ($P = 0.01$) and with a dominant effect model ($P = 0.004$). As with the original Asian study, overrepresentation of the T-allele in the OA individuals was identified as the risk allele. Stratification by both joint site and sex revealed no significant increase in the association. The second study utilized a Greek population ($n = 519$) of radiographically assessed knee OA cases and controls in which no association was identified with either allele frequency data or genotype data. There were also no differences detected when stratification for sex was considered. Interestingly, an increased frequency of the T-allele in the case individuals (62.9%) versus the controls (60.3%) was noted, which is comparable with that observed in the pooled UK and Spanish sample (63.3% cases versus 61.0% controls). It is possible that the predisposing risk factor of *GDF5* has a modest effect in European populations and hence these studies were of inadequate sample size.

In this article, meta-analyses were performed to assess the association of *GDF5* on OA. The analysis incorporates unpublished data from the Netherlands and UK (Manchester and Oxford) to add to previously published results from Japan, China, the UK (Oxford), Spain and Greece (17–19).

## RESULTS

Table 1 shows the allele counts and genotypes for the SNP rs143383 in cases and controls from Europe and Asia. It includes both the published and unpublished data. The total data for each study are presented along with the stratification into hip, knee and hand OA.

### Allele frequency model

Initially, the analysis was conducted on the allele data following the initial finding from the Asian populations of the association of the T-allele of *GDF5* and OA (Table 2). The forest plots for the allele model are shown in Figure 1. There was very significant heterogeneity when all the studies were combined for all the cases ($I^2 = 84.8\%$). This heterogeneity was also present in the combined hip OA cases ($I^2 = 88.3\%$), but

**Table 1.** Allele counts and genotypes for the SNP rs143383 in cases and controls for eight studies from Europe and Asia

| Country (study) | Case Allele T | Count (%) C | Control Allele T | Count (%) C | Case Genotype TT | TC | CC | Total | Control Genotype TT | TC | CC | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| All cases | | | | | | | | | | | | |
| UK [Southam *et al*. (18)][a] | 3138 (65.0) | 1688 (35.0) | 1020 (62.0) | 624 (38.0) | 1005 | 1128 | 280 | 2413 | 324 | 372 | 126 | 822 |
| Spain [Southam *et al*. (18)] | 1002 (61.20 | 634 (38.8) | 1441 (60.2) | 951 (39.8) | 302 | 398 | 118 | 818 | 439 | 563 | 194 | 1196 |
| Greece [Tsezou *et al*. (19)] | 316 (62.9) | 186 (37.1) | 323 (60.3) | 213 (39.7) | 95 | 126 | 30 | 251 | 99 | 125 | 44 | 268 |
| Netherlands[b] | 431 (59.4) | 295 (40.6) | 909 (62.8) | 539 (37.2) | 121 | 189 | 53 | 363 | 289 | 331 | 104 | 724 |
| Japan—Hip1 [Miyamoto *et al*. (17)] | 386 (81.1) | 90 (18.9) | 375 (73.0) | 139 (27.0) | 160 | 66 | 12 | 238 | 135 | 105 | 17 | 257 |
| Japan—Hip2 [Miyamoto *et al*. (17)] | 1282 (84.3) | 238 (15.7) | 1080 (74.4) | 372 (25.6) | 541 | 200 | 19 | 760 | 407 | 266 | 53 | 726 |
| Japan—Knee [Miyamoto *et al*. (17)] | 1131 (78.8) | 305 (21.2) | 1276 (74.1) | 446 (25.9) | 444 | 243 | 31 | 718 | 473 | 330 | 58 | 861 |
| China [Miyamoto *et al*. (17)] | 491 (78.4) | 135 (21.6) | 681 (70.2) | 289 (29.8) | 197 | 97 | 19 | 313 | 244 | 193 | 48 | 485 |
| Total | | | | | | | | 5874 | | | | 5339 |
| Hip OA | | | | | | | | | | | | |
| UK [Southam *et al*. (18)] | 1645 (63.8) | 935 (36.2) | 1020 (62.0) | 624 (38.0) | 519 | 607 | 164 | 1290 | 324 | 372 | 126 | 822 |
| Spain [Southam *et al*. (18)] | 361 (59.4) | 247 (40.6) | 1441 (60.2) | 951 (39.8) | 102 | 157 | 45 | 304 | 439 | 563 | 194 | 1196 |
| Netherlands[b] | 136 (64.2) | 76 (35.8) | 909 (62.8) | 539 (37.2) | 43 | 50 | 13 | 106 | 289 | 331 | 104 | 724 |
| Japan—Hip1 [Miyamoto *et al*. (17)] | 386 (81.1) | 90 (18.9) | 375 (73.0) | 139 (27.0) | 160 | 66 | 12 | 238 | 135 | 105 | 17 | 257 |
| Japan—Hip2 [Miyamoto *et al*. (17)] | 1282 (84.3) | 238 (15.7) | 1080 (74.4) | 372 (25.6) | 541 | 200 | 19 | 760 | 407 | 266 | 53 | 726 |
| Total | | | | | | | | 2698 | | | | 3725 |
| Knee OA | | | | | | | | | | | | |
| UK [Southam *et al*. (18)] | 676 (66.4) | 342 (33.6) | 1020 (62.0) | 624 (38.0) | 219 | 238 | 52 | 509 | 324 | 372 | 126 | 822 |
| Spain [Southam *et al*. (18)] | 340 (62.0) | 208 (38.0) | 1441 (60.2) | 951 (39.8) | 102 | 136 | 36 | 274 | 439 | 563 | 194 | 1196 |
| Greece [Tsezou *et al*. (19)] | 316 (62.9) | 186 (37.1) | 323 (60.3) | 213 (39.7) | 95 | 126 | 30 | 251 | 99 | 125 | 44 | 268 |
| Netherlands[b] | 180 (63.4) | 104 (36.6) | 909 (62.8) | 539 (37.2) | 54 | 72 | 16 | 142 | 289 | 331 | 104 | 724 |
| Japan—Knee [Miyamoto *et al*. (17)] | 1131 (78.8) | 305 (21.2) | 1276 (74.1) | 446 (25.9) | 444 | 243 | 31 | 718 | 473 | 330 | 58 | 861 |
| China [Miyamoto *et al*. (17)] | 491 (78.4) | 135 (21.6) | 681 (70.2) | 289 (29.8) | 197 | 97 | 19 | 313 | 244 | 193 | 48 | 485 |
| Total | | | | | | | | 2207 | | | | 4356 |
| Hand OA[c] | | | | | | | | | | | | |
| UK[d] | 692 (67.2) | 338 (32.8) | 1020 (62.0) | 624 (38.0) | 233 | 226 | 56 | 515 | 324 | 372 | 126 | 822 |
| Spain [Southam *et al*. (18)] | 301 (62.7) | 179 (37.3) | 1441 (60.2) | 951 (39.8) | 98 | 105 | 37 | 240 | 439 | 563 | 194 | 1196 |
| Netherlands[a] | 239 (59.8) | 161 (40.2) | 909 (62.8) | 539 (37.2) | 64 | 111 | 25 | 200 | 289 | 331 | 104 | 724 |
| Total | | | | | | | | 955 | | | | 2742 |

Data are presented unstratified and then subdivided by joint (study names are defined in Figure 1). Some cases from the UK and Spain were excluded from the stratified groups because they were diagnosed as having OA at more than one major site.
[a]Analysis includes unpublished data in addition to data presented in Southam *et al*. (18).
[b]Not previously published data. Cases are subjects of the GARP sibling pair study. To adjust for family relationships between subjects of the GARP study, standard errors were estimated from the variance between sibling pairs (robust standard errors) and used in each analyses (31,32).
[c]In Spain, hand OA was defined by ACR hand criteria. In the UK, hand OA was defined as nodal OA. In the Netherlands, hand OA was defined as radiographic OA at three or more joint sites out of 20 scored.
[d]Unpublished data compared against UK controls of Southam *et al*. (18).

**Table 2.** Random-effects meta-analysis of eight studies from Europe and Asia for the association of *GDF5* (allele frequency and dominant model) with OA

| Summary (number of studies) | Allele frequency model OR (95% CI) | *P*-value for the test of combined OR | *P*-value for the test of heterogeneity ($I^2$%) | Dominant model OR (95% CI) | *P*-value for the test of combined OR | *P*-value for the test of heterogeneity ($I^2$%) |
|---|---|---|---|---|---|---|
| All OA studies combined (8) | 1.26 (1.07–1.48) | 0.006 | <0.0000001 (84.8) | 1.43 (1.16–1.76) | 0.0007 | 0.042 (51.9) |
| European (4) | 1.05 (0.94–1.17) | 0.400 | 0.144 (44.6) | 1.24 (1.07–1.44) | 0.0045 | 0.403 (0) |
| Asian (4) | 1.55 (1.30–1.85) | 0.000001 | 0.042 (63.5) | 1.87 (1.33–2.65) | 0.0004 | 0.199 (35.5) |
| All Hip studies combined (5) | 1.26 (0.97–1.64) | 0.083 | <0.0001 (88.3) | 1.43 (1.03–2.00) | 0.0352 | 0.029 (62.8) |
| European (3) | 1.04 (0.94–1.15) | 0.438 | 0.621 (0) | 1.20 (0.99–1.46) | 0.069 | 0.885 (0) |
| Asian Hip (2) | 1.78 (1.52–2.08) | <0.0000001 | 0.389 (NA) | 2.12 (0.94–4.78) | 0.070 | 0.078 (NA) |
| All Knee studies combined (6) | 1.21 (1.09–1.34) | 0.0004 | 0.160 (36.9) | 1.48 (1.24–1.78) | <0.0001 | 0.941 (0) |
| European (4) | 1.13 (1.02–1.25) | 0.0222 | 0.714 (0) | 1.43 (1.15–1.76) | 0.001 | 0.860 (0) |
| Asian (2) | 1.39 (1.17–1.64) | 0.00012 | 0.234 (NA) | 1.64 (1.16–2.32) | 0.005 | 0.869 (NA) |
| All Hand OA studies combined (3) | 1.08 (0.89–1.32) | 0.4392 | 0.045 (67.8) | 1.26 (1.01–1.58) | 0.0446 | 0.417 (0) |

Heterogeneity was evaluated using the Cochran *Q*-test. $I^2$ percentage values are also given (%).
NA, not available.

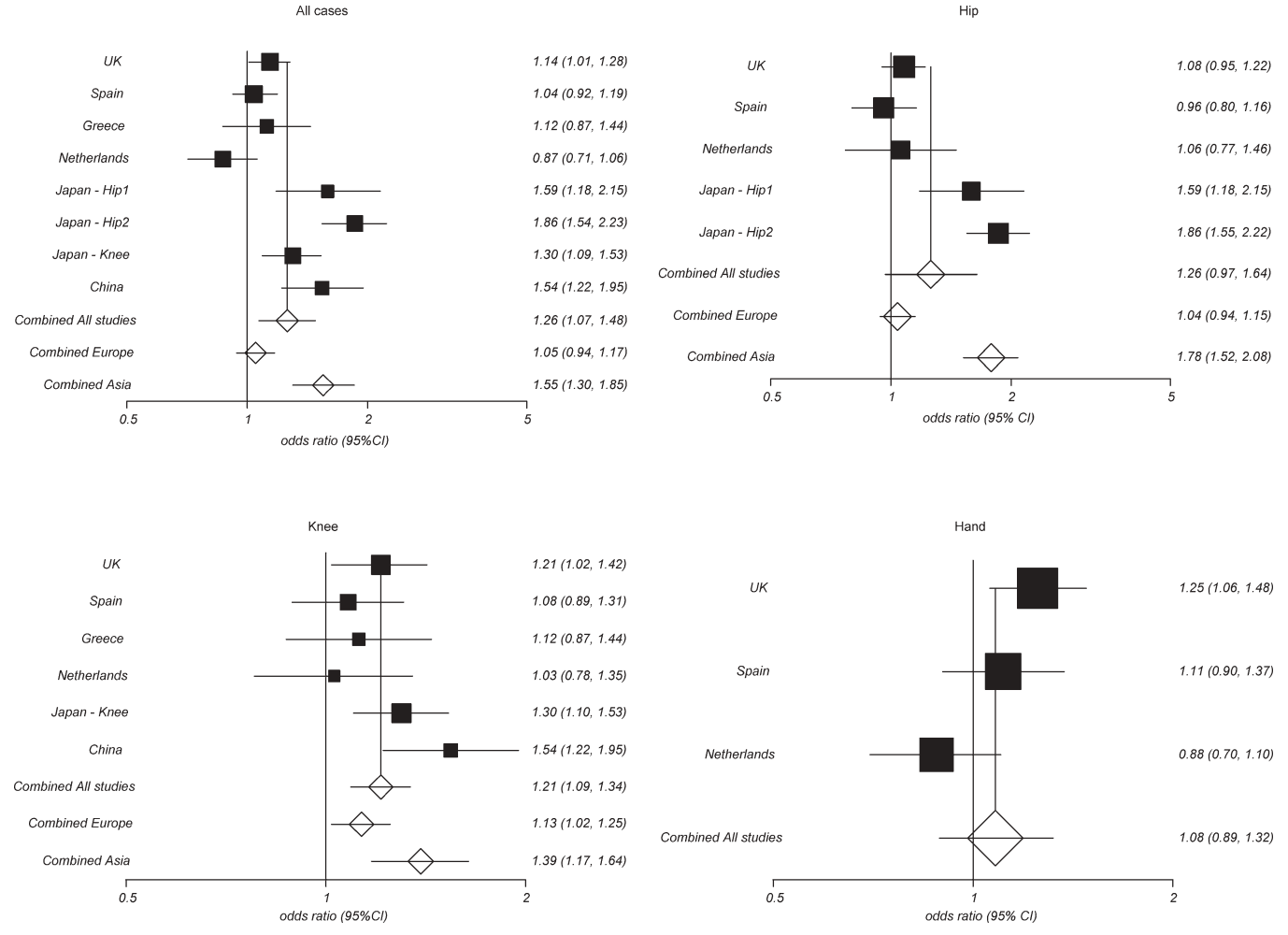


**Figure 1.** DerSimonian–Laird OR meta-analysis (random effects) of the association between *GDF5* SNP rs143383 and OA as measured by allele frequency data. Black squares (OR) and 95% CI (bar) are shown for each study. The pooled ORs and their 95% CIs are represented by the unshaded diamonds. Summary ORs are given for each ethnic group as well as all groups combined. The left-hand column lists the individual studies, each one defined by country of origin. The three cohorts from Japan are defined as follows: Japan—Hip1 represents the first Japanese cohort of hip OA; Japan—Hip2 is the second (replicating) Japanese hip OA cohort; Japan—Knee represents the Japanese cohort of knee OA.

was less apparent when only the knee studies were pooled ($I^2 = 36.9\%$). The summary OR for all cases was significant ($P = 0.006$) but the exclusion of the European studies increased the significance considerably (OR = 1.55, $P = 0.000001$), and no association was detected when the pooled European data were considered alone. The most significant result and strongest summary OR are obtained when just the two Asian hip cohorts are combined (OR = 1.78, $P < 0.0000001$), with the European studies showing no significant association in these strata. The four combined European studies for knee OA, however, demonstrate significant association ($P = 0.022$), as well as the pooled two Asian studies ($P = 0.00012$). Interestingly, there is no significant heterogeneity in the knee strata either between or within the two ethnic groups, and the combined OR for Asian and European populations is highly significant (OR = 1.21, $P = 0.0004$). These results indicate that the initial finding of a strong association in the Japanese and Chinese populations of knee OA with *GDF5* is independently replicated in European groups. This result is not seen with European hip OA and hand OA cases however.

## Dominant model

We then examined the effects of genotype differences following a dominant model, in response to the previous findings from the UK and Spanish cohorts. Their results suggest that the T-allele has a dominant effect on OA in European populations. This analysis indicates very strong association for both ethnic groups (Table 2, Fig. 2). For all cases, the association is highly significant for Europeans ($P = 0.0045$), Asians ($P = 0.0004$) and the combined group of Europeans plus Asians ($P = 0.0007$). Moderate but significant heterogeneity was seen when these studies were combined ($I^2 = 51.9\%$, $P = 0.042$) but was not apparent when the groups were considered separately. The summary ORs for the Asian group is much stronger compared with the Europeans (1.87 and 1.24, respectively), indicating perhaps the more moderate role

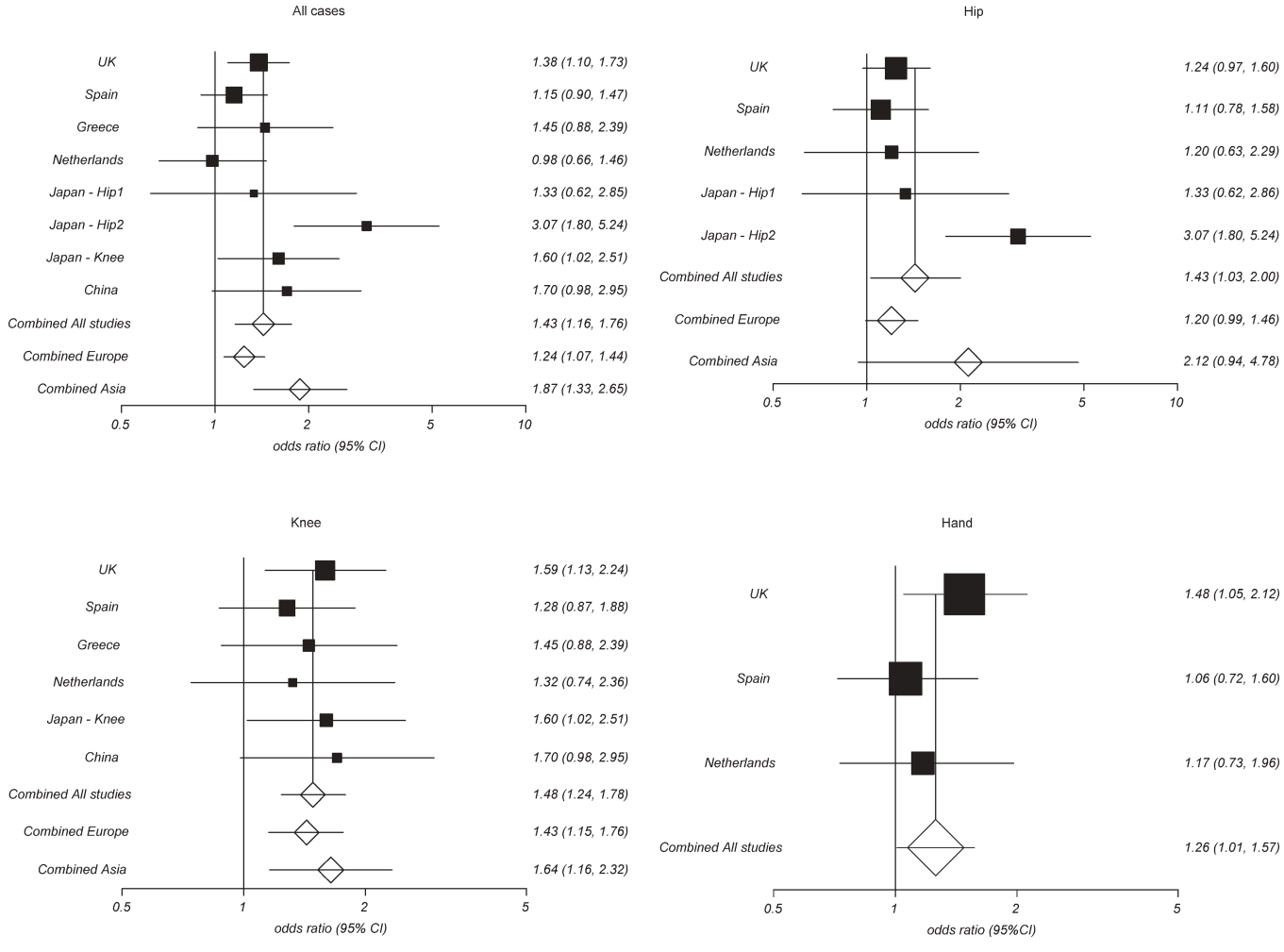


**Figure 2.** DerSimonian–Laird OR meta-analysis (random effects) of the association between *GDF5* SNP rs143383 and OA as measured by genotype data assuming a dominant mode of inheritance. Black squares (OR) and 95% CIs (bar) are shown for each study. The pooled ORs and their 95% CIs are represented by the unshaded diamonds. Summary ORs are given for each ethnic group as well as all groups combined. The left-hand column lists the individual studies as defined in Figure 1.

*GDF5* plays in populations of European ancestry. For hip OA, the association is only significant when the two ethnic groups are combined ($P = 0.035$), but again significant heterogeneity is detected. The trend of the ORs for each of the European hip cohorts of the UK, Spain and the Netherlands is similar (Fig. 2). It may be that the sample size is too small to detect the seemingly weak genetic effect of *GDF5* with hip OA. However, Europeans and Asians do show significant association for *GDF5* with knee OA individually (Europeans, $P = 0.001$; Asians, $P = 0.005$). Combining all studies of Europeans and Asians together increases the significance of association ($P < 0.0001$) without any evidence for heterogeneity. The consistency of these results is underlined by the fact that all the CIs across individual studies overlap and contain the summary OR estimate of the meta-analysis (Fig. 2). The OR for knee OA shows that the risk allele for *GDF5* has a moderate-to-strong effect in both ethnic groups [OR = 1.43 (Europeans); 1.64 (Asians); 1.48 (both)].

The combined OR for hand OA is also significant ($P = 0.045$, OR = 1.26). This was the smallest category ($n = 955$ cases) in the analysis in which just three European studies were combined and so this result may well be more significant with an increase in sample size to increase statistical power. In addition, there were differences in the clinical definition of hand OA in the three cohorts and hence future studies may need to address whether association may be to a specific hand OA trait.

## DISCUSSION

GDF5 plays a major role in joint formation and articular cartilage homeostasis (3). The rs143383 T-allele, associated with increased risk for OA, has been demonstrated to show decreased transcriptional activity in chondrogenic cells (17). In addition, *in vivo* allelic expression data have demonstrated that the *GDF5* gene is expressed in the cartilage of elderly adults with a small but statistically significant decrease in the expression of the T-allele relative to the C-allele (18). Recently, the *GDF5* polymorphism has been reported to be

associated with a decrease in human height in both males and females (20). This interesting finding indicates that the reduced expression of *GDF5* which renders an individual susceptible to OA in later life may also result in a reduction in limb bone growth during the earlier stage of life.

We performed a meta-analysis because of the concern that the individual studies may have insufficient power to detect the small effect of the functional polymorphism of *GDF5* on OA susceptibility in populations of European ancestry. Combining eight studies from six countries across two continents, we were able to perform an analysis that included 11 213 individuals. The results confirm the strong effect that the rs143383 polymorphism of *GDF5* plays in OA susceptibility in Asians, with very highly significant association for both the hip and knee OA strata as measured by allele frequency comparison. The effect appears to be strongest in the hip OA category in which the OR = 1.78 ($P < 0.0000001$). This association was not detected in the hip OA stratum for the European studies, as measured by allele frequency and when considering the dominant model. Significant heterogeneity was measured between the ethnic groups for this category however making conclusions difficult although the trend does indicate that there would be significant association for the dominant model in European ($P = 0.069$) and Asian ($P = 0.070$) groups alone if the sample size was increased further. It is interesting that there is heterogeneity between the hip and knee strata for the Asian group alone ($P = 0.042$). This may reflect a differential effect of the risk allele between the two joint strata. All centres defined their cases as having a KL grade $\geq 2$. The UK, Spanish and Greek centres' cases also had a total knee or hip replacement. Although it is possible that the differences found between the populations for the risk association are the result of the OA phenotypes defined within each centre, it is also likely that it reflects differences in environmental influences or even in the genetic background of the two groups.

In contrast, the highly significant association, as measured by allele frequencies, in the Asian populations for knee OA cases, was detected in the Europeans. The combined summary OR was also highly significant ($P = 0.0004$). This result is also seen when the dominant model for carriers of the T-allele [(TT) + (TC)] is considered. In contrast to the hip OA stratum, the knee stratum was found to have no significant heterogeneity (Table 2).

Of final interest is the association in the hand OA stratum. Association has been reported between the presence of hand OA (particularly Heberden's nodes) and radiographic knee OA (21,22); it cannot be excluded at this stage that the association seen in the hand OA category may be the results of concordant knee OA, which was not scored in the UK and Spanish study. In fact, in the GARP study, in which OA was assessed at four joint locations, the largest effect was found in subjects with OA at multiple joint locations involving at least one large joint stratum (knee or hip) and hand (results not shown). The effect in GARP among subjects without large joint involvement stayed behind as reflected in the 'all cases' analyses in Figures 1 and 2.

Together, we found evidence that the functional polymorphism in the 5′-UTR of *GDF5* plays a significant role in OA susceptibility in European as well as Asian populations. Overall significance for the dominant model was observed for knee, hip and hand OA cases, which indicates that the *GDF5* variant contributes to OA susceptibility at different joint locations and across different ethnic groups.

## MATERIALS AND METHODS

### New European data

The Netherlands data consist of 724 independent controls (aged 30–80 years) and 191 Dutch sibling pairs (aged 40–70 years) from the GARP study (23). Subjects of the GARP study were included with symptomatic and radiographic OA in two or more joint sites of the hand, spine (cervical or lumbar), knee or hip. Patients with secondary OA and familial syndromes were excluded. Radiographic OA at knee, hip, hand and spine was assessed according to the Kellgren/Lawrence (KL) scale (0–4) (24), whereas symptomatic OA of those joints was defined according to the American College of Rheumatology (ACR) recommendations (25–27). As such, the GARP study is the only one in which OA was assessed in four different joint locations. In the current analyses, GARP knee and hip OA was defined as radiographic OA, whereas hand OA was defined as radiographic OA in three or more hand joints out of 20 scored irrespective of the OA at other joint locations. In the 'all OA' analyses, all sibling pairs of GARP ($n = 91$ pairs) were included. The new UK (Manchester) data consisted of 515 unrelated cases, with hand OA defined as clinical evidence of Heberden's nodes on at least two or more distal interphalangeal joints of each hand (28). The new UK (Oxford) cases were ascertained through the Nuffield Orthopaedic Centre in Oxford and had undergone a total joint replacement of the hip (THR, $n = 69$) or of a knee (TKR, $n = 160$). The ascertainment criteria for the new hip and knee cases were as previously published for the Oxford, UK (18) cohort. The new cases increased the UK study population from 1669 to 2413 individuals. These were compared against the published UK controls of 822 individuals.

### Published data

A PubMed database search for OA and *GDF5* revealed just three relevant articles. These were published between April and September 2007: a study from Greece (19), another which combined UK (Oxford) and Spanish populations (18) and a third from Asia that initially reported the finding in two independent Japanese cohorts with replication in a Chinese population (17). The Japanese and Chinese cases were defined radiographically with a KL $\geq 2$. The Greek cases had undergone a total knee replacement. Anteroposterior weight-bearing radiographs were obtained for all the Greek individuals (cases and controls). Cases were defined as having a KL score $> 2$. The UK (Oxford) cases were ascertained using the criteria of signs and symptoms of OA sufficiently severe to require joint replacement surgery. The radiological stage of the disease was a KL grade $> 2$ in all cases, with $> 90\%$ being grade 3 or 4 (29). The Spanish hip and knee cases were also defined by total hip or knee replacement. Radiographs were used to verify that the OA was

primary. The hand OA was defined using clinical ACR criteria (30). Collaboration was established between the authors of these papers and the groups responsible for the new data. The subsequent meta-analysis combined data from eight independent populations from Europe and Asia to give a total of 5874 cases and 5339 controls (Table 1).

### Statistical analysis

Odd ratios were estimated for all the studies in the standard way except for the Netherlands data. In this case, to adjust for the family relationship among sibling pairs, standard errors were estimated from the variance between the sibling pairs (robust standard errors) and used in all the comparisons between the GARP subjects and controls, including the meta-analyses (31,32). Meta-analyses were performed using the random-effects model of DerSimonian and Laird (DSL) (33). The random-effects model anticipates that the studies may have genuine differences in their results which are not due to chance alone and thus incorporates a between-study variance in its estimate. It was found that there was highly significant heterogeneity between the frequencies of the common T-allele between the control populations of the two ethnic groups, as measured by Fisher's exact test ($P = 0.0000001$). Consequently, a DSL model was deemed to be the most appropriate method even though it generally gives wider CIs than a fixed-effect model and is thus considered to be more conservative (34). In the absence of any between-data set heterogeneity, fixed-effect model estimates are identical to random-effect model estimates.

Heterogeneity between studies was evaluated using the $I^2$ statistic for inconsistency (35) and the $\chi^2$ distributed Cochran $Q$-statistic (36). $I^2$ is given by $100\% \times (Q - \mathrm{df})/Q$, where df denotes degrees of freedom. It describes the proportion of variation that is unlikely to be due to chance and is considered large for values $>50\%$ (37). The $Q$-test is the most widely used test for heterogeneity, but is recognized to have poor power when there are few studies. Hence $Q$ is considered statistically significant for $P < 0.10$ (35,36). However $I^2$ is unaffected by the number of studies and consequently is useful when comparing subgroups within the overall study. The $I^2$ estimate and the $Q$-statistic were computed for all study comparisons.

We estimated summary ORs and 95% CIs for each ethnic group as well as for the entire set. We also subdivided for knee, hip and hand OA, following the findings of previous studies. Finally, we examined the effects of allele frequency differences as well as genotype differences (following a dominant model) in response to the previous findings from the UK and Spanish cohort. All statistical analyses were performed using the R software (http//www.R-project.org) (38) and StatsDirect version 2.6.3 (http//www.statsdirect.com) (39).

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