

Variation at the ANP32A Gene Is Associated With Risk of Hip Osteoarthritis in Women

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Objective. The ANP32A gene encodes a tumor suppressor molecule that plays a regulatory role in apoptosis and interferes with canonical Wnt signaling in vitro. We undertook this study to test whether genetic variation at ANP32A was associated with osteoarthritis (OA) in women.

Methods. Single-nucleotide polymorphisms (SNPs) in the ANP32A gene were genotyped in 438 control women, 425 women with total knee replacements (TKRs), and 537 women with total hip replacements (THRs) from the Nottingham case-control study as well as in 820 women from the population-based Chingford Study cohort for whom hip and knee radiographs were available. The most highly associated SNP was further tested in women from the Rotterdam Study (131 with THRs, 633 with knee OA, and 1,567 controls) and the TwinsUK Study cohort (67 with THRs, 43 with TKRs, and 358 controls), for a total of 2,170 patients with OA and 2,849 controls.

Results. The ANP32A transcript was abundantly expressed in normal and OA articular cartilage. Three

SNPs in the ANP32A gene were significantly associated in Nottingham patients with hip OA, but not knee OA. One of these (rs7164503) was associated with hip and knee OA in the Chingford Study cohort and with THR in the TwinsUK Study cohort, but the association was not statistically significant in the Rotterdam Study. When we combined hip data from all 4 cohorts, we found that the minor allele of rs7164503 was associated with a significantly lower risk of hip OA (Mantel-Haenszel odds ratio 0.67 [95% confidence interval 0.53–0.84], $P < 3.8 \times 10^{-4}$) and that a similar trend was observed for knee OA (Mantel-Haenszel odds ratio 0.87 [95% confidence interval 0.73–1.01], $P < 0.055$).

Conclusion. Our results provide evidence suggesting that ANP32A is involved in the pathogenesis of OA of the hip.

Osteoarthritis (OA) is the most common joint disorder in the US and Western Europe and is the leading cause of disability in the elderly (1). OA is thought to result from failed repair of damage in joint tissues, which in turn produces degradation of the articular cartilage in a localized, nonuniform manner accompanied by thickening of the subchondral bone, new bony outgrowths at joint margins, and mild-to-moderate synovial inflammation (2). The initiating events that lead to OA are not clearly established but are probably due to abnormal signals that alter the chondrocyte phenotype so that it synthesizes proteins that degrade the matrix and cause the joint to degenerate (3,4).

Several lines of work, including familial aggregation studies, twin studies, linkage analysis, and genetic association studies, have shown that OA has a significant genetic component (for review, see ref. 5). A recent genome-wide association study identified several regions of the genome that are potentially implicated in knee

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OA in women. One of the markers identified was located in chromosome 15 (6), and although the association was modest ($P < 0.08$ in replication samples), it mapped 46 kb 3' of the acidic leucine-rich nuclear phosphoprotein 32 (ANP32A) gene. ANP32A encodes a tumor suppressor (7) known to associate with both axin 1 (8,9) and phosphatase 2A (PPP2CA [10]), both of which are crucial for regulating the Wnt pathway (8,11). Wnt/ β -catenin signaling molecules play a critical role in chondrocyte proliferation, differentiation, and apoptosis during development and growth (12,13), and recent data also suggest a role for Wnt signaling molecules in the homeostasis of the articular chondrocytes (14).

We hypothesized that genetic variation at ANP32A could be involved in susceptibility to OA. In order to test this hypothesis, we genotyped polymorphisms encompassing the genetic variation in this gene in 2 independent UK cohorts and further assessed the role of the top genetic association in an additional UK cohort and in a cohort from The Netherlands (total of 2,170 patients with OA and 2,849 controls).

SUBJECTS AND METHODS

Study subjects. *The Nottingham case-control study.* Patients with hip and knee OA were recruited from hospital orthopedic surgery lists (current and for the previous 5 years). All research participants gave written informed consent. Approval for recruitment of patients with knee and hip OA was obtained from the North Nottinghamshire Research Ethics Committee and from the Leicestershire, Northamptonshire, and Rutland Research Ethics Committee. All patients had been referred to the hospital because of symptomatic, clinically severe hip or knee OA, and the majority had undergone unilateral or bilateral total hip replacement (THR) or total knee replacement (TKR) within the previous 5 years. Only female cases and controls were included in the present study. Subjects were excluded from the study if they had other major arthropathy (e.g., rheumatoid arthritis, ankylosing spondylitis), Paget's disease of bone affecting the pelvis or femur, overt childhood hip disease (e.g., Legg-Calvé-Perthes disease, slipped femoral epiphysis, severe acetabular dysplasia), THR due to trauma or avascular necrosis of the femoral head, or terminal illness. We included patients only if they had a diagnostic code of primary OA, and we excluded patients over the age of 90 years or those who had had joint replacement surgery when younger than age 40 years to further ensure the exclusion of traumatic lesions. The case status of patients was further characterized by enquiry, examination, and investigation. Height and weight were measured to calculate body mass index (BMI). Preoperative knee or pelvis radiographs of patients with knee or hip OA were examined to confirm the diagnosis and to grade for changes of OA (15,16).

All pelvis and knee radiographs were scored for individual radiographic features of OA by a single observer and

were graded 0–3 according to a standard atlas using the Kellgren/Lawrence (K/L) scale for the tibiofemoral compartments of each knee and the femoroacetabular compartment of each hip (17,18). Self-reported ethnicity was assessed by a nurse-administered questionnaire, and only individuals of European descent were included in the genetic study.

Unaffected siblings of probands who had undergone joint replacement, but had no evidence of radiographic OA and were over the age of 45 years, were considered controls. In addition, subjects age 45–85 years who had previously undergone intravenous urography in the same hospital were assumed to have the same average genetic susceptibility as the general population and were recruited as unrelated controls. These subjects and the unaffected siblings of patients were then assessed radiographically for OA, and those did not have OA and were of Caucasian origin were included as controls in the present study. Hip OA was defined as K/L grade ≥ 2 for one or both hips, and knee OA was defined as K/L grade ≥ 2 for the tibiofemoral compartment (17,18). A maximum of 1 unaffected sibling per family was included among the controls, and the allele frequencies between unaffected siblings and unrelated controls were compared to ensure that the use of family-based controls did not bias the association results. No significant differences in allele frequencies were found between these 2 groups of controls.

The Chingford Study. The Chingford Study is a prospective, population-based longitudinal cohort study that comprises 1,003 Caucasian women who are similar in most demographic variables to the general UK population and representative of it in terms of weight, height, and smoking characteristics. These women are derived from the age/sex register of a large general practice in North London. The study design and rationale have been described in detail elsewhere (19). The Guy's and St Thomas' Trust and Waltham Forest Trust Ethics Committees approved the study protocol. After study procedures were explained to participants, they gave written consent.

The K/L scale was used to grade the tibiofemoral compartments of each knee (17,18). Knee OA was defined as a K/L grade ≥ 2 for the tibiofemoral compartment at either the right or left knee (possible joint space narrowing and definite osteophytes). Pelvic radiographs were also obtained with the subject in the supine anteroposterior position, and films were scored for the following radiographic features: minimum joint space width, the presence of osteophytes, maximum thickness of subchondral sclerosis, and cyst formation. Hip OA was defined as definite joint space narrowing and a K/L grade ≥ 2 for one or both hips. Controls were individuals from the population without hip OA or knee OA as defined above. A total of 21 patients with radiographic knee OA had undergone a TKR, and a total of 18 patients with radiographic hip OA had undergone a THR. Because of the small number of individuals with clinical OA, these samples were not analyzed separately. Individuals from the Chingford Study with a K/L grade < 2 for the tibiofemoral compartment at both knees as well as a K/L grade < 2 for both hips were used as controls for the present study.

The TwinsUK Study. A case-control substudy of OA was derived from the TwinsUK Adult Twin Registry, a sample

consisting of volunteers that was initially developed to study the heritability and genetics of age-related diseases. Without selecting for particular diseases or traits, these twins were recruited from the general population through a series of national media campaigns in the UK. Overall, the TwinsUK Study cohort comprises both men and women, but only women participated in a radiographic study to investigate the heritability of radiographic OA. From those participating in that study, we selected as controls for genotyping in the present study 358 unrelated Caucasian women (only 1 twin from each pair) age ≥ 55 years at the time of the visit with K/L grades < 2 at both the femoroacetabular (hip) and tibiofemoral (knee) compartments. Patients were unrelated Caucasian women from the same cohort with severe hip or knee OA who had undergone unilateral or bilateral THR (n = 67) or TKR (n = 43). The study was approved by St Thomas' Hospital Research Ethics Committee, and all participants provided informed written consent.

The Rotterdam Study. The Rotterdam Study is a prospective, population-based cohort study investigating determinants, incidence, and progression of chronic disabling diseases of the elderly. Participants are individuals age ≥ 55 years, and further details of recruitment can be found elsewhere (20). The Medical Ethics Committee of Erasmus University Medical School approved the study, and written informed consent was obtained from each participant. Only women were included in the present study. The description of scoring of the radiographs is described elsewhere in detail (21). Briefly, knee radiographs were scored for the presence of radiographic OA using the K/L scale (17,18) and the presence of a TKR. Pelvis radiographs were scored for the presence of a THR. Knee OA was defined as a K/L grade ≥ 2 at the tibiofemoral compartment of one or both knees. The presence of a TKR was also scored, but only 19 patients with knee OA had undergone a TKR; therefore, these patients were not analyzed separately from patients with radiographic knee OA alone. Controls were individuals from the same cohort with no radiographic evidence of hip OA or knee OA (K/L grade < 2) who had not undergone either THR or TKR.

Laboratory methods. Genomic DNA was extracted from peripheral blood leukocytes of affected individuals and controls using standard protocols. Individual genotyping on all samples except those from the Rotterdam Study cohort was carried out by KBiosciences (Hoddesdon, UK). Single-nucleotide polymorphisms (SNPs) were genotyped using the KASPar assay system, which is a competitive allele-specific polymerase chain reaction (PCR) SNP genotyping system using fluorescence resonance energy transfer quencher cassette oligos (http://www.kbioscience.co.uk/genotyping/genotyping_chemistry.html). As determined from the genotype concordance between duplicate samples, genotyping accuracy was 99.8%. All polymorphisms were in Hardy-Weinberg equilibrium in controls (all $P > 0.05$). Rotterdam Study genotypes for SNP rs7164503 were extracted from the genome-wide association data available using the HumanHap 500K array (Illumina, San Diego, CA). Quality control procedures are described elsewhere in detail (22).

Tag SNP selection. Eight SNPs were selected using the tag SNP picker from HapMap B35 (www.HapMap.org) using a

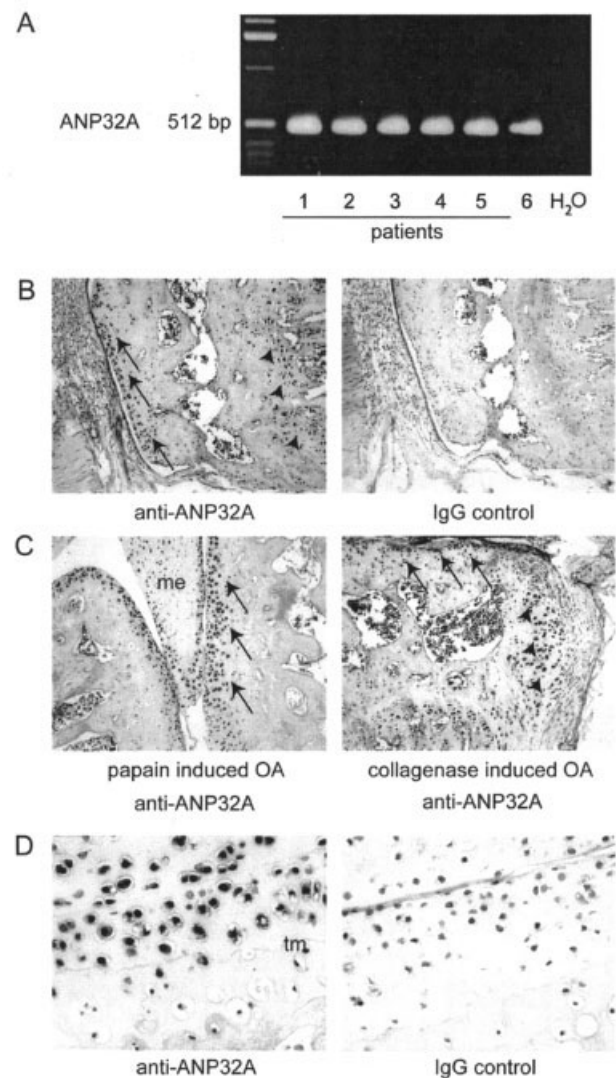


Figure 1. Expression of ANP32A in cartilage. **A**, ANP32A gene expression tested by reverse transcription–polymerase chain reaction on mRNA obtained from freshly isolated knee cartilage chondrocytes from 5 patients with osteoarthritis (OA). Lane 6 represents a positive control tissue. Water was used as a negative control. **B**, Immunohistochemistry for ANP32A in the healthy knee of a mouse. **Arrows** indicate ANP32A-positive articular chondrocytes. **Arrowheads** indicate ANP32A-positive cells in the growth plate. **C**, Immunohistochemistry for ANP32A in papain- and collagenase-induced OA in the mouse knee (at 7 days and 21 days, respectively). **Arrows** indicate ANP32A-positive articular chondrocytes. **Arrowheads** indicate ANP32A-positive chondrocytes in a developing osteophyte. **me** = meniscus. **D**, Detail of ANP32A-positive articular chondrocytes from the papain-induced OA model. Both nuclear and weaker cytoplasmic staining are present. **tm** = tidemark. (Original magnification $\times 100$ in **B** and **C**; $\times 400$ in **D**.)

cutoff value of $r^2 > 0.85$. The tag SNPs selected capture 17 alleles within the ANP32A gene with a mean $r^2 > 0.986$.

Table 1. Descriptive statistics on the study cohorts*

Subject group, characteristic	Nottingham Study, UK	Chingford Study, UK	TwinsUK Study	Rotterdam Study, The Netherlands
Unaffected controls†				
Sample size	438	486	358	1,567
BMI, mean \pm SD kg/m ²	26.5 \pm 4.2	26.2 \pm 4.2	25.2 \pm 4.2	25.9 \pm 3.8
Age, mean \pm SD years	66.2 \pm 9.7	63.2 \pm 5.9	67.6 \pm 5.6	67.7 \pm 7.7
Patients with TKR				
Sample size	425	–	43	–
BMI, mean \pm SD kg/m ²	30.2 \pm 5.6	–	27.1 \pm 3.2	–
Age, mean \pm SD years	69.2 \pm 8.7	–	65.9 \pm 9.9	–
Patients with THR				
Sample size	537	–	67	131
BMI, mean \pm SD kg/m ²	27.1 \pm 4.7	–	26.2 \pm 4.0	27.5 \pm 4.3
Age, mean \pm SD years	67.7 \pm 9.2	–	64.3 \pm 7.9	70.4 \pm 7.2
Patients with radiographic knee OA (K/L grade \geq2)				
Sample size	–	258‡	–	633‡
BMI, mean \pm SD kg/m ²	–	28.4 \pm 5.3	–	28.1 \pm 4.3
Age, mean \pm SD years	–	66.4 \pm 6.4	–	70.7 \pm 8.1
Patients with radiographic hip OA (definite JSN)				
Sample size	–	76‡	–	–
BMI, mean \pm SD kg/m ²	–	25.5 \pm 4.7	–	–
Age, mean \pm SD years	–	67.4 \pm 6.8	–	–

* All subjects in all studies were Caucasian. The Nottingham Study was a case-control study using a clinical definition of osteoarthritis (OA), while the Chingford and Rotterdam Studies were population-based studies using radiographic definitions of OA. While the TwinsUK Study cohort was originally population based, for this analysis a subset of participants, all unrelated, was selected either for not having radiographic OA (controls) or for having undergone a total joint replacement. Hence, for the purposes of the present study, the TwinsUK Study is described as a case-control study and uses a clinical definition of OA. BMI = body mass index; JSN = joint space narrowing.

† Individuals with Kellgren/Lawrence (K/L) grade $<$ 2 for the tibiofemoral compartment of both knees and K/L grade $<$ 2 for both hips.

‡ A small number of individuals with total knee replacements (TKRs) or total hip replacements (THRs) were included (see Subjects and Methods).

Gene expression. Articular chondrocytes were isolated from femoral cartilage obtained from patients with OA undergoing knee replacement surgery as described previously (23). Procedures were approved by the Ethics Committee for Clinical Research at Katholieke Universiteit Leuven, Leuven, Belgium. RNA from freshly isolated chondrocytes was obtained using an RNeasy kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol. After reverse transcription using a RevertAid H Minus complementary DNA (cDNA) synthesis kit (Fermentas, St. Leon-Rot, Germany), cDNA templates were used for PCR. Primers were designed using Vector NTI (Invitrogen, Carlsbad, CA). The expression of the ANP32A gene was investigated using the following primer sequences: 5'-TAACCAACCTGAACGACTACCG-3' (sense) and 5'-CCTCTCGTCCACAGCAA-3' (antisense). Complementary DNA was mixed with 0.5 units of *Taq* polymerase (Eurogentec, Seraing, Belgium), 0.2 mM dNTP, 0.5M specific primers, and 1.5 mM MgCl₂. An annealing temperature of 55°C was used. PCRs were run for 30 cycles, and products were visualized on 1.2% agarose gels.

Immunohistochemistry of mouse joint tissues. Papain- and collagenase-induced arthritis were induced as previously described (14). Animal experiments were approved by the

Ethics Committee for Animal Research at Katholieke Universiteit Leuven. Contralateral knees were used as controls. Immunohistochemistry of paraffin-embedded EDTA-decalcified knee sections was performed with 4 μ g/ml goat anti-ANP32A antibody (sc-5652; Santa Cruz Biotechnology, Santa Cruz, CA). After overnight incubation of the sections at 4°C, a 1:100 dilution of peroxidase donkey anti-goat IgG (Jackson ImmunoResearch, Suffolk, UK) was applied for 30 minutes, and peroxidase activity was determined using diaminobenzidine. Goat IgG (Santa Cruz Biotechnology) was used as a negative control.

Statistical analysis. *Individual polymorphism genetic associations.* The association between individual SNP genotypes and OA was tested by comparing SNP allele frequencies among cases and controls using Pearson's chi-square test. Odds ratios (ORs) with corresponding 95% confidence intervals were also computed.

Fixed-effects meta-analyses. To assess the overall evidence of association, we constructed a Mantel-Haenszel meta-analysis of data from all cohorts. The Mantel-Haenszel chi-square test and the Mantel-Haenszel estimate of the OR were used to provide a summary test and an OR. Heterogeneity of effect size between studies was assessed with the I² statistic for

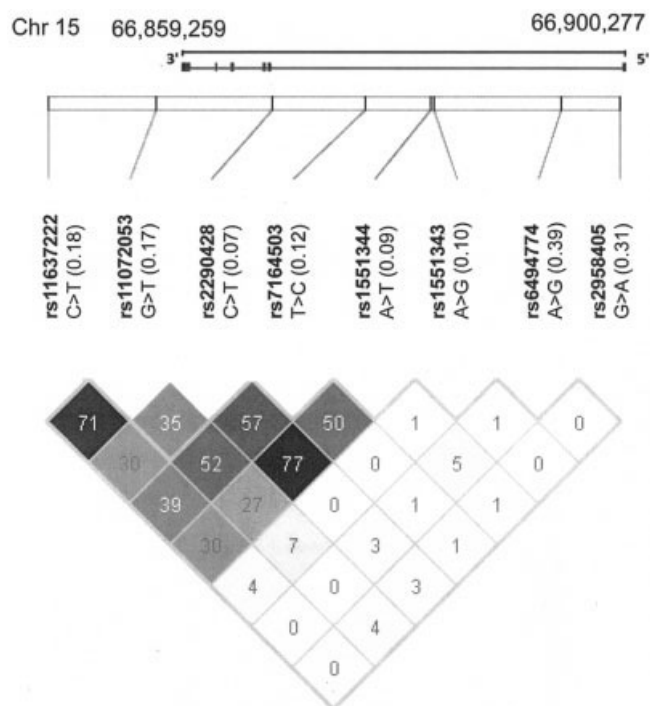


Figure 2. Single-nucleotide polymorphisms in the ANP32A gene studied. Linkage disequilibrium measured as the square of the correlation coefficient (r^2) is shown, along with the minor allele frequency in UK controls and the allele change involved. Chr = chromosome.

inconsistency and with Cochran’s Q statistic (24). StatsDirect software, version 2.7 (StatsDirect, Altricham, UK) was used.

RESULTS

We first reviewed the integrated cartilage gene database iCartiGD (<http://bioinfo.hku.hk/iCartiGD/main?db=iCartiGD> PMID=17316453) for the 2 genes flanking rs7172123, CORO2B and ANP32A. While CORO2B was found to be poorly expressed only in a chondrosarcoma library, ANP32A was also found to be expressed in normal cartilage in a microarray experiment. Based on these data, and given evidence in the literature for an involvement of ANP32A in apoptosis and Wnt signaling, we proceeded to confirm experimentally whether the gene was expressed in an OA-relevant tissue. We found that the ANP32A transcript was abundantly expressed in tissues from all OA patients tested (Figure 1A). Moreover, immunohistochemistry showed that ANP32A was found in healthy (Figure 1B) and OA (Figure 1C) articular cartilage in mice. ANP32A was also found in growth plate chondrocytes (Figure 1B) and in cartilage cells in osteophytes (Figure 1C). ANP32A

was mainly localized in the nucleus, but some cytoplasmic staining was also detected (Figure 1D).

Having confirmed the expression of this gene in chondrocytes and taking into account what is known about ANP32A biology, we proceeded to carry out a genetic association analysis with ANP32A genetic variants. The descriptive statistics on the test and replication study cohorts are presented in Table 1.

Eight tag SNPs were initially tested for genetic association with knee and hip OA (Figure 2). The SNP rs7172123 identified by pooled genome-wide association scan of knee OA (6) was not in very high linkage disequilibrium (LD) with 2 of the tag SNPs selected (rs2290428 and rs7164503), but was in significant LD

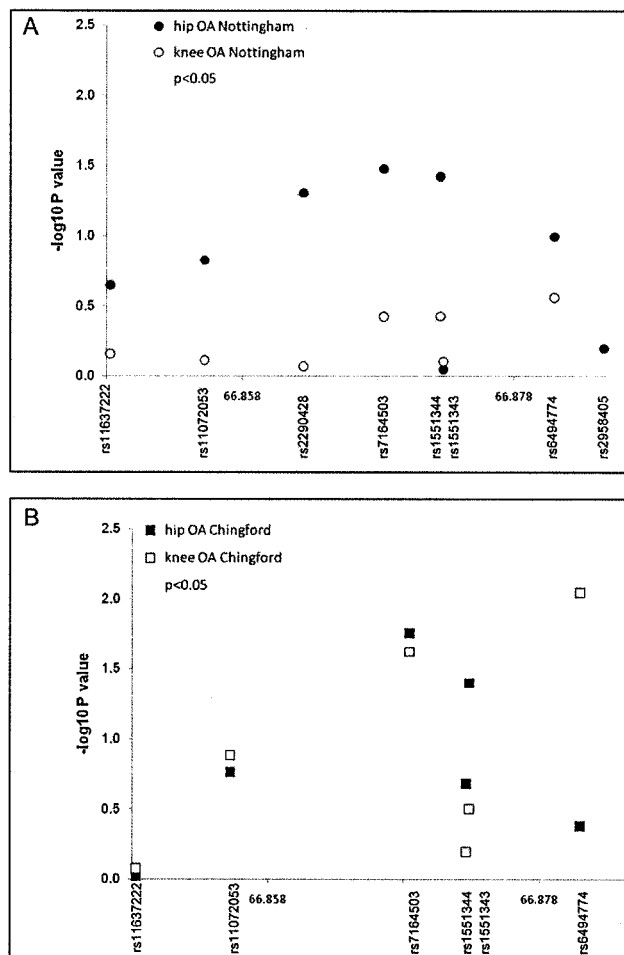


Figure 3. Genetic association between ANP32A single-nucleotide polymorphisms and osteoarthritis (OA) in the Nottingham Study (A) and in the Chingford Study (B). The position along chromosome 15 is shown on the x-axes.

Table 2. Association of rs7164503 with hip and knee OA in 4 studies from the UK and The Netherlands*

Study group, genotype, allele	Nottingham Study, UK	Chingford Study, UK	TwinsUK Study	Rotterdam Study, The Netherlands
Controls				
No. with CC/CT/TT genotype	4/97/337	5/104/377	3/63/292	18/200/916
No. (%) with C allele	105 (12.0)	114 (11.7)	69 (9.6)	236 (10.4)
No. (%) with T allele	771 (88.0)	858 (88.3)	647 (90.4)	2,032 (89.6)
Patients with radiographic knee OA				
No. with CC/CT/TT genotype	–	2/37/219	–	7/110/516
No. (%) with C allele	–	41 (7.9)	–	124 (9.8)
No. (%) with T allele	–	475 (92.1)	–	1,142 (90.2)
OR (95% CI)†	–	0.65 (0.44–0.94)	–	0.93 (0.74–1.17)
<i>P</i>	–	0.023	–	0.56
Patients with TKR				
No. with CC/CT/TT genotype	6/83/335	–	0/5/36	–
No. (%) with C allele	95 (11.2)	–	5 (6.1)	–
No. (%) with T allele	753 (88.8)	–	77 (93.9)	–
OR (95% CI)†	0.93 (0.68–1.24)	–	0.61 (0.23–1.55)	–
<i>P</i>	0.61	–	0.29	–
Patients with radiographic hip OA				
No. with CC/CT/TT genotype	–	0/8/68	–	–
No. (%) with C allele	–	8 (5.3)	–	–
No. (%) with T allele	–	144 (94.7)	–	–
OR (95% CI)†	–	0.42 (0.19–0.87)	–	–
<i>P</i>	–	0.017	–	–
Patients with THR				
No. with CC/CT/TT genotype	7/83/447	–	0/5/62	3/17/111
No. (%) with C allele	97 (9.0)	–	5 (3.7)	23 (8.8)
No. (%) with T allele	977 (91.0)	–	129 (96.3)	239 (91.2)
OR (95% CI)†	0.73 (0.54–0.97)	–	0.36 (0.14–0.91)	0.82 (0.52–1.29)
<i>P</i>	0.033	–	0.026	0.41

* See Table 1 for other definitions.

† Odds ratio (ORs) and 95% confidence interval (95% CI) for the minor allele.

with 6 of the 8 tested with r^2 values ranging from 0.05 to 0.20 (data from HapMap B35 [www.HapMap.org]). Of the SNPs tested, 2 were not in LD with rs7172123: rs2290428 and rs7164503.

P values for the association with hip and knee OA in the Nottingham case–control study are shown in Figure 3A. Three SNPs (rs2290428, rs7164503, and rs1551344) were significantly associated with hip OA, but none of the markers showed a significant association with knee OA. Six of the 8 SNPs were then tested in the population-based Chingford Study cohort with regard to knee and hip OA (Figure 3B). In this cohort, we found that rs7164503 was significantly associated with the risk of both knee and hip OA, rs1551343 was associated only with hip OA, and rs6494774 was associated with knee OA.

Since the only SNP associated across both cohorts was rs7164503, this marker was then tested in the replication samples from the Rotterdam Study and the TwinsUK Study (Table 2). The minor allele (C) was consistently found at a lower frequency among OA patients

than among controls in all the cohorts studied. However, the difference was statistically significant only for hip OA in the 3 UK cohorts and for knee OA only in the Chingford Study.

To assess the overall evidence of association for this polymorphism, we combined the data in a fixed-effects meta-analysis (Figure 4) and found that the C allele was associated with a significantly lower risk of hip OA (Mantel-Haenszel OR 0.67; $P < 3.8 \times 10^{-4}$) (Figure 4A). No significant evidence of heterogeneity between cohorts was observed ($I^2 = 16\%$, $P = 0.31$). A similar trend was observed for knee OA; however, in this case the result did not achieve statistical significance (Mantel-Haenszel OR 0.87; $P < 0.055$, $I^2 = 22\%$, $P = 0.28$) (Figure 4B).

DISCUSSION

In this study, we found that genetic variation at the gene implicated in apoptosis and Wnt signaling, ANP32A, is significantly associated with risk of hip OA in women and that it shows a trend in the same direction

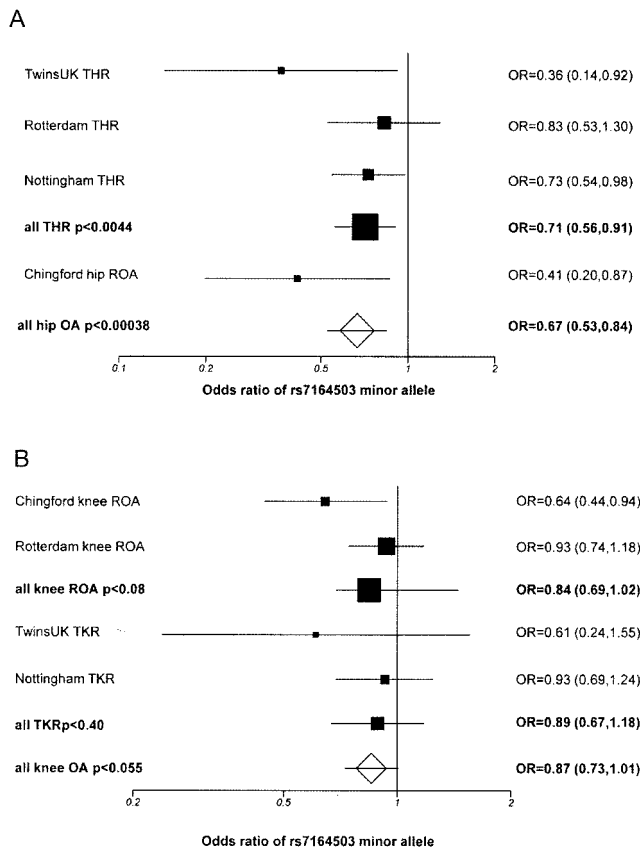


Figure 4. Forest plot of study-specific estimates and fixed-effects summary odds ratios (ORs) and 95% confidence intervals for the association of the minor allele of the ANP32A intronic single-nucleotide polymorphism rs7164503 with hip osteoarthritis (OA) (A) and knee OA (B). Summary effects are shown in boldface. THR = total hip replacement; ROA = radiographic OA; TKR = total knee replacement.

for knee OA. The gene was also found to be abundantly expressed in healthy, OA, growth plate, and remodeling cartilage.

Stelzl and coworkers (9) showed that ANP32A interferes with canonical Wnt signaling in vitro, suggesting that it may be a functional modulator of the Wnt pathway also in vivo. ANP32A has been shown to enhance caspase 9 and caspase 3 activation, thus inducing apoptosis (25). These known effects of the ANP32A gene product suggest that variation in this gene could play a role in increased chondrocyte apoptosis, and, given the importance of Wnt signaling in cartilage metabolism in mouse models (14), in cartilage and bone biology as well (26). The abundant expression of ANP32A in chondrocytes from OA patients and the

genetic association of this gene with hip OA that we found suggest that this molecule could indeed play a relevant role in the pathogenesis of OA.

We note that our study has several limitations. Our data apply only to women of European descent. Differences in the strength or even the presence of genetic associations with OA between sexes have previously been reported, as have those between Asian and Caucasian patients (27,28); therefore, it is possible that this gene may not be associated with OA in men or in other ethnic groups.

The SNP that we find associated with hip OA is an intronic SNP with no obvious function, and this marker is in an LD block with several other SNPs in the same gene. In addition, the fact that the association did not reach statistical significance in the study from The Netherlands could be explained if rs7164503 is in stronger LD with the functional variant in the UK samples than in the Dutch ones. Hence, further research is needed to identify the functional variant involved. More importantly, the SNP identified in this study is not in LD ($r^2 = 0.002$, $P < 0.9$ [not significant]) with the SNP located between the ANP32A and CORO2B genes identified by pooled genome-wide association scan of knee OA (6); therefore, this association would appear to be independent of the previously reported one. Our knowledge of ANP32A biology is still limited, and the gene has not been studied in a disease context. The findings reported here provide an impetus for the development of translational research projects elucidating the potential function of ANP32A in OA, using animal models and in vitro/ex vivo analysis of the articular cartilage and subchondral bone. Furthermore, the impact of OA susceptibility genes on extraarticular factors influencing OA may be underestimated (29). The expression of ANP32A in brain tissues also indicates that this molecule may play a neurologic role in OA. Its expression in the cerebellum could indicate a role in the coordination of movements, for instance, or in proprioception (30).

Another study limitation is that of the heterogeneity between cohorts and the mixture of radiographic and clinical phenotypes. The data in the present study derive from a case-control study, 2 population-based cohorts, and a case-control study derived from a larger population-based cohort of twins. In terms of the genetic effects, we find no evidence of heterogeneity between cohorts, and the effect sizes are extremely similar either if only clinical data (total joint replacement) are used or if radiographic data are also included. We therefore

conclude that this has not biased the overall result. One of the studies is derived from a twin population, but the subjects from this cohort have been shown to be equivalent to the general UK population in terms of socio-economic, lifestyle, anthropometric, and physiologic measures (31).

The effect that we find is stronger for hip OA than for knee OA. Other genes that have been reproducibly associated with OA have shown stronger associations with knee OA than with hip OA (e.g., GDF5) (28), suggesting that genetic factors may play a different role depending on the site involved. One possible explanation for these differences may lie in different etiopathogenesis of OA of the hip and OA of the knee. Some investigators claim that primary hip OA is secondary to developmental abnormalities (32), with a subtle deformity of the proximal femur being responsible for the subsequent development of hip OA. A recent case-control study found that both the femoral head shape and the femoral neck shaft angle (33) are very strongly associated with the risk of hip OA, indicating that a nonspherical femoral head shape not only occurs as a consequence of OA, but may itself be a morphologic risk factor for the development of hip OA. Considering the fundamental role of Wnt genes in morphogenesis and vertebrate skeletogenesis, it may indeed be possible that through its role in the regulation of β -catenin signaling, ANP32A has a more relevant role in affecting OA of the hip than OA of the knee. At the same time, there are risk factors such as BMI that have a strong influence on knee OA, but that have a much smaller effect on hip OA (34). Understanding the genetic factors that influence hip OA more than knee OA will shed light on the differences in pathogenesis of OA at these 2 anatomic sites, and Wnt-related molecules may hold some clues to those differences.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Valdes 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 conception and design. Valdes, Lories, Kerkhof, Hofman, Zhang, Luyten, Uitterlinden, Spector.

Acquisition of data. Valdes, Lories, S. Doherty, Zhang, Luyten, Uitterlinden, M. Doherty.

Analysis and interpretation of data. Valdes, Lories, Van Meurs, Hart, Zhang, Luyten, Uitterlinden.

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