

A Genome-Wide Association Study Identifies an Osteoarthritis Susceptibility Locus on Chromosome 7q22

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Objective. To identify novel genes involved in osteoarthritis (OA), by means of a genome-wide association study.

Points of view or opinions in this paper are those of the authors and do not necessarily represent the official position or policies of the Tufts Clinical and Translational Science Institute.

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Methods. We tested 500,510 single-nucleotide polymorphisms (SNPs) in 1,341 Dutch Caucasian OA cases and 3,496 Dutch Caucasian controls. SNPs associated with at least 2 OA phenotypes were analyzed in 14,938 OA cases and ~39,000 controls. Meta-analyses were performed using the program Comprehensive Meta-analysis, with P values $<1 \times 10^{-7}$ considered genome-wide significant.

Results. The C allele of rs3815148 on chromosome 7q22 (minor allele frequency 23%; intron 12 of the *COG5* gene) was associated with a 1.14-fold increased risk (95% confidence interval 1.09–1.19) of knee and/or hand OA ($P = 8 \times 10^{-8}$) and also with a 30% increased risk of knee OA progression (95% confidence interval 1.03–1.64) ($P = 0.03$). This SNP is in almost complete linkage disequilibrium with rs3757713 (68 kb upstream of *GPR22*), which is associated with *GPR22* expression levels in lymphoblast cell lines ($P = 4 \times 10^{-12}$). Immunohistochemistry experiments revealed that G protein-coupled receptor protein 22 (GPR22) was absent in normal mouse articular cartilage or synovium. However, GPR22-positive chondrocytes were found in the upper layers of the articular cartilage of mouse knee joints that were challenged with *in vivo* papain treatment or methylated bovine serum albumin treatment. GPR22-positive chondrocyte-like cells were also found in osteophytes in instability-induced OA.

Conclusion. Our findings identify a novel common variant on chromosome 7q22 that influences susceptibility to prevalence and progression of OA. Since

the *GPR22* gene encodes a G protein-coupled receptor, this is potentially an interesting therapeutic target.

Osteoarthritis (OA; MIM no. 165720), the most common age-related degenerative disease of the synovial joints, is commonly characterized by cartilage degradation, formation of osteophytes, and subchondral sclerosis. There is currently no cure for OA, and there are few good options for treatment of symptoms. OA is a complex disease in which both environmental and genetic factors play an important role. Primary OA has an estimated heritability of 40% for the knee, 60% for the hip, and 65% for the hand (1). Identifying the genes underlying the genetic background could provide new insights into the pathophysiology of OA and could potentially lead to new therapeutic targets.

To date, investigations of OA genetics have focused mainly on genome-wide linkage and candidate gene studies. Results of these studies have been controversial due to lack of power and replication. Currently, only 2 genes have been found to be consistently associated with OA in Caucasians: growth differentiation factor 5 (*GDF5*) (2) and iodothyronine deiodinase enzyme type 2 (*DIO2*) (3). Recently, a novel approach to searching for important risk genes involved in complex traits has become available: the hypothesis-free genome-wide association study (GWAS). The first pooled-DNA GWAS in OA showed the C allele of single-nucleotide polymorphism (SNP) rs4140564 (minor allele frequency [MAF] 8%), 75 kb upstream of the prostaglandin-endoperoxide synthase 2 gene (*PTGS2*), to be associated with prevalent knee OA in Caucasian women ($n = 387$ cases and 255 controls in pooled GWAS and 1,177 cases and 2,372 controls in the replication studies) (4). In addition, findings by Miyamoto et al (5) indicated that SNP rs7639618 in the double von Willebrand factor A gene (*DVWA*) influences susceptibility to knee OA (using 94 cases and 658 controls in the discovery study), but this could not be replicated in Caucasian populations with a total of 2,119 knee OA cases, 2,325 hip OA cases, and 3,313 controls (6,7).

Herein we report the findings of a GWAS of hip, knee, and hand OA using, as the discovery population, subjects in the population-based prospective Rotterdam Study (8), with replication in 12 additional study populations for a total of 14,938 cases and ~39,000 controls (depending on the OA phenotype studied). Since OA can occur at different joint sites, we tried to identify SNPs that showed a consistent pattern of association across multiple sites. This method will likely identify more general susceptibility genes for OA, rather than

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Ms Kerkhof and Drs. Uitterlinden and van Meurs have a patent pending in the field of disease diagnostics, including classification and prognosis of osteoarthritis by use of genetic markers. Drs. Jonsdottir, Ingvarsson, Thorleifsson, Halldorsson, Styrkarsdottir, and Stefansson own stock or stock options in deCODE Genetics. Dr. van de Putte owns stock or stock options in TiGenix and is coholder of a patent on a transgenic animal with controllable hyperproliferation and a skin inflammation phenotype.

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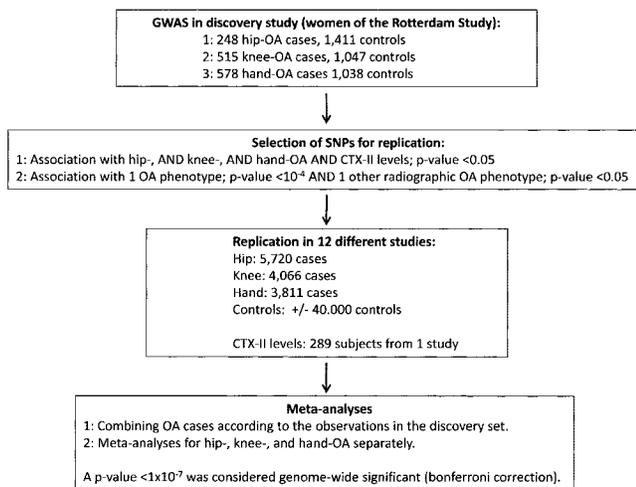


Figure 1. Flow chart of the study design. GWAS = genome-wide association study; OA = osteoarthritis; SNPs = single-nucleotide polymorphisms; CTX-II = C-terminal crosslinked telopeptide of type II collagen.

site-specific genes. In addition to the relationship with prevalence of OA, we examined the relationship between the genome-wide significant SNP(s) and progression of OA in the knee.

PATIENTS AND METHODS

Study design. A flow chart of the study design is presented in Figure 1. We used a 2-stage design to test the association of 500,510 SNPs with hip, knee, and hand OA and with urinary levels of C-terminal crosslinked telopeptide of type II collagen (CTX-II), a marker for cartilage degradation. In the first stage, the SNPs were tested for association with the 4 OA phenotypes in women from the Rotterdam Study, to identify signals that were consistent across multiple OA phenotypes. Data were available on genotypes and phenotypes in 248 hip OA cases and 1,411 controls, 515 knee OA cases and 1,047 controls, and 578 hand OA cases and 1,038 controls. If one of the following criteria was fulfilled, the SNP was followed up into the replication stage: 1) hip and knee and hand OA and CTX-II levels with a P value of <0.05 , or 2) one of the radiographic OA phenotypes with a P value of $<10^{-4}$ and one other radiographic OA phenotype with a P value of <0.05 . The direction of the association had to be the same for the different phenotypes, i.e., if a SNP was associated with an increased risk of knee OA, there also had to be an association with an increased risk of hand OA and/or hip OA. In total, 12 loci were selected on the basis of these criteria and were tested for association with OA in 12 additional studies. In total, there were 5,968 hip OA cases and 40,420 controls, 4,581 knee OA cases and 38,730 controls, and 4,389 hand OA cases and 35,694 controls available for association analysis.

Phenotype definitions. For this study, radiographic definitions of OA were standardized as follows: knee OA was

defined as a Kellgren/Lawrence (K/L) score (9) of ≥ 2 (definite osteophytes and possible joint space narrowing), hand OA as 2 of 3 hand joint groups (distal interphalangeal joints/proximal interphalangeal joints/carpometacarpal joints) affected with a K/L score of ≥ 2 (definite osteophyte), and hip OA as definite joint space narrowing. These are the definitions as they were originally described by Kellgren and Lawrence. Controls (knee and hip) were defined as having a K/L score of <2 for the knee and hip, respectively. Subjects with hand data were controls if they did not fulfill the criteria for hand OA. Subjects affected with hand OA but not with knee OA were included as controls for the knee OA analyses. Clinical studies all used total joint replacement of the hip or knee as outcome to define hip and knee OA. The American College of Rheumatology criteria (10) were used to define hand OA for the Spanish cases; the deCODE study (11,12) used a definition of either squaring or dislocation of the thumb or at least 2 nodes at the distal interphalangeal joints. In the Rotterdam Study and the Chingford Study (13–15), progression of knee OA was defined as a K/L grade of ≥ 1 at baseline and an increase in the K/L grade by at least 1 during followup.

Study populations. The study population from the Rotterdam Study (8) was the discovery population used in the present investigation. Populations from the deCODE study (11,12), the TwinsUK Study (16), the Framingham Osteoarthritis Study (17,18), the Chingford Study (13–15), Oxford cohorts (2), the Nottingham Case–Control Study (7), a Greek total joint replacement (TJR) study (19), a Spanish TJR and hand OA study (20), the Genetics Osteoarthritis and Progression (GARP) Study (21), the Study of Osteoporotic Fractures (SOF) (22), and the Osteoporotic Fractures in Men (MrOS) study (23,24) were used as the replication populations. Table 1 provides an overview of all study populations included in this study. An extensive description of each of the studies used for this GWAS is provided in the supplementary material, available in the online version of this article at <http://www3.interscience.wiley.com/journal/76509746/home>.

Biochemical measurements. Urinary CTX-II, a marker of degradation of mineralized cartilage, was measured as described previously (25,26). The concentration of CTX-II (ng/liter) was standardized to the total urine creatinine content (mmoles/liter).

Laboratory methods and quality control. *Discovery study (Rotterdam Study population).* Genotyping of the samples with the Illumina HumanHap 550v3 Genotyping BeadChip was carried out at the Genetic Laboratory of the Department of Internal Medicine, Erasmus Medical Center. The Beadstudio GenCall algorithm was used for genotype calling and quality control procedures, as described previously (27). For this study, the following quality control filters were applied: SNP call rate $\geq 95\%$, MAF $\geq 5\%$, P for Hardy-Weinberg equilibrium $\geq 1 \times 10^{-6}$. After quality control procedures, 500,510 SNPs remained for association analyses. The intensity cluster plots were visually inspected for the top hits from the Rotterdam Study, and no abnormalities were discovered. Genomic inflation factors were calculated for all analyses, and there was no evidence of population stratification, with lambda values of 1.01 for hip and hand OA, 1.00 for knee OA, and 1.02 for CTX-II levels.

Replication cohorts. Detailed information on the laboratory and quality control methods in the replication studies is

Table 1. Studies included in the genome-wide association study meta-analysis of OA phenotypes*

	Replication studies (ref.)											
	Discovery study (Rotterdam Study) [ref. 8]†	deCODE (11,12)	TwinsUK (16)	Framingham OA Study (17,18)	Chingford Study (13–15)	GARP (21)	Spanish cases (20)	Greek TJR cases (19)	Oxford cohorts (2)	Nottingham case-control (7)	MrOS (23,24)	
No. of hip OA cases/controls	413/2,474	1,630/31,654	33/425	NA	97/685	109/720	307/294	110/344	1,402/871	929/236	541/1,602	397/1,540
No. of knee OA cases/controls	698/1,893	1,068/31,654	53/476	419/1,674	269/568	154/720	262/294	368/344	555/871	735/236	NA	NA
No. of hand OA cases/controls	832/2,055	2,725/31,654	64/425	NA	286/546	241/720	241/294	NA	NA	NA	NA	NA
No. studied for CTX-II	1,100	NA	NA	NA	289	NA	NA	NA	NA	NA	NA	NA
Case-control or cohort design	Cohort	Case-control	Cohort	Cohort	Cohort	Case-control	Case-control	Case-control	Case-control	Case-control	Cohort	Cohort
OA definition	Radiographic	Clinical	Radiographic	Radiographic	Radiographic	Radiographic/clinical	Clinical	Clinical	Clinical	Clinical	Radiographic	Radiographic
Age, mean (range)	67 (55–94)	69 (19–99)	54 (37–76)	64 (29–93)	54 (44–67)	60 (30–79)	66 (32–94)	61 (20–90)	67 (55–89)	68 (40–92)	70 (65–90)	73 (65–91)
BMI, mean (range)	26 (16–56)	26 (14–60)	25 (15–51)	26 (14–54)	26 (17–47)	27 (19–47)	31 (18–53)	26 (17–34)	NA	28 (15–52)	27 (15–58)	28 (18–51)
% women	59	58	100	56	100	63	66	72	55	62	100	0
Genome-wide association platform	Illumina HumanHap 550v3	Infimum HumanHap 300	Infimum HumanHap 300	Affymetrix GeneChip Human Mapping	NA	NA	NA	NA	NA	NA	NA	NA

* OA = osteoarthritis; GARP = Genetics Osteoarthritis and Progression Study; TJR = total joint replacement; SOF = Study of Osteoporotic Fractures; MrOS = Osteoporotic Fractures in Men; NA = not applicable; CTX-II = C-terminal crosslinked telopeptide of type II collagen; BMI = body mass index.

† Number of cases and controls shown includes both men and women. For the discovery set, only women were analyzed; men were analyzed as a replication study.

available in the supplementary material (<http://www3.interscience.wiley.com/journal/76509746/home>). In summary, all samples from the deCODE study were assayed with the Infinium HumanHap 300 or humanCNV370 SNP chips (Illumina, San Diego, CA). Samples from the TwinsUK Study were genotyped with the Infinium HumanHap 300 assay (Illumina) at the Duke University Genotyping Center (Durham, NC), Helsinki University (Helsinki, Finland), and the Wellcome Trust Sanger Institute (Cambridge, UK). In the Framingham Osteoarthritis Study, all individuals were genotyped using the Affymetrix GeneChip Human Mapping 500K array set and the 50K supplemental array set, focused on coding SNPs and SNPs tagging protein-coding genes (Santa Clara, CA) as part of the SNP Health Association Resource initiative. In all other studies, genotyping was performed using MassArray iPLEX Gold (Sequenom, San Diego, CA) for replication of the top hits from the Rotterdam Study, except for the SOF and the MrOS study, in which genotyping was performed using TaqMan. An overview of all Hardy-Weinberg equilibrium *P* values, minor alleles, and minor allele frequencies in the controls from all participating studies is shown in Supplementary Table 1 (available in the online version of this article at <http://www3.interscience.wiley.com/journal/76509746/home>).

Statistical analysis. *Association analyses.* All associations with OA phenotypes for all nonfamilial studies were tested using an allelic chi-square test (1 df) assuming an additive effect for each SNP tested. Analyses for the Rotterdam Study, the Chingford Study, the Oxford cohorts, the Nottingham case-control study, the Spanish TJR and hand OA study, and the Greek TJR study were carried out using Plink version 1.02 software (28). CTX-II levels were log-transformed and converted to Z scores adjusted for age, and subsequently analyzed using an allelic chi-square test (1 df). In the SOF and the MrOS study, logistic regression analyses were performed using the program SAS version 9. In the deCODE study, a previously described likelihood procedure (29) was used for the association analyses; a 2-sided *P* value and odds ratio (OR) was calculated for each individual allele, assuming a multiplicative model for risk, i.e., that the risk multiplies with carriage of 2 copies of the allele. Some of the individuals in the Icelandic case-control groups (deCODE study) were related to one another, causing the chi-square test statistic to have a mean of >1 and median of >0.675². In addition, the inflation factor was estimated, using genomic controls, by computing the median of the 300,754 chi-square statistics and dividing it by 0.675², as previously described (30). For all analyses from the deCODE study, adjustments were made for the inflation factor.

For the TwinsUK study, robust standard errors were calculated and logistic regression was performed using Stata version 10 (StataCorp, College Station, TX). The analysis used in the Framingham Osteoarthritis Study was a logistic regression using generalized estimating equations to control for dependency among family members. Analyses were performed using R software. In the GARP study, standard errors were estimated from the variance between the sibling pairs (robust standard errors) to adjust for the family relationship among sibling pairs (2). Robust standard error analyses were performed using Stata SE8 (StataCorp).

In the Rotterdam Study and the Chingford Study, the association of SNP rs3815148 with progression of knee OA was

also tested. These analyses were performed using a logistic regression model adjusted for sex and followup time, with SPSS version 15.0.

Meta-analysis. A SNP-specific meta-analysis was performed for the combined and individual phenotypes, using the program Comprehensive Meta-analysis (www.meta-analysis.com). For the combined phenotype meta-analyses, the analyses were performed on, for example, hand and/or knee OA cases versus controls who had neither hand nor knee OA, if in the discovery study a SNP was associated with knee and hand OA using the criteria specified above. With this method of analysis, there was no overlap between cases and controls. All OA cases from the replication studies were included in the meta-analyses when applicable. For example, if a study had hip, knee, and hand OA cases and in the discovery study a SNP was associated with only hip and knee OA, then the hip and knee OA cases were used as cases in the meta-analyses. If the heterogeneity metric *I*² exceeded 25%, a random-effects model (DerSimonian and Laird) was also used for the analysis; otherwise, only a fixed-effects model (inverse variance method) was applied. The analyses were performed on the total population from all studies and were subsequently stratified by sex to reveal sex-specific associations (if any). For family-based studies, association results from men and women combined are shown because of relatedness among men and women in the study. *P* values less than 1×10^{-7} were considered genome-wide significant (0.05/500,510 after Bonferroni correction).

Association analyses with previously published SNPs. Within the Rotterdam Study, allelic chi-square tests (1 df) assuming an additive model were performed for the SNPs discovered in previous studies (see above). SNPs rs225014 and rs12885300 of the *DIO2* gene, rs4140564 75kb upstream of the *PTGS2* gene, rs7639618 of the *DVWA* gene, and rs6088813 of the *GDF5* gene were tested for association with hip, knee, and hand OA. *P* values less than 0.05 were considered significant.

Messenger RNA (mRNA) expression analyses and immunohistochemistry experiments. Synovial biopsy specimens from patients undergoing arthroscopy of the knee for meniscal and cartilage injury at the Division of Orthopedics, University Hospitals, Leuven, Belgium were cultured in vitro in Dulbecco's modified Eagle's medium/10% serum, using plastic adherence as described by Lories et al (31). Histologic and flow cytometric analysis revealed that the synovia did not show signs of chronic inflammation. Articular chondrocytes and meniscal cells were isolated from knee cartilage and meniscus obtained from patients with OA undergoing knee replacement surgery as described previously (32). All procedures were approved by the ethics committee for clinical research at Katholieke Universiteit Leuven, and informed consent was obtained from all patients. Synovial fibroblast-like cells at passage 4 and freshly isolated chondrocytes (P0) were used for quantitative polymerase chain reaction (PCR) using Assays-on-Demand (Applied Biosystems, Foster City, CA). Gene expression levels relative to the housekeeping gene β -actin were determined by the $\Delta\Delta C_t$ method. Immunohistochemistry studies were performed on mouse knee tissue after induction of arthritis by papain treatment, methylated bovine serum albumin treatment, or induction of medial meniscus instability as described previously (33,34). Sections were

Table 2. Identified top hits from the genome-wide association study using the population from the Rotterdam Study*

rs number	Locus	Chromosome	Position, bp	MAF, %	Nearest gene	Annotation location	Radiographic OA									
							Knee		Hip		Hand		CTX-II			
							P†	OR	P†	OR	P†	OR	P†	OR	P†	OR
Novel loci																
rs10465850	1	1	65471791	46	<i>JAK1</i>	-347 kb	0.009	1.22	0.02	1.26	0.01	1.20	0.02	1.07	0.02	1.07
rs12402320	2	1	152968030	30	<i>KCNN3</i>	Intron	0.03	1.19	0.04	1.23	0.01	1.22	0.01	1.07	0.01	1.07
rs4656364	3	1	160545479	10	<i>NOS1AP</i>	Intron	0.04	1.29	9×10^{-6}	1.88	0.96	1.01	0.49	1.02	0.49	1.02
rs3963342	4	4	43995005	33	<i>KCTD8</i>	Intron	0.04	1.18	0.02	1.26	0.03	1.18	0.04	1.05	0.04	1.05
rs10248619	5	7	50718584	21	<i>GRB10</i>	Intron	0.002	1.33	0.03	1.28	0.04	1.21	0.01	1.10	0.01	1.10
rs7791286	5	7	50824286	17	<i>GRB10</i>	Intron	0.02	1.25	0.02	1.33	0.01	1.28	0.03	1.07	0.03	1.07
rs3815148	6	7	106725656	23	<i>COG5</i>	Intron	7×10^{-5}	1.42	0.81	1.03	0.05	1.18	0.96	1.00	0.96	1.00
rs1548524	6	7	106731799	24	<i>COG5</i>	Intron	9×10^{-5}	1.41	0.72	1.05	0.04	1.19	0.96	1.00	0.96	1.00
rs997311	6	7	106740269	24	<i>COG5</i>	Intron	8×10^{-5}	1.41	0.74	1.04	0.03	1.20	0.96	1.00	0.96	1.00
rs959396	7	9	37001289	38	<i>PAX5</i>	Intron	3×10^{-6}	0.69	0.04	0.81	0.93	0.99	0.91	0.95	0.91	0.95
rs12352822	8	9	119587103	15	<i>TLR4</i>	-69 kb	2×10^{-5}	0.60	0.001	0.57	0.30	0.89	0.19	0.95	0.19	0.95
rs873598	9	10	120413174	45	<i>GPR10</i>	-68 kb	0.002	0.79	0.004	0.75	0.007	0.82	0.02	0.95	0.02	0.95
rs880844	10	12	107486497	24	<i>ISCU</i>	Intron	0.02	1.23	3×10^{-5}	1.56	0.16	1.13	0.18	1.05	0.18	1.05
rs741542	10	12	107488184	24	<i>ISCU</i>	3' downstream	0.02	1.23	3×10^{-5}	1.56	0.16	1.13	0.18	1.05	0.18	1.05
rs11651351	11	17	71478457	07	<i>ACOX1</i>	Intron	0.02	0.68	0.02	0.57	0.03	0.70	0.01	0.89	0.01	0.89
Known loci																
rs4911494	12	20	33435328	40	<i>GDF5/UQCC</i>	Coding/intron	0.03	0.84	0.91	1.01	2×10^{-5}	0.72	0.40	0.98	0.40	0.98
rs6088813	12	20	33438595	40	<i>GDF5/UQCC</i>	Intron	0.02	0.84	0.91	1.01	1×10^{-5}	0.72	0.40	0.98	0.40	0.98
rs6087705	12	20	33464664	40	<i>GDF5/UQCC</i>	5' upstream	0.03	0.84	0.91	1.01	1×10^{-5}	0.72	0.40	0.98	0.40	0.98

* MAF = minor allele frequency; OA = osteoarthritis; OR = odds ratio; CTX-II = C-terminal crosslinked telopeptide of type II collagen.

† From allele-based association analysis.

Table 3. Meta-analysis results, showing the association of the 12 top hits with OA phenotypes*

SNP	Chromosome	Minor allele	MAF, %	Annotation		Association with OA		CTX-II	
				Nearest gene	Location	OR (95% CI)	<i>P</i>	β	<i>P</i>
rs10465850	1	T	43	<i>JAK1</i>	−347 kb	1.02 (0.98–1.05)†	0.42	−0.99‡	0.82
rs12402320	1	T	27	<i>KCNN3</i>	Intron	1.05 (0.99–1.12)†	0.12	1.07	0.005
rs4656364	1	C	7	<i>NOS1AP</i>	Intron	1.00 (0.89–1.13)‡§	0.95	1.00	0.97
rs3963342	4	C	25	<i>KCTD8</i>	Intron	1.04 (0.97–1.12)†‡	0.29	1.05	0.07
rs10248619	7	T	19	<i>GRB10</i>	Intron	1.09 (1.04–1.14)†	4×10^{-4}	1.05‡	0.26
rs3815148	7	C	23	<i>COG5</i>	Intron	1.14 (1.09–1.19)¶	8×10^{-8}	0.98‡	0.37
rs959396	9	G	38	<i>PAX5</i>	Intron	0.94 (0.86–1.04)‡§	0.26	0.95	0.01
rs12352822	9	G	10	<i>TLR4</i>	−69 kb	0.99 (0.86–1.15)‡§	0.93	1.01‡	0.89
rs873598	10	T	49	<i>GPR10</i>	−68 kb	0.98 (0.91–1.06)†‡	0.65	0.95	0.03
rs741542	12	T	24	<i>ISCU</i>	3' downstream	1.03 (0.91–1.16)‡§	0.65	1.02	0.43
rs11651351	17	T	7	<i>ACOX1</i>	Intron	0.89 (0.74–1.08)†‡	0.24	0.91‡	0.33
rs6088813	20	C	35	<i>GDF5</i>	Intron	0.91 (0.84–0.98)‡¶	0.01	0.99	0.65

* SNP = single-nucleotide polymorphism; 95% CI = 95% confidence interval (see Table 2 for other definitions).

† Association with hand and/or hip and/or knee OA.

‡ Random-effects model.

§ Association with hip and/or knee OA.

¶ Association with hand and/or knee OA.

stained overnight with rabbit anti-G protein-coupled receptor protein 22 (anti-GPR22) antibody (4 μ g/ml in phosphate buffered saline; Sigma-Aldrich, Bornem, Belgium). Secondary antibody was peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, Suffolk, UK).

RESULTS

Association results. In the first stage of the present analysis, 500,510 SNPs were tested for association with OA in women from the Rotterdam Study. Because power to identify significant genome-wide associations with individual phenotypes was limited, we applied a strategy that takes advantage of the multiple phenotypes available in our discovery study (see Patients and Methods) to search for common variants showing consistent associations with OA at multiple joint sites. In total, 18 SNPs in 12 loci were identified using this approach (Table 2).

In the second stage, these 12 loci were tested for association with OA in 12 additional studies. The 12 loci were tested for association with the OA phenotype that formed the basis for selection in the discovery phase. In total, there were 5,968 hip OA cases and 40,420 controls, 4,581 knee OA cases and 38,730 controls, and 4,389 hand OA cases and 35,694 controls available for association analysis.

The results of the meta-analysis for these 12 SNPs for the combined phenotypes are shown in Table 3, and those for the individual phenotypes are shown in Supplementary Table 2 (available in the online version of this article at <http://www3.interscience.wiley.com/>

journal/76509746/home). From these 18 SNPs, we observed 3 loci to be significantly associated with OA. The C allele of SNP rs3815148 (A>C; MAF 23%), annotated in intron 12 of the component of oligomeric Golgi complex 5 gene (*COG5*), was associated with knee and/or hand OA in the discovery study and also in the meta-analysis (Table 3), with an OR of 1.14, a 95% confidence interval (95% CI) of 1.09–1.19, a *P* value of 8×10^{-8} , and consistent results across the data sets for knee and/or hand OA (*P* value Q statistic for heterogeneity 0.39, $I^2 = 5\%$). SNPs rs10248619 (T>C; MAF 19%), situated in the *GRB10* gene; and rs6088813 (A>C; MAF 35%), situated in the promoter region of the *GDF5* gene; were both associated with OA in the meta-analysis, but did not reach genome-wide significance (*P* = 4×10^{-4} and *P* = 0.01 respectively). There were no sex-specific associations observed. Given its significant associations with 2 OA phenotypes in the discovery study and the meta-analysis (knee and/or hand OA), we further focused on SNP rs3815148.

Figure 2 shows a forest plot of the association of the C allele of rs3815148 with knee and/or hand OA. The estimated effect sizes were similar for knee OA alone (OR 1.16) and hand OA alone (OR 1.14). Interestingly, this SNP was also associated with progression of knee OA in the Rotterdam Study (OR 1.31, 95% CI 1.01–1.69), and a nonsignificant trend in the same direction was observed in the Chingford Study (OR 1.26, 95% CI 0.73–2.17) (see Supplementary Figure 1, available in the online version of this article at <http://www3.interscience.wiley.com/journal/76509746/home>).

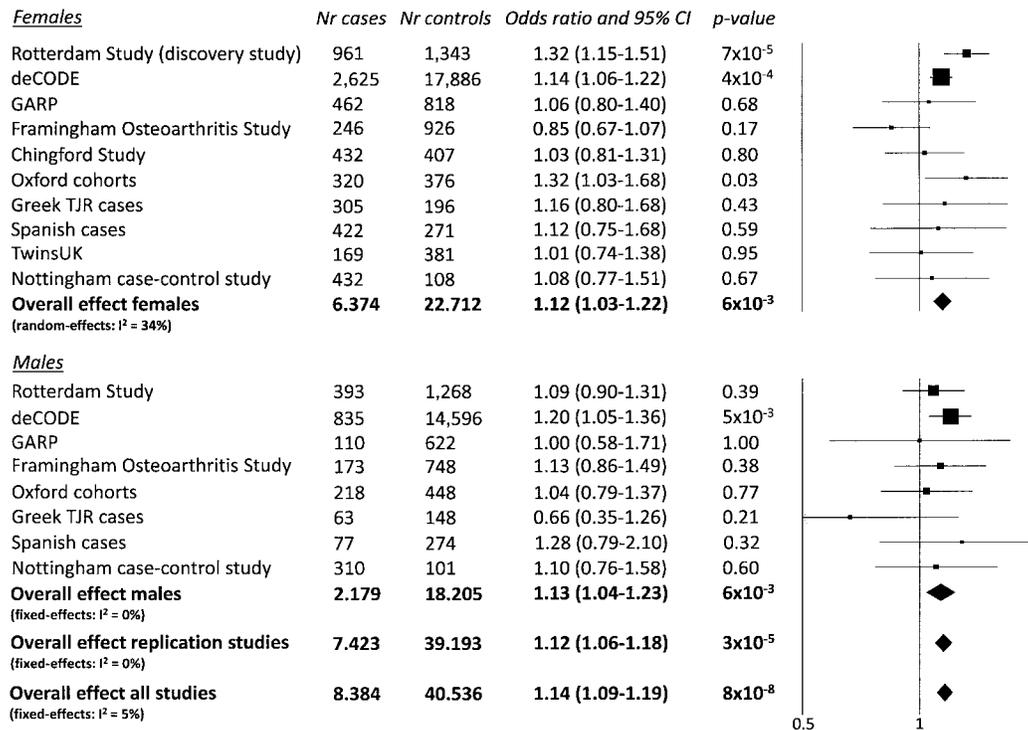


Figure 2. Forest plot of the association of the rs3815148 single-nucleotide polymorphism (*GPR22* locus) with hand and/or knee osteoarthritis (allelic model). Nr = number; 95% CI = 95% confidence interval; GARP = Genetics Osteoarthritis and Progression Study; TJR = total joint replacement.

The meta-analysis for progression of knee OA combining the results of the Rotterdam Study and the Chingford Study showed an OR of 1.30 per C allele (95% CI 1.03–1.64) ($P = 0.03$). We performed a sensitivity analysis in which the Rotterdam Study (as the discovery study) was excluded from the meta-analysis of SNP rs3815148 and found that the association remained highly significant (OR 1.12 [95% CI 1.06–1.18], $P = 3 \times 10^{-5}$).

Functional analysis for SNP rs3815148. Although SNP rs3815148 is annotated in the *COG5* gene, there is a very large linkage disequilibrium (LD) block of >500 kb in which this SNP resides with >400 SNPs that are highly correlated (see Supplementary Figure 2, <http://www3.interscience.wiley.com/journal/76509746/home>). (35). The functional variant is likely to be one of the variants in LD with rs3815148 and could be located in any of the 5 genes annotated within this region, i.e., *COG5*, high mobility group box transcription factor 1 (*HBPI*), dihydrouridine synthase 4-like (*DUS4L*), protein kinase, cAMP-dependent, regulatory, type II, beta (*PRKAR2B*), or *GPR22*. Therefore, we performed in silico and in vitro functional analysis to try to identify the gene underlying this association.

There are no mutations in the human *COG5*, *HBPI*, *DUS4L*, *PRKAR2B*, and *GPR22* genes that have been reported to result in clinical phenotypes. Expression databases show that *DUS4L*, *COG5*, and *HBPI* are ubiquitously expressed, with *PRKAR2B* expressed especially in the immune system and brain and *GPR22* mainly in the heart and brain (<http://www.genecards.org/>). Furthermore, *DUS4L*, *COG5*, and *HBPI* are expressed in cartilage, according to the integrated cartilage gene database (<http://bioinfo.hku.hk/iCartiGD/main>), while *GPR22* and *PRKAR2B* are not included in this database.

For the *GPR22* gene and the *PRKAR2B* gene, animal models are described in the literature, but skeletal and joint tissues have not been studied in depth. It is known that mice with knockout of the *PRKAR2B* gene have diminished white adipose tissue (36) and show decreased physical activity (37), while *GPR22*-knockout mice develop heart failure in response to induced stress (38).

Also, we tested whether rs3815148 or linked SNPs affected expression of any of the 5 genes in lymphoblast cell lines ($n = 400$) (39) and/or in the cortex of the brain ($n = 193$) (40), using publicly available

databases. In the expression database for the brain, only the *DUS4L* and *COG5* genes were represented, and no clear significant associations were observed between SNPs in the region surrounding SNP rs3815148 and expression levels of those 2 genes. In the lymphoblast cell lines, there was a significant association between rs3757713 ($D' = 1.0$, $r^2 = 0.95$ with rs3815148) and expression levels of *GPR22* ($P = 4 \times 10^{-12}$) (Supplementary Figure 3, <http://www3.interscience.wiley.com/journal/76509746/home>).

We also studied expression levels of mRNA for the 5 genes of interest in cells derived from several tissues of the joint. Expression analyses by quantitative PCR in cultured synovial fibroblasts and primary chondrocyte cultures showed detectable transcripts of all 5 genes, with *COG5* and *HBP1* being most abundantly expressed, while levels of *DUS4L*, *PRKAR2B*, and *GPR22* were very low in all cell types (Figures 3A–C).

Because *GPR22* showed the only notable association with altered expression in the lymphoblast cell lines, we studied the presence of *GPR22* protein in knee joints from normal and osteoarthritic mice (Figures 3D–I). Immunohistochemistry analysis did not reveal

GPR22-positive cells in normal mouse articular cartilage or synovium (Figure 3D). However, *GPR22*-positive chondrocytes were found in the upper layers of the articular cartilage of mouse knee joints that were challenged by in vivo papain treatment (Figure 3E) or methylated bovine serum albumin treatment (Figure 3F). *GPR22*-positive chondrocyte-like cells were also found in osteophytes from animals with instability-induced OA (Figure 3G).

Analysis of SNPs shown to be associated with OA (from the literature). SNPs rs225014 and rs12885300 situated in the *DIO2* gene, rs4140564 of the *PTGS2* gene, and rs7639618 of the *DVWA* gene were tested for association with hip, knee, and hand OA in the Rotterdam Study. Results for these SNPs and for the *GDF5* gene (rs6088813), which was also selected for replication in this GWAS, are shown in Supplementary Table 3 (<http://www3.interscience.wiley.com/journal/76509746/home>). The T allele of SNP rs12885300 (*DIO2* gene) showed a trend toward a protective effect in hip OA (OR 0.84 [95% CI 0.68–1.03]). The T allele of SNP rs7639618 (*DVWA* gene) was associated with an increased risk of hand OA (OR 1.18 [95% CI 1.02–1.38]). The C allele of rs6088813 (*GDF5*) was associated with a

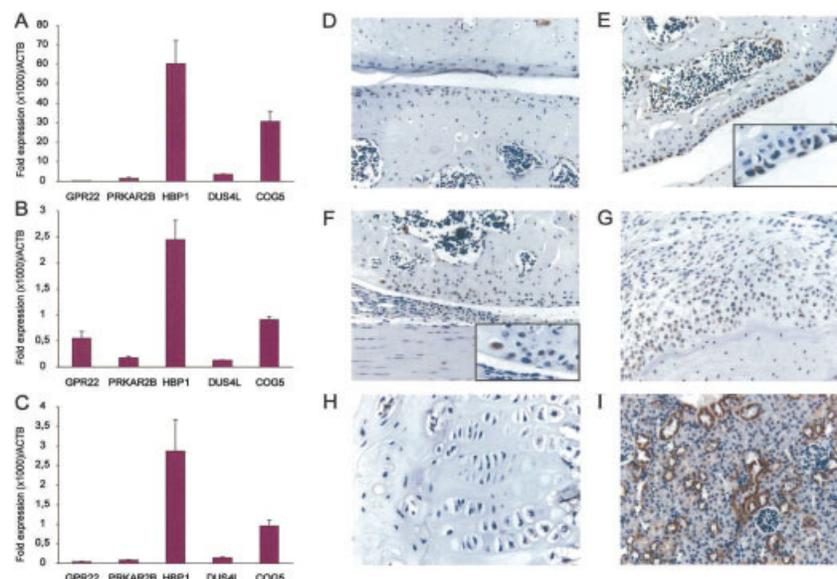


Figure 3. A–C, Quantitative polymerase chain reaction analysis of gene expression levels in expanded synovial fibroblasts (A) ($n = 3$ sets), freshly isolated chondrocytes (B) ($n = 5$ sets), and meniscal cells (C) ($n = 3$ sets). Values are the mean and SEM. ACTB = β -actin. D–I, Immunohistochemical analysis. G protein receptor–coupled protein 22 (GPR22) was absent in normal articular cartilage (D). GPR22-positive chondrocytes (brown cytoplasm) were found in articular chondrocytes of mice with papain-induced osteoarthritis (OA) (E) and mice with methylated bovine serum albumin–induced arthritis (F), and in developing osteophytes of mice with instability-induced OA (G). No GPR22 signal was found in the synovium. Growth plate chondrocytes were negative for GPR22 (H). Kidney sections were used as a positive control (I). (Original magnification $\times 200$ in D, E, F, and I; $\times 100$ in G; $\times 400$ in H; $\times 1,000$ in insets in E and F.)

decreased risk of hand OA (OR 0.82 [95% CI 0.73–0.92]) and a nonsignificant trend in the same direction for knee OA (OR 0.79 [95% CI 0.89–1.01]). No other statistically significant associations were observed.

DISCUSSION

We conducted a genome-wide association study of OA to identify DNA sequence variation associated with OA at multiple joint sites, and with progression of knee OA. The C allele of SNP rs3815148 was associated with a 14% increase in risk of knee and hand OA and was also associated with progression of knee OA (increased risk of 30% per allele). This DNA variant is located in intron 12 of the *COG5* gene, while there is high LD in the surrounding region (Supplementary Figure 2, <http://www3.interscience.wiley.com/journal/76509746/home>).

In silico and in vitro functional experiments showed that the T allele of SNP rs3757713 ($D' = 1.0$, $r^2 = 0.95$ with rs3815148) was associated with expression levels of *GPR22* in lymphoblasts ($P = 4 \times 10^{-12}$), and immunohistochemistry experiments showed presence of the *GPR22* protein in cartilage and osteophytes in mouse OA models, while this protein was absent in normal cartilage. We believe this indicates that *GPR22* might be the causal gene in the association found. *GPR22*^{-/-} mice were previously tested only for heart-specific outcomes (36); it would be interesting to study their skeletal phenotype as well. To our knowledge, ligands that may bind to *GPR22* have not yet been identified, which makes this receptor a so-called “orphan receptor.” Since the *GPR22* gene encodes a G protein-coupled receptor, this is potentially an interesting therapeutic target. Although our results suggest that *GPR22* is the gene underlying the genetic association, understanding of the precise mechanism remains unknown, and it is possible that one or more of the other genes in the same LD block are important in OA. Further research into the other genes in this locus is necessary to assess whether *GPR22* is the true underlying gene.

We focused on a spectrum of OA phenotypes, for several reasons. Because multiple joint sites can be affected by OA, we searched for loci that showed association with more than one OA phenotype. This method is likely to identify more general susceptibility genes for OA, rather than site-specific aspects. In addition, our discovery study had limited power to identify modest associations of joint-specific alleles, such as the *GPR22* SNP, with OA. We realize that our broad

phenotype approach can introduce heterogeneity and could have increased the chances of missing true OA susceptibility alleles that are site specific.

In studies in which the OA phenotype was defined as clinical OA, radiographic information was unavailable for many of the controls, and some could have had asymptomatic OA. In theory, it is possible that this might influence results, and some associations could have been missed in studies in which radiographic information on controls was not available. This, however, would only lead to an underestimation of our results, and thus only false-negatives would be a concern. Therefore, we cannot fully exclude other SNPs, except SNP rs3815148, as being associated with OA. Unfortunately, subjects in only one replication study, the Chingford Study, were genotyped for all SNPs and had data on CTX-II levels available for analysis, and therefore power was too low to obtain genome-wide significant results on CTX-II for any of the SNPs.

The consistent and robust pattern of association we observed for the *GPR22* locus, together with the expression data, provides sufficient evidence to implicate this locus in OA etiology. However, the expression patterns observed did not clarify the exact underlying mechanism, and more experiments are therefore warranted. While we observed several other potentially interesting loci, these did not reach genome-wide significance; larger GWAS sample sizes and meta-analyses will be useful to identify further OA susceptibility loci, including those that might act at a joint site-specific level.

To date, genome-wide linkage and association studies have shown that there are DNA variants in 4 genes found to be consistently associated with OA (2–5,41). These are SNPs rs225014 and rs12885300 of the *DIO2* gene, rs4140564 of the *PTGS2* gene, rs7639618 of the *DVWA* gene, and rs143383 (rs6088813 proxy SNP with $D' = 1.0$ and $r^2 = 0.93$) of the *GDF5* gene. In Supplementary Table 3 (<http://www3.interscience.wiley.com/journal/76509746/home>), the association results for those 5 SNPs are shown for the Rotterdam Study, based on the GWAS data set.

The 2 SNPs in the *DIO2* gene found to be associated with hip OA in women were discovered through a linkage study (3). Those 2 SNPs were not associated with hip OA in the Rotterdam Study, although SNP rs12885300 showed a trend toward a decreased risk in female carriers of the T allele, which is the same direction as the significant difference in risk described in a report by Meulenbelt et al (3).

The SNP in the *PTGS2* gene was not associated

with knee OA in women in the Rotterdam Study. In the reports by Valdes et al (4) and Meulenbelt et al (3), an association with a more severe OA phenotype (total joint replacement or a K/L grade of ≥ 3) was described, which could be an explanation for the lack of replication in the Rotterdam Study, in which less severe radiographic knee OA (K/L grade ≥ 2) was included.

In addition, the *DVWA* SNP was not associated with knee OA in our study population. An increased risk of hand OA was observed with the T allele of SNP rs7639618 (*DVWA*); however, this effect is in the opposite direction from the association observed in the GWAS of knee OA by Miyamoto et al (5). This SNP was also not associated with knee OA in other Caucasian studies (6,7), which could indicate that it has different effects in different ethnic populations.

The *GDF5* gene, identified with our search strategy, showed an association with knee and/or hand OA, with an OR of 0.91 and a 95% CI of 0.84–0.98 in the meta-analysis. This was previously also shown by Miyamoto et al (41), Chapman et al (2), Vaes et al (42), and in a large meta-analysis (n = 5,085 cases and 8,135 controls) performed within the Translational Research in Europe Applied Technologies for OsteoArthritis consortium (43) for knee OA. Although the *GDF5* locus does not reach genome-wide significance, it is considered a true and consistent association in the OA field.

In conclusion, we have identified a novel locus, SNP rs3815148 close to the *GPR22* gene, involved in susceptibility to prevalence and progression of OA. Based on this finding, the G protein-coupled receptor may warrant study as a potential therapeutic target.

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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. Kerkhof 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.

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Analysis and interpretation of data. Kerkhof, Lories, Meulenbelt, Jonsdottir, Valdes, Ingvarsson, Jonsson, Stolk, Thorleifsson, Zhai, Zhang, Zhu, van der Breggen, M. Doherty, Halldorsson, Hart, Hauks-son, Ioannidis, Lane, Loughlin, Luyten, Parimi, Pols, Rivadeneira, Slagboom, Styrkársdóttir, Tsezou, van de Putte, Spector, Stefansson, Uitterlinden, van Meurs.

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