

Reproducible Genetic Associations Between Candidate Genes and Clinical Knee Osteoarthritis in Men and Women

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Objective. Osteoarthritis (OA) is recognized to have a genetic component, and in this study, we aimed to replicate in a case–control study of men and women with clinical knee OA genetic associations in 12 candidate genes previously reported to be associated with OA.

Methods. Twenty-five single-nucleotide polymorphisms were genotyped in 298 men and 305 women ages 50–86 who were diagnosed as having knee OA, as assessed both clinically and radiographically, and in 297 men and 299 women matched for age and ethnicity (controls). Standardized anteroposterior radiographs of the knee in extension were performed on each of the cases, and all cases met the American College of Rheumatology criteria for OA of the knee. Genotype and haplotype frequencies in cases and controls were compared separately in men and women. The 12 genes tested were *AACT*, *ADAM12*, *BMP2*, *CD36*, *CILP*, *COX2*, *ESR1*, *NCOR2*, *OPG*, *TNA*, *TNFAIP6*, and *VDR*.

Results. Eight of the candidate genes were associated in women and 5 in men, and only 3 genes (*TNFAIP6*, *NCOR2*, and *CD36*) were not significantly associated in either sex. The strongest associations in terms of odds ratios (ORs) were a haplotype in *ADAM12* (OR 7.1 [95% confidence interval (95% CI) 3.3–33.8]) and a haplotype in *ESR1* (OR 3.6 [95% CI 1.18–10.98]) in women. The same *ADAM12* haplotype (OR 2.54 [95% CI 1.2–5.4]) and a haplotype in the *CILP* gene

(OR 0.38 [95% CI 0.23–0.62]) were the strongest associations in men.

Conclusion. We found that genes previously identified by their association with subclinical features of knee OA or progression were also associated with clinical knee OA. These genetic associations may identify individuals at a particularly high risk of developing knee OA.

Osteoarthritis (OA) is a prevalent joint disease that primarily affects the knees, hips, hands, and spine and is characterized by late-onset degeneration of articular cartilage, which is marked by the breakdown of matrix proteins (1). This leads to the development of fibrillations, fissures, and ulcerations at the articular cartilage surface. Cartilage degradation is mediated by metalloproteinases, which are classified into 2 groups: matrix metalloproteinases and a family of metalloproteinases with thrombospondin motifs (also called aggrecanases) (2). Several factors play a role in OA risk, including age, sex, genetics, ethnicity, behavioral influences, obesity, and occupation (1). In addition, epidemiologic studies in women suggest that estrogen loss may be accompanied by an increase in the prevalence and incidence of knee and hip OA (3), which may help explain the sex differences in disease prevalence.

A genetic contribution to OA has been suggested in several epidemiologic studies (4). Twin studies, segregation analyses, linkage analyses, and candidate gene association studies have generated important information about inheritance patterns and the genome location of potentially causative mutations, although the results across studies have thus far been inconsistent. Linkage and family studies have suggested that there are likely to be both sex-specific and anatomical site-specific genes that influence OA (4).

Recently, we reported genetic associations between several genes, which had been selected by their

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differential expression in healthy and OA cartilage and synovium, and the prevalence and progression of radiographic knee OA (5). The genetics of OA is complex and is not completely understood, and to assess the validity of reported genetic associations, the best strategy is to reproduce those associations in independent cohorts, preferably focusing on the most clinically relevant phenotypes. Thus, in this study, we genotyped variants of the 12 genes previously reported by our research group to be associated with radiographic features of knee OA, comparing genotype and haplotype frequencies among patients with clinical OA and age-matched controls, both in men and women. We hypothesized that variations in genes selected both on the basis of being differentially expressed in healthy and OA cartilage and synovium (for review, see ref. 5) and being associated with genetic susceptibility to, and/or progression of, radiographic OA of the knee should also influence the risk of more severe clinical knee OA in a case-control setting.

PATIENTS AND METHODS

Subjects. A total of 603 Caucasian patients with knee OA (298 men and 305 women; age range 50–86 years) were recruited from families with a history of OA and from clinic populations in Nottingham. In addition, 596 Caucasian age-matched controls without signs or symptoms of OA (age range 50–80 years) were also recruited from 2 centers: Nottingham (111 women and 50 men) and Oxford (185 women and 250 men). The mean \pm SD age of the female cases was 73.5 ± 7.16 years and that of female controls was 72.1 ± 8.5 years. The mean age of the male cases was 72.1 ± 6.9 years and that of male controls was 71.0 ± 7.8 years. OA was assessed clinically and radiographically, and each study participant underwent radiography of both knees. Standardized anteroposterior views were obtained with the subject standing and bearing weight.

Allele frequencies for each single-nucleotide polymorphism (SNP) were compared between the Oxford and Nottingham control samples and between male and female controls. No significant differences between male and female controls were found. Although overall differences between the control groups from the 2 centers were not significant, we found that for 2 genes, *COX2* and *TNA*, the allele frequencies were significantly different ($P < 0.05$ unadjusted for multiple comparisons). As a precaution, only controls from Nottingham ($n = 161$ male and female subjects) were used for assessment of these 2 genes.

Genotyping. A total of 25 SNPs were genotyped from 12 genes: *AACT*, *ADAM12*, *BMP2*, *CD36*, *CILP*, *COX2*, *ESR1*, *NCOR2*, *OPG*, *TNA*, *TNFAIP6*, and *VDR*. Multiplex polymerase chain reaction (PCR) and SNP analyses were performed using the GenomeLab SNPstream Genotyping System (Beckman Coulter, Fullerton, CA) and its accompanying automated SNPstream software suite. Primers for the multiplex PCR and single-base extension reactions were optimally designed using

Web-based software provided by Beckman Coulter (available at www.autoprimer.com).

Following a multiplex PCR, the PCR-amplified fragments were treated with a mixture of exonuclease I and shrimp alkaline phosphatase to degrade unincorporated PCR primers and dNTPs. The tagged extension primers were extended using single-labeled TAMRA-fluorescein or BODIPY-fluorescein nucleotide-terminator reactions and spatially resolved by hybridization to the complementary oligonucleotides arrayed on the 384-well microplates (SNPware Tag Array). The Tag Array plates were imaged using a 2-laser, 2-color CCD-based imager (GenomeLab SNPstream Array Imager). The individual SNPs within each multiplex were identified according to the position of the arrayed oligonucleotides within each well. Individual sample genotype data were generated on the basis of the relative fluorescence intensities for each spot and processed for graphic review using the automated SNPstream software suite. The genotyping success rate was 97.4% (range 93.8–100%). Internal genotyping controls were included on each plate, with a concordance rate of 100%. Genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium among controls ($P > 0.10$).

Statistical methods. *Individual polymorphism genetic associations.* The association between individual SNP genotypes and OA was tested by comparing SNP genotype frequencies among cases and controls using Wald's chi-square test derived from a univariate logistic regression model.

Except where explicitly noted, only an additive genetic model was tested, where genotypes were coded 0, 1, or 2 depending on the number of minor alleles carried by a subject. Thus, the reported odds ratios (ORs) refer to the increased or decreased odds of disease in a person carrying 1 copy versus no copies or 2 copies versus 1 copy of the minor allele. ORs for this model with the corresponding 95% confidence intervals (95% CIs) were also computed (Table 1).

For each sex and with the use of SNPs that have a minor allele frequency ranging from 5.5% to 50%, the current study is powered (80% power at $P < 0.05$) to detect ORs ranging from 1.39 to 1.70 (OR range 0.72–0.58 if allele is protective) under an additive (codominant) genetic model, which is equivalent to comparing allele frequencies among pools of chromosomes. This study is powered to replicate the size of effects on radiographic knee OA that were previously observed if the genetic associations are real. The power calculations using a binomial approximation were carried out using S-Plus 6.0 software (Insightful, Seattle, WA). Since this was a replication study with a priori hypotheses, no adjustment for multiple tests was performed, which would otherwise have been performed if this were a de novo study rather than a replication study.

Haplotype frequency estimation and haplotype genetic associations. Two methods were used to estimate haplotype frequency among female and male OA cases and controls. Maximum likelihood haplotype frequencies were computed using an expectation-maximization algorithm, as implemented by the Arlequin software program (available at <http://lgb.unige.ch/arlequin/>). In addition, the program Phase version 2.02, which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data (available at <http://www.stat.washington.edu/stephens/software.html>), was also used to confirm haplotype frequency

Table 1. Association of individual SNPs with knee OA in women and men*

Gene	SNP alias	Reference SNP	SNP description	DNA change	Minor allele frequency in controls, %	Women (n = 305 with knee OA; n = 296 controls)		Men (n = 298 with knee OA; n = 300 controls)	
						OR (95% CI)	P	OR (95% CI)	P
<i>AACT</i>	aact	rs4934	Ala ⁹ Thr	GA	49.1	0.75 (0.59–0.94)	<0.014†	1.05 (0.83–1.32)	NS
<i>ADAM12</i>	adam_48	rs3740199	Gly ⁴⁸ Arg	CG	47.6	1.11 (0.88–1.39)	NS	1.12 (0.89–1.41)	NS
	adam_504	rs1278279	Asn ⁵⁰⁴ Asn	GA	21.6	1.13 (0.86–1.49)	NS	0.98 (0.75–1.28)	NS
	adam_825	rs1044122	Ala ⁸²⁵ Ala	TC	26.9	0.85 (0.65–1.11)	NS	1.02 (0.79–1.32)	NS
<i>BMP2</i>	adam_int	rs1871054	Intron	GA	43.9	1.12 (0.89–1.41)	NS	1.24 (0.97–1.57)	<0.080
	bmp2_87	rs1049007	Ser ¹⁹⁰ Arg	TC	35.7	1.27 (1.00–1.62)	<0.053	1.13 (0.90–1.44)	NS
	bmp2_190	rs235768	Ser ⁸⁷ Ser	TA	37.5	1.20 (0.96–1.52)	NS	1.11 (0.88–1.40)	NS
<i>CD36</i>	cd36_5p	rs1049654	5'-UTR	CA	45.1	0.85 (0.68–1.07)	NS	1.07 (0.85–1.34)	NS
	cd36_int	rs3211822	Intron	GA	40.6	0.86 (0.68–1.08)	NS	1.02 (0.81–1.29)	NS
<i>CILP</i>	cilp_3p	rs1561888	3'-UTR	GA	29.4	1.27 (0.98–1.64)	<0.070	1.61 (1.24–2.08)	<0.0003†
	cilp_395	rs2073711	Thr ³⁹⁵ Ile	CT	42.1	0.86 (0.68–1.08)	NS	1.08 (0.85–1.36)	NS
<i>COX2</i>	cox2_102	rs5277	Val ¹⁰² Val	CG	18.4‡	1.23 (0.86–1.78)	NS	1.50 (1.02–2.20)	<0.039†
	cox2_3p	rs689470	3'-UTR	GA	3.7†	0.52 (0.23–1.18)	NS	0.65 (0.30–1.43)	NS
<i>ESR1</i>	esr_325	rs1801132	Pro ³²⁵ Pro	CG	21.6	0.86 (0.65–1.15)	NS	0.86 (0.64–1.15)	NS
	esr_594	rs2228480	Thr ⁵⁹⁴ Thr	GA	16.8	1.34 (1.00–1.79)	<0.052	0.86 (0.64–1.17)	NS
	esr_int1	rs2234693	Intron	TC	46.1	0.93 (0.73–1.17)	NS	1.00 (0.80–1.26)	NS
	esr_int2	rs827421	Intron	GA	49.6	1.02 (0.80–1.29)	NS	1.05 (0.83–1.32)	NS
<i>NCOR2</i>	ncor_1699	rs2229840	Thr ¹⁶⁹⁹ Ala	GA	18.0	0.96 (0.73–1.27)	NS	0.89 (0.67–1.20)	NS
<i>OPG</i>	opg_5p	rs1564858	5'-UTR	CT	11.7	1.08 (0.74–1.57)	NS	0.76 (0.53–1.09)	NS
	opg_3	rs2073618	Asn ³ Lys	CG	44.9	0.91 (0.72–1.15)	NS	0.92 (0.74–1.15)	NS
<i>TNA</i>	tna_106	rs13963	Ser ¹⁰⁶ Gly	GA	31.1‡	1.49 (1.11–2.01)	<0.009†	1.45 (1.07–1.96)	<0.016†
	tna_int	rs939309	Intron	TC	41.9‡	1.35 (1.02–1.78)	<0.036†	1.34 (1.01–1.77)	<0.044†
<i>TNFAIP6</i>	tnfaip	rs1046668	Arg ¹⁴⁴ Gln	GA	12.1	0.81 (0.56–1.16)	NS	0.90 (0.64–1.26)	NS
<i>VDR</i>	vdr_1	rs10735810	FokI start codon	GA	36.3	0.99 (0.79–1.24)	NS	1.15 (0.90–1.47)	NS
	vdr_365	rs731236	Ile ³⁶⁵ Ile	TC	37.4	1.14 (0.90–1.46)	NS	1.19 (0.95–1.50)	NS

* SNP = single-nucleotide polymorphism; OA = osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant.

† Statistically significant *P* value.

‡ Only control subjects from Nottingham were used for these comparisons.

estimates in each of the 4 study groups (male and female OA cases and controls). Haplotype frequencies estimated by both methods were very similar and were always within the standard error of the estimate. Contingency tables were generated by multiplying the number of chromosomes in OA cases and controls for each sex by the haplotype frequency estimate. Haplotype frequencies between knee OA cases and controls were then compared using Pearson's chi-square statistic.

RESULTS

Individual polymorphisms in genes *AACT* and *TNA* were found to be significantly associated ($P < 0.05$) in women with knee OA, and in genes *CILP*, *COX2*, and *TNA* in men with knee OA (Table 1). In every case, the same allele that we had previously reported to be associated with a higher progression or increased risk of radiographic knee OA was the one found to be associated with a higher risk of knee OA.

We examined associations with genes for which we genotyped 2 or more SNPs, and found that 1 or more haplotypes were associated with disease risk, even if none of the individual SNPs was associated. Table 2

shows the haplotypes for the 2 genes for which 4 polymorphisms were typed and for 4 genes for which a haplotype association was found but no individual SNP has yet been found to be associated. Although haplotype frequencies were estimated for all genes with 2 or more SNPs, the ORs and *P* values of the genes that were not included in Table 2 were either the same or were less significant for the haplotypes than for the individual SNPs and, thus, are not shown.

Of particular note are 3 haplotypes in the *ADAM12* gene (Table 2). Haplotype CAAT was associated with an increased risk of knee OA in men (OR 2.54) as well as in women (OR 7.10), whereas haplotype CGAT was associated with a reduced risk of OA in both sexes (OR 0.52 in men; OR 0.53 in women). Haplotype CGGT was also associated with a reduced risk of knee OA, although the difference was statistically significant only in men (OR 0.51). Also remarkable is the association of the *ESR1* haplotypes. Only the synonymous polymorphism in codon 325 associated with allele G resulted in reduced OA risk. Yet, 2 haplotypes, CGCA and TAGA, carrying different alleles at *esr_325* were

Table 2. Estimated haplotype frequencies of *ADAM12*, *BMP2*, *CILP*, *ESR1*, *OPG*, and *VDR* among knee OA cases and controls, and their association with knee OA*

Gene, SNP	Haplotype	Women				Men			
		Frequency		OR (95% CI)	P	Frequency		OR (95% CI)	P
		Controls	OA cases			Controls	OA cases		
<i>ADAM12</i>									
adam_48; adam_int; adam_504; adam_825	CAAT	0.9	6.1	7.10 (3.31–33.8)	<1 × 10 ⁻⁶ †	1.7	4.2	2.54 (1.20–5.40)	<0.014†
	CAGT	13.6	11.2	0.79 (0.56–1.13)	NS	12.2	16.0	1.37 (0.98–1.92)	<0.091
	CGAT	8.8	4.8	0.53 (0.31–0.81)	<0.006†	6.4	3.3	0.52 (0.31–0.99)	<0.047†
	CGGT	12.2	10.2	0.82 (0.56–1.19)	NS	12.4	6.7	0.51 (0.34–0.77)	<0.002†
	GAGT	16.6	19.3	1.20 (0.88–1.63)	NS	17.1	16.3	0.94 (0.69–1.29)	NS
	GGGT	11.4	11.7	1.09 (0.75–1.58)	NS	11.2	14.2	1.23 (0.87–1.74)	NS
	Other	35.6	36.0	1.02 (0.79–1.30)	NS	38.7	39.3	1.03 (0.81–1.30)	NS
	All				<2 × 10 ⁻⁶ †				<0.003†
<i>BMP2</i>									
bmp2_87; bmp2_190	CA	35.5	41.2	1.27 (0.99–1.60)	NS	37.1	40.4	1.15 (0.91–1.45)	NS
	CT	0.7	0.8	1.27 (0.34–4.78)	NS	0.7	0.2	0.24 (0.03–2.11)	NS
	TA	2.4	0.8	0.35 (0.12–0.96)	<0.035†	2.1	1.2	0.56 (0.22–1.42)	NS
	TT	61.4	57.2	0.84 (0.67–1.06)	NS	60.1	58.2	0.93 (0.73–1.17)	NS
	All				<0.048†				NS
<i>CILP</i>									
cilp_395; cilp_3p	CA	28.4	33.3	1.26 (0.98–1.64)	NS	27.6	37.2	1.55 (1.2–1.99)	<0.005†
	TA	0.7	0.7	1.05 (0.25–4.36)	NS	0.6	1.3	2.03 (0.55–7.44)	NS
	CG	12.5	6.1	0.45 (0.30–0.69)	<4 × 10 ⁻⁴ †	9.9	4.0	0.38 (0.23–0.62)	<1 × 10 ⁻⁴ †
	TG	58.4	59.8	1.06 (0.83–1.35)	NS	61.8	57.6	0.84 (0.66–1.07)	NS
	All				<0.002†				<8 × 10 ⁻⁷ †
<i>ESR1</i>									
esr_int1; esr_int2; esr_325; esr_594	CGCA	7.6	12.3	1.70 (1.12–2.58)	<0.017†	6.4	8.9	1.44 (0.93–2.25)	NS
	CGCG	30.9	27.6	0.85 (0.65–1.11)	NS	31.8	29.2	0.89 (0.69–1.14)	NS
	TACG	25.5	29.1	1.20 (0.92–1.58)	NS	26.9	31.9	1.27 (0.98–1.60)	NS
	TAGA	0.8	2.8	3.61 (1.18–10.98)	<0.017†	1.2	0.7	0.59 (0.14–1.53)	NS
	TAGG	12.1	9.0	0.72 (0.48–1.06)	NS	11.8	10.1	0.84 (0.58–1.22)	NS
	TGCA	1.2	0.0	0.04 (0–1.84)	NS	0.9	0.5	0.5 (0.15–3.67)	NS
	Other	21.8	19.1	0.85 (0.63–1.14)	NS	20.9	18.7	0.87 (0.65–1.16)	NS
	All				<0.002†				NS
<i>OPG</i>									
opg_5p; opg_3	TC	5.6	2.8	0.49 (0.27–0.92)	<0.026†	5.2	3.2	0.61 (0.34–1.09)	<0.097
	TG	6.0	9.4	1.61 (1.01–2.57)	<0.050†	7.5	7.4	0.99 (0.64–1.53)	NS
	CC	39.6	44.5	1.23 (0.96–1.56)	NS	39.5	43.7	1.19 (0.94–1.5)	NS
	CG	48.8	43.3	0.8 (0.63–1.02)	NS	47.7	45.6	0.92 (0.73–1.16)	NS
	All				<0.007†				NS
<i>VDR</i>									
vdr_1; vdr_365	AC	13.1	16.7	1.33 (0.96–1.85)	NS	11.7	20.9	1.99 (1.46–2.78)	<9 × 10 ⁻⁵ †
	AT	24.0	21.3	0.85 (0.65–1.12)	NS	23.1	16.8	0.67 (0.50–0.90)	0.016
	GC	24.6	25.4	1.04 (0.80–1.36)	NS	24.8	19.8	0.75 (0.57–0.99)	0.073
	GT	38.3	36.6	0.93 (0.73–1.18)	NS	40.6	42.6	1.09 (0.86–1.37)	NS
	All				NS				<2 × 10 ⁻⁵ †

* OA = osteoarthritis; SNP = single-nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant.

† Statistically significant *P* value.

found to increase the risk of knee OA in women. However, no individual polymorphism or haplotype at this gene was associated with OA in men.

We found that a haplotype formed by the two *BMP2* SNPs (TA) was associated with a reduced risk of knee OA in women (Table 2). For this gene, we also investigated a genetic model in which the risk in individuals carrying the genotype with the rare homozygote was compared with that in individuals carrying 1 or 2

copies of the common allele. Both genotype CC at *bmp2_87* (OR 1.75 [95% CI 1.09–2.82], *P* < 0.021) and genotype AA at *bmp2_190* (OR 1.61 [95% CI 1.03–2.50], *P* < 0.035) were associated with increased susceptibility to knee OA according to this model. These results are consistent with our previous findings for *BMP2* (5).

One of the *OPG* haplotypes formed by the 5'-UTR and the coding change at codon 3, polymorphism

TC, was associated with decreased risk of knee OA in women (OR 0.49, $P < 0.026$) (Table 2). A similar trend, although not statistically significant, was seen in men (OR 0.61, $P < 0.097$). Another haplotype, TG, which carries the minor allele at the 5'-UTR SNP, was associated with an increased risk of knee OA in women (OR 1.61). In our previous study (5), the minor allele at this SNP was found to increase the progression in osteophyte grade. The contrasting effect on OA risk that is dependent upon the haplotype context can explain why the individual SNP was not associated with knee OA risk in this study.

Neither of the *CILP* SNPs was significantly associated with knee OA in women. The *CILP*-coding SNP that we had previously reported to be associated with the progression of knee OA was found not to be associated with knee OA in men or women; however, the 3'-UTR SNP was found to be associated with a risk of knee OA in men. However, haplotype CG was found to be strongly protective both in men and in women ($P < 0.0005$) (Table 2). In our previous study (5), women carrying 2 CC alleles at codon 395 had lower radiographic progression, which is consistent with the current findings.

We found that the haplotype formed by the 2 minor alleles at codon positions 1 and 365 of the *VDR* gene was associated with a significantly higher risk of knee OA in men (OR 1.99). A similar trend (OR 1.33) was seen for that same haplotype in women, although we were not able to detect a significant association between the *VDR* genetic variation and knee OA in women.

Only 3 genes, *TNFAIP6* (encodes tumor necrosis factor α -induced protein 6), *NCOR2* (encodes nuclear receptor corepressor 2), and *CD36* (encodes the CD36 antigen [type II collagen receptor, thrombospondin receptor]), were not found to be significantly associated with knee OA in either sex.

DISCUSSION

The results of this study show that the majority of candidate genes from cartilage expression libraries and from our initial association study of subclinical OA are also associated with the risk of clinical knee OA. The study is important in determining the likelihood of genuine gene associations because, for most complex traits, a large proportion of initial associations turn out to be false positives (6).

Three of the genetic associations that we identified were seen both in men and in women, most notably, the association of *ADAM12*, which encodes a metalloproteinase involved in osteoclast formation and cell-cell

fusion (for review, see ref. 5). We found 1 haplotype that increased the risk of knee OA (CAAT) and 2 haplotypes that reduced the risk of knee OA (CGAT and CGGT), all of which carry the same allele (C) in codon 48, which results in an arginine residue instead of a glycine residue. This might explain why this polymorphism, which we previously found to be strongly associated with the prevalence and progression of radiographic OA (5), was not by itself associated with knee OA in either sex. It also suggests that, by itself, this coding SNP does not affect knee OA susceptibility, but acts only in the presence of other alleles. However, the results presented here, together with the previous genetic association results and the known biology of *ADAM12*, strongly support a role for this molecule in the pathogenesis of knee OA.

Tetranectin (*TNA* or *CLEC3B*), a C-type lectin, is a plasminogen binding protein that is present in the mammalian musculoskeletal system and is involved in osteogenesis and bone mineralization. Modulation of plasminogen receptors mediates the degradation of the extracellular matrix and has been implicated in the destruction of cartilage and bone in pathologic processes such as arthritis (7). We found that the same SNP allele that we had previously found to be associated with a higher progression of radiographic knee OA in women (allele A, resulting in a glycine at codon 106) was also associated with an increased risk of knee OA both in men and in women. A weaker association (in terms of the OR) was seen with an intronic SNP in the same gene. No *TNA* haplotype had a higher OR.

CILP, which encodes the cartilage intermediate protein, was also associated in both sexes. *CILP* has been reported to inhibit the transforming growth factor β 1 (TGF β 1)-mediated induction of cartilage matrix genes through direct interaction with TGF β 1 and inhibition of TGF β 1 signaling (8). The T allele at *cilp*_395 has been shown to increase the binding and inhibition of TGF β 1, indicating that the extracellular matrix protein *CILP* regulates TGF β 1 signaling. In a Japanese case-control study of lumbar disk disease, the T allele at this polymorphism was also implicated in an increased risk of lumbar disk disease (8). In the present study, 1 of the haplotypes carrying the C allele at position 395 was associated with a greatly reduced risk of knee OA. This finding is consistent with the results of our previous study, in which we found that women carrying 2 CC alleles had lower radiographic progression (5), as well as the Japanese lumbar disk disease study.

Most genes, however, were only significantly associated in one of the sexes. Osteoprotegerin plays an

important role in regulating osteoclastogenesis (9). Our finding of a similar trend in the association of *OPG* SNPs with knee OA risk in both sexes, which was significant only in women, suggests that the effect of genetic variation in the *OPG* gene might be similar in both sexes, although perhaps stronger in women.

BMP2 encodes the bone morphogenetic protein 2, a growth factor involved in chondrogenesis and osteogenesis (10), and variations in this gene have also been implicated in bone mineral density (5). The same alleles that we had previously reported to be associated with an increase in joint space narrowing were also found to increase the risk of OA, but only in women.

AACT, the gene that encodes α_1 -antichymotrypsin, is the natural inhibitor of cathepsin G, a proteinase involved in the degradation of cartilage proteoglycan (11). The same allele that we had previously found to be associated with a lower rate of change in joint space narrowing over time (allele A, resulting in threonine at codon position 9) was associated in this study with a decreased risk of knee OA, but only in women. No trend was seen in men for this polymorphism.

The production of prostaglandin E₂ (PGE₂) is increased in OA cartilage, and cyclooxygenase 2 is the rate-limiting enzyme of PGE₂ synthesis (12). Thus, genetic variations in the *COX2* gene may contribute to the pathogenesis of knee OA. However, we did not identify an association in women, and the association in men was fairly modest. Thus, more evidence on the role of this gene in knee OA is needed.

Polymorphisms in the *VDR* gene have previously been implicated in the risk of OA as well as in the occurrence of osteophytes (5). The results of the present study suggest that *VDR* may indeed affect knee OA susceptibility, but the effect size is probably relatively modest and difficult to replicate.

Polymorphisms considered as haplotype combinations for the *ESR1* gene, which encodes estrogen receptor α (ER α), were associated with radiographic OA of the knee in the Rotterdam cohort of elderly patients (13), in particular, with the presence of osteophytes. In addition, we have previously reported a weak association of the *ESR1* gene with the presence of osteophytes (5). The estrogen receptors ER α and ER β have been identified in normal and OA cartilage, indicating that cartilage can respond to estrogens. Indeed, estrogen added in combination with interleukin-1 β to cartilage cells in vitro modulates proteoglycan degradation as well as matrix metalloproteinase messenger RNA expression (3). A role of estrogens in OA is consistent with the greater increases in women than in men of the

incidence and prevalence of hip, knee, and finger OA after the age of 50 years. These facts are all consistent with our findings that genetic variations in *ESR1* affect the risk of knee OA in women but not in men.

Although sex differences in the genetics of OA have not been fully elucidated, in addition to the effect of estrogen, a higher heritability of knee OA has been reported for women than for men (14), as well as sex differences in cartilage volume (15). All of these observations may help explain the different genetic associations between women and men that were found in our study.

It is of interest to note that in the present study, we found that testing for individual SNPs did not enable us to detect genetic associations for several genes. It was necessary to investigate associations with haplotypes to confirm the role of some of these genes in susceptibility to knee OA.

There are several limitations to the present study. First, we did not fully cover all genetic variation in the genes analyzed, and only common variants were studied. Therefore, if rare variants (or rare haplotypes) of these genes are involved in the pathogenesis of OA, we would have missed them. Second, we had only limited data on the controls, which did not allow us to test possible confounders such as obesity. However, since previous studies have shown no genetic correlations, but only environmental correlations, of knee OA with obesity (16), it is unlikely that we have merely confirmed obesity genes. Finally, these results apply only to Caucasian patients, and these associations might not be found in patients of Asian or African ancestry.

In conclusion, we found that of the 12 genes previously identified by their association with subclinical features of knee OA, 9 were associated with susceptibility to knee OA in one or both sexes. These replicated genetic associations are likely to represent real disease associations. These genes can now be pursued with confidence in research seeking to uncover new mechanisms of OA, and individually or in combination, they might be helpful in identifying women who are at high risk of developing knee OA.

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