

Association Between a Variation in *LRCHI* and Knee Osteoarthritis

A Genome-Wide Single-Nucleotide Polymorphism Association Study Using DNA Pooling

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Objective. To perform a large-scale association analysis of single-nucleotide polymorphisms (SNPs) in patients with radiographically defined osteoarthritis (OA) of the knee.

Methods. We examined >25,000 SNPs located within ~14,000 genes for associations with radiographically defined knee OA, using polymerase chain reaction and MassExtend amplification techniques. Allele frequencies were estimated initially in DNA pools from 335 female patients with knee OA and 335 asymptomatic and radiographically negative female control subjects. All were of northern European ancestry. Significant allele frequency differences were validated by genotyping of individual DNA samples. Confirmed significant findings were verified in 2 additional case–control samples from the UK (443 cases and 303 controls) and Newfoundland (346 cases and 264 controls). Chondrosarcoma cell lines were used to test for potential differences in gene expression.

Results. The marker most strongly associated with the risk of knee OA was rs912428, a C/T polymorphism in intron 1 of *LRCHI*, a gene on chromosome 13q14 that encodes a novel protein of as-yet-unknown function. The frequency of the T allele compared with controls was consistently increased by 40% across all 3 case–control groups. Additional subanalyses in case–control samples with hip OA and hand OA suggested similar trends, but did not reach statistical significance. Association fine-mapping using 10 additional SNPs in *LRCHI* confirmed intron 1 as the region of highest association but failed to reveal variations with significance stronger than the marker SNP, as did the haplotype analysis. *LRCHI* was not up-regulated or overexpressed in chondrosarcoma cell lines exposed to inflammatory stimuli, suggesting a possible structural role.

Conclusion. A genetic variant in *LRCHI* was consistently associated with knee OA in 3 samples from 2 populations. Our results also suggest that the same association with OA may exist at other sites. Additional genetic and experimental work is needed to elucidate the precise mechanism by which the *LRCHI* gene influences OA risk.

Osteoarthritis (OA) is a degenerative joint disease that primarily affects the knees, hips, hands, and spine (1). The biology and epidemiology of OA seem to differ between sites, with a different balance of risk factors and age at onset for different sites. For genetic purposes, site-specific classification is likely to be important, with differential linkage and association results for the hip and the spine (2).

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Submitted for publication May 12, 2005; accepted in revised form November 3, 2005.

The prevalence of OA increases with age (1). In addition to age, body weight, occupational history, trauma, and sex are major risk factors for OA (1). Genetic factors also contribute to the risk of OA, with heritability estimates at different sites ranging from 39% to 65%, as determined in studies of twins (3–5). The genetic component may be greater in women than in men (4).

A number of linkage studies of OA at different sites have been performed in single large pedigrees and in multiple families (6–14). The genes responsible for the observed linkage peaks have not yet been clearly determined or validated, and results from different linkage studies show little overlap. Candidate gene studies, meanwhile, have focused mainly on such genes as those encoding collagens, matrix metalloproteinases (MMPs), noncollagenous matrix proteins, cytokines, and the vitamin D receptor, with few showing strong or consistent evidence of association with OA in different samples (15). A recent Japanese candidate gene study, however, has implicated the asporin gene in both hip and knee OA, although it is still unclear whether the findings are generalizable to other groups (16). Therefore, since few validated genes have been found and since the ones that have been found account for only a small proportion of the genetic effect, it is highly likely that as-yet-unknown genes also contribute to OA susceptibility (2).

Studies that rely on direct association approaches based on linkage disequilibrium within populations, although prone to bias, have potentially greater statistical power and may be more feasible than traditional linkage studies for the identification of common variations that influence widespread complex traits such as OA (17). Recently, there has been increasing interest in the use of whole-genome association methods to identify genes that are involved in complex trait variation. To date, however, few such large-scale studies have been reported. In an effort to identify genes and variants that influence risk of OA of the knee, we conducted a large-scale study using more than 25,000 single-nucleotide polymorphisms (SNPs) located within ~14,000 genes in pools of DNA from unrelated females with and without knee OA. We report herein the most consistent association, which was observed with a SNP in *LRCHI* (*CHDC1*), a gene encoding a novel, not well-characterized protein.

SUBJECTS AND METHODS

Three case–control collections were used in the present study, 1 for the initial genome-wide study and the other

2 for validation of the positive findings. All were comprised of individuals of northern European ancestry. All subjects provided written consent for the study, and the study was approved by the ethics committee at each of the clinical centers.

UK discovery sample used for initial large-scale association analysis. The case group in the initial sample was derived from a sibling study of knee OA in Nottingham, UK (18). Index cases were those who had undergone or were awaiting knee replacement surgery. Siblings of the index cases were recruited for study and underwent knee radiography; their status as affected or unaffected was then assigned. In all, 1,157 individuals were available for analysis.

In order to create same-sex pools of appropriate sizes, 335 unrelated women with knee OA were selected as cases. There were insufficient unaffected and unrelated individuals in the Nottingham study to allow the formation of a control group from that collection. Instead, we created from participants in the TwinsUK study (19) a control group of 335 unrelated female subjects who had normal findings on knee radiographs, no other indications of OA, and no family history of OA. Basic characteristics of the 2 groups are reported in Table 1.

UK replication sample. The first replication sample consisted of 246 female patients with knee OA (Kellgren/Lawrence [K/L] grade ≥ 2 in either knee). These cases were drawn from 2 large population-based studies in the UK: the TwinsUK study ($n = 148$) (3) and the longitudinal Chingford Study ($n = 98$) (20). Unrelated female subjects with a K/L score of 2 or more in one or both knees were identified and designated as cases. Controls were selected from among female subjects in the TwinsUK study who were unrelated to the cases and had no symptoms of knee OA ($n = 303$). In order to explore allele and genotype frequencies in men with knee OA, 197 unrelated male subjects with knee OA who were part of the Nottingham study were also genotyped (Table 1).

Newfoundland replication sample. As a second replication sample, we genotyped 378 individuals from Newfoundland, Canada, who had definite OA according to the American College of Rheumatology criteria, and compared them with 264 controls without signs or symptoms of OA at any site, based on their medical history and the findings of a thorough examination conducted by an experienced rheumatologist (PR). There were 3 individuals with OA of the knee, hand, and hip, 35 with OA of the knee and hand, 12 with OA of the knee and hip, 5 with OA of the hand and hip, 161 with knee OA only, 56 with hand OA only, and 48 with hip OA only. This sums to 211 individuals with knee OA, 99 with hand OA, and 68 with hip OA. Some of the summary characteristics of this sample are reported in Table 1.

SNP markers and genotyping. A set of 25,494 SNP markers was selected from a collection of 125,799 experimentally validated polymorphic variations (21). This set was limited to SNPs located within gene regions, minor allele frequencies >0.02 (95% with frequencies >0.1), and a target intermarker spacing of 40 kb. SNP annotation was based on the National Center for Biotechnology Information (NCBI) dbSNP database, reference SNP (refSNP), build 118. Genomic annotation was based on NCBI genome build 34. Gene annotation was based on Entrez Gene genes for which NCBI was providing positions on the Map Viewer FTP site.

For pooled DNA assays, 25 ng of case and control DNA pools was used for amplification at each site. All

Table 1. Characteristics of the study samples*

Sample, study group	No. of subjects	Age, median (IQR) years	Weight, median (IQR) kg
UK discovery sample			
Female cases with knee OA	335	70 (65–75)	76 (65–85)
Female controls	335	57 (53–71)	64 (57–71)
UK replication sample			
Female cases with knee OA	246	59 (52–63)	68 (61–76)
Male cases with knee OA	197	66 (62–69)	86 (78–95)
Female controls	303	55 (53–59)	64 (59–71)
Newfoundland replication sample			
Female cases with any OA	227	63 (54–71)	81 (70–95)
Male cases with any OA	119	63 (54–71)	83 (70–98)
Female and male cases with knee OA	211	61 (55–71)	74 (66–86)
Female and male cases with hand OA	99	64 (56–72)	81 (68–91)
Female and male cases with hip OA	68	62 (53–70)	88 (75–103)
Female and male controls	264	62 (53–70)	88 (75–103)

* Some subjects had osteoarthritis (OA) at multiple sites. IQR = interquartile range.

polymerase chain reaction (PCR) and MassExtend (Sequenom, San Diego, CA) amplifications were conducted under standard conditions (21). Relative allele frequency estimates were derived from area under the peak calculations of mass spectrometry measurements from 4 analyte aliquots as described elsewhere (22). For individual genotyping, the same procedure was applied, except that only 2.5 ng of DNA was used and only 1 mass spectrometry measurement was taken. For genotyping of rs912428, the following primers were used: PCR primer 1, 5'-TCAGATCAGAGTGAGTTTAG-3'; PCR primer 2, 5'-ACTACATCCATTCCAGGGAG-3'; and extension primer, 5'-CCATTCCAGGGAGACTCCCA-3'.

Cell culture and measurement of *LRCH1* expression in human chondrosarcoma cells. SW1353 human chondrosarcoma cells (HTB-94; American Type Culture Collection [ATCC], Rockville, MD) were propagated in Leibovitz's L-15 medium supplemented with 2 mM L-glutamine, 10% fetal calf serum, and penicillin/streptomycin (100 units/ml) according to the ATCC protocol. Confluent SW1353 cells were prepared as single-cell suspensions by treatment with trypsin-EDTA and were resuspended in 1.2% alginate (Keltone LVCR; Kelco, Chicago, IL) in 0.9% NaCl at a density of 4×10^6 cells/ml (10 million cells per stimulus).

Alginate beads of uniform diameter were prepared by dispensing the cell-alginate suspension dropwise through a 22-gauge needle into 100 mM CaCl₂ from a height of ~2 cm. After polymerization (10 minutes), the beads were washed 3 times with phosphate buffered saline and then once with medium. The encapsulated cells were differentiated in a 24-well plate (10 beads/well; 25,000–50,000 cells/bead) for 2 weeks under standard conditions, with medium changes every 3 days. At the end of 14 days, a few randomly selected beads were stained with Alcian blue to identify the presence of glycosaminoglycans, suggesting a chondrocyte phenotype (23).

After 14 days, the alginate-cultured cells were stimulated with either recombinant human interleukin-1 β (IL-1 β ; R&D Systems, Abingdon, UK) or phorbol 12-myristate 15-acetate (PMA; Sigma, Poole, UK) alongside serum-starved controls for 3 hours (PMA) or for 24 hours (IL-1 β). Similar experimental conditions were used with confluent plates of

undifferentiated SW1353 cells to compare the effects of monolayer culture and alginate culture on gene expression.

Encapsulated cells were released from the alginate beads by treatment with sodium citrate (55 mM in 0.15M NaCl), and the expression of target genes plus control genes (MMPs 8 and 13) was determined by messenger RNA isolation (Dynabeads oligo-[dT]₂₅; Dynal Biotech, Wirral, UK), followed by complementary DNA (cDNA) synthesis (Superscript II; Invitrogen, Paisley, UK) and semiquantitative PCR. Semiquantitative PCR was performed by initially determining the linear range for each gene using cDNA from SW1353 cells. To determine the linear range for each gene, identical PCR reactions were amplified, collected at increasing amplification cycles, and run on an agarose gel that was stained with ethidium bromide for visualization. After identifying the linear range, a common cycle number within the linear range for all genes was chosen and used for the final comparative analysis.

The PCR program consisted of the following steps. The first step was an initial denaturation in which the reaction was incubated for 10 minutes at 95°C. In the second step, DNA was amplified for 22 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. PCR was performed using a standard protocol of 30 cycles. The following primer pairs were used: for *LRCH1*, 5'-CCAAAGATCAGGACATGGATA-3' (forward) and 5'-TGCTGTTTGTGGTAGGAGAG-3' (reverse); for *MMP-8*, 5'-CAATACTGGGCTCTGAGTGG-3' (forward) and 5'-GGAAAGGCACCTGATATGC-3' (reverse); for *MMP-13*, 5'-ATATCTGAACTGGGTCTTCC-3' (forward) and 5'-GACAGCATCTACTTTATCACC-3' (reverse); and for *GAPDH*, 5'-ATCATCTCTGCCCTCTG-3' (forward) and 5'-GAGGCATTGCTGATGATCTTTG-3' (reverse). Single-band PCR products were resolved on 2% agarose gels and visualized by ethidium bromide staining. Levels of cDNA were normalized for differences in cell number by the housekeeping gene *GAPDH*. Control cDNA was composed of an equimolar mixture of 56 cDNA preparations from various human cell lines and was used to verify that the selected primers amplified only a single predicted product.

Statistical analysis. Tests of association between OA affected status and each SNP, using pooled DNA, were performed as described elsewhere (22). Sources of measurement variation included pool formation, PCR/mass extension, and chip measurement. When 3 or more replicate measurements of a SNP were available within a model level, the corresponding variance component was estimated from the data. Otherwise, the following historical laboratory averages were used: pool formation = 5.0×10^{-5} , PCR/mass extension = 1.7×10^{-4} , and chip measurement = 1.0×10^{-4} . All genotypes were tested for Hardy-Weinberg equilibrium. Tests of association using individual genotypes were performed using a chi-square test of heterogeneity based on allele and genotype frequencies. *P* values were derived using the log odds of each contrast and their standard errors. No attempt was made to correct *P* values for multiple testing, which would diminish the chances of finding or distinguishing a real initial result, since the subsequent robustness was determined by multiple replication (22). Rather, *P* values are provided to compare the relative strength of association.

RESULTS

Initial association study of the UK discovery sample. We performed a large-scale association study using 25,494 SNPs located within 10 kb of 13,735 Entrez Gene annotated genes. An overview of the investigative process is shown in Figure 1. The basic design was a 2-group study of subjects with and without OA of the knee. To facilitate the screening of such a large number of SNPs, we utilized a high-throughput approach using DNA pools, chip-based mass spectrometry (24–26), and a 3-step SNP selection strategy (27). In the first step, we performed a single PCR and primer extension reaction for each SNP on 2 DNA pools consisting of equimolar amounts of DNA from each subject in each study group (knee OA and control). Relative allele frequencies obtained from 4 mass spectrometry measurements of the extension products were compared between pools. In the second step, the 1,420 SNPs (6%) with the most statistically significant associations were re-measured in triplicate on each DNA pool. In the third step, we genotyped in individuals, rather than pools, the 104 most significant SNPs from step 2 (7%) in all subjects comprising the pools. Based on the genotype results, 82 SNPs were confirmed to have statistically significant allele frequency differences between cases and controls at a level of $P < 0.05$ (31 SNPs showed $P < 10^{-3}$ and 11 SNPs showed $P < 10^{-4}$).

Among the 31 SNPs at the 10^{-3} significance level was the rs912428a C/T polymorphism within intron 1 of *LRCH1* on chromosome 13. Frequencies of the T allele based on genotyping were 0.228 in the knee OA group and 0.170 in the unaffected group (odds ratio [OR] 1.44,

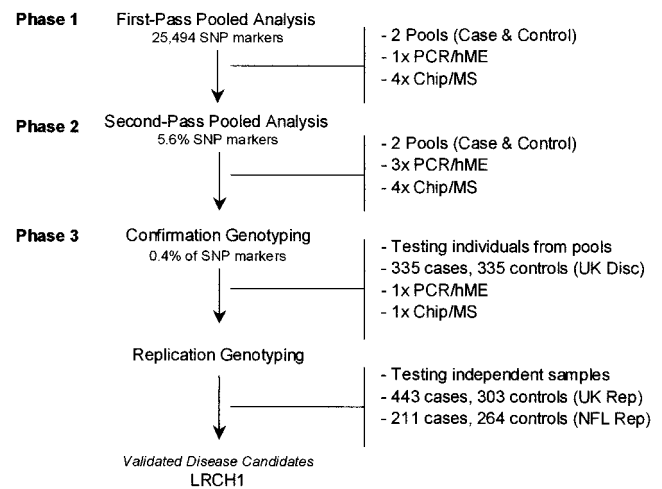


Figure 1. Schematic presentation of the genome-wide association study of osteoarthritis, from the pool-based screening to the replicated candidate genes. Phases 1 and 2 were performed using DNA pools (see Subjects and Methods for details). All subsequent steps involved genotyping of individual samples. SNP = single-nucleotide polymorphism; PCR = polymerase chain reaction; hME = homogeneous MassExtend; MS = mass spectrometry; Disc = discovery sample; Rep = replication sample; NFL = Newfoundland.

$P = 0.0078$) (Table 2). To determine whether this selected result represents a true genetic effect or simply a spurious association owing to the liberal statistical selection criteria, the rs912428 SNP was genotyped in individuals in 2 additional replication case–control samples. Although several SNPs in the discovery sample showed higher statistical significance ($P < 10^{-4}$), rs912428 revealed the strongest and most consistent effect in comparisons of the 3 independent sample sets (see below).

Genotyping of rs912428 in replication samples.

In the UK replication sample, the T allele was more common in the OA cases (20.1%) than in the controls (14.0%) with a *P* value of 0.0025 (OR 1.54). There was no evidence of heterogeneity of the genetic effect between the female and male OA case subsamples, as evidenced by similar allele and genotype frequencies (Table 2).

In the sample from Newfoundland, the T allele was also increased in knee OA cases (24.4%) compared with controls (18.4%) with a *P* value of 0.0234 (OR 1.43). For the subsamples with hand and hip OA, an increased frequency of the T allele relative to the controls was also observed (21.7% and 23.5%, respectively), yet the difference did not reach statistical significance. We also compared allele and genotype frequencies in the combined group of Newfoundland subjects

Table 2. Allele and genotype frequencies of single-nucleotide polymorphism rs912428 in knee OA cases and controls*

Sample, study group, and allele/genotype	No. of chromosomes		No. (%) of subjects		OR for T allele (95% CI)	P
	OA cases	Controls	OA cases	Controls		
UK discovery sample, knee OA						
Females	670	664				
T			153 (22.8)	113 (17.0)	1.44 (1.10–1.89)	0.0078
TT			15 (4.5)	8 (2.4)		0.0258
TC			123 (36.7)	97 (29.2)		
CC			197 (58.8)	227 (68.4)		
UK replication sample, knee OA						
Females and males	886	606				
T			178 (20.1)	85 (14.0)	1.54 (1.16–2.04)	0.0025
TT			12 (2.7)	8 (2.6)		0.0019
TC			154 (34.8)	69 (22.8)		
CC			277 (62.5)	226 (74.6)		
Females	492	606				
T			95 (19.3)	–	1.47 (1.06–2.02)	0.0187
TT			5 (2.0)	–		0.0093
TC			85 (34.6)	–		
CC			156 (63.4)	–		
Males	394	606				
T			83 (21.1)	–	1.64 (1.17–2.28)	0.0036
TT			7 (3.6)	–		0.0074
TC			69 (35.0)	–		
CC			121 (61.4)	–		
Newfoundland replication sample						
Knee OA						
Females and males	422	528				
T			103 (24.4)	97 (18.4)	1.43 (1.05–1.96)	0.0234
TT			12 (5.7)	7 (2.7)		0.0643
TC			79 (37.4)	83 (31.4)		
CC			120 (56.9)	174 (65.9)		
Hand OA						
Females and males	198	528				
T			43 (21.7)	–	1.23 (0.82–1.84)	0.3088
TT			5 (5.1)	–		0.4632
TC			33 (33.3)	–		
CC			61 (61.6)	–		
Hip OA						
Females and males	136	528				
T			32 (23.5)	–	1.37 (0.87–2.15)	0.1752
TT			3 (4.4)	–		0.3802
TC			26 (38.2)	–		
CC			39 (57.4)	–		
Any OA						
Females and males	640	528				
T			155 (24.2)	–	1.42 (1.07–1.89)	0.0156
TT			16 (5.0)	–		0.0470
TC			123 (38.4)	–		
CC			181 (56.6)	–		

* OA = osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.

with OA at any of the 3 sites with the frequencies in the unaffected individuals. These results were similar to those for knee OA, with 24.2% of subjects with any OA having the T allele ($P = 0.0156$, OR 1.42) (Table 2).

Combining all 3 data sets (UK discovery, UK replication, and Newfoundland replication) and adjusting for study of origin and sex, an overall OR for knee

OA of 1.45 (95% confidence interval 1.18–1.79) was computed for the T allele ($P < 5 \times 10^{-4}$).

Association fine-mapping. In order to define the region of association, we selected 10 informative SNPs in a 140-kb window surrounding the original marker rs912428 SNP on chromosome 13 (Figure 2). These SNPs were hand selected using the data available from

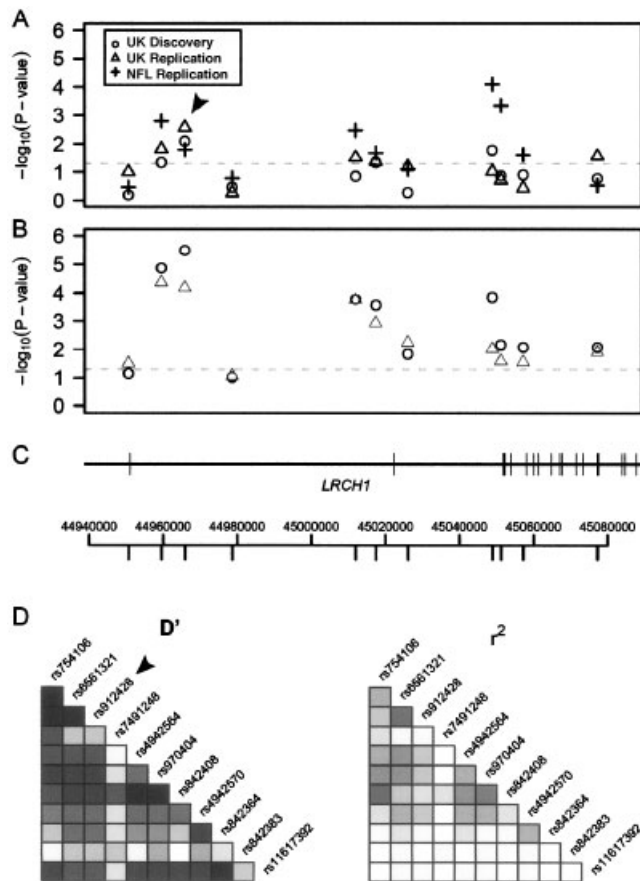


Figure 2. Genotype analysis of 11 single-nucleotide polymorphisms (SNPs) in a 130-kb window around the marker SNP rs912428 (arrowhead). Broken line represents a nominal significance level ($P = 0.05$). **A**, P values for each SNP in the 3 osteoarthritis (OA) samples. NFL = Newfoundland. **B**, Combined P values from the OA replication samples (triangles) and from all 3 study samples (circles). **C**, Gene map and chromosome positions, with SNP locations indicated. **D**, Estimates of linkage disequilibrium in the combined OA sample. Arrowhead indicates the rs912428 SNP. In this gray-scale representation, intermediate shades between white (linkage disequilibrium 0) and black (linkage disequilibrium 1) indicate linkage disequilibrium increments of 0.1.

the HapMap project (28) to be maximally informative on the basis of patterns of linkage disequilibrium. The location of these SNPs within the *LRCH1* gene, their association with OA, and the linkage disequilibrium between them are shown in Figure 2.

We obtained a mixed and moderate pattern of linkage disequilibrium, suggesting that most SNPs were informative (Figure 2D). Although 2 markers in intron 2 of *LRCH1* were statistically more significant in the Newfoundland sample (rs4942570 and rs842364), these were not as significant in the UK groups (Figure 2A).

Combined analyses of the replication data and of all subjects were most significant in the 5' region of intron 1 (Figure 2B). Therefore, the region of highest association seemed to span intron 1, with the significance declining to both sides. Overall, there were no SNPs in the region that exhibited stronger association with the trait than the marker SNP rs912428. Haplotype analysis failed to demonstrate any particular combination of SNPs with a consistently significant association that was greater than that of the individual SNPs.

Expression of *LRCH1* in human chondrosarcoma cells. Human chondrosarcoma cells (SW1353) were cultured either in monolayers or in a solid alginate matrix to address the possibility that chondrocytes would de-differentiate in monolayer culture but would retain a chondrocyte phenotype in matrix environments (29). Figure 3 shows the results of the analysis of *LRCH1* expression in alginate-cultured human chondrosarcoma cells treated with the inflammatory stimuli IL-1 β and PMA. This analysis revealed substantial increases in the expression of the IL-1 β -responsive genes *MMP8* (30) and *MMP13* (31) in IL-1 β -stimulated SW1353 cells. Interestingly, PMA had no obvious effect on the expression of either *MMP8* or *MMP13*. *LRCH1* expression after IL-1 β and PMA stimulation was unchanged from that in controls. This suggests that the effect of *LRCH1* on the pathogenesis of OA may be via an inflammatory-

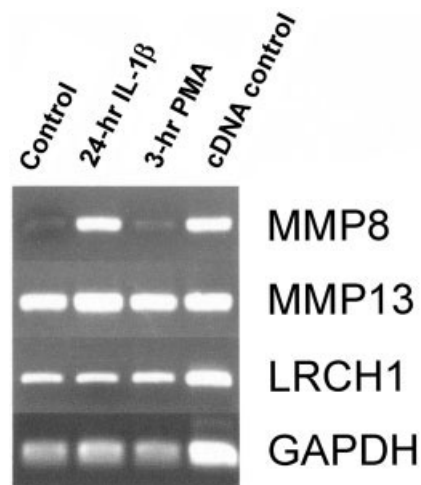


Figure 3. Expression of *LRCH1* in cultured human chondrosarcoma cells. SW1353 cells grown on alginate beads were stimulated with either interleukin-1 β (IL-1 β) or phorbol 12-myristate 15-acetate (PMA) alongside untreated controls for 3 hours (PMA) or 24 hours (IL-1 β). The expression of target genes plus control genes (matrix metalloproteinases [MMPs]) was determined by mRNA isolation followed by cDNA synthesis and semiquantitative polymerase chain reaction techniques.

independent mechanism, possibly involving compressive stress. SW1353 cells have been used by other researchers for the investigation of OA-related pathways (32,33). Although it was not the optimal cell line for the experiments we performed, these observations are interesting and should be validated in followup experiments using models that are more appropriate.

DISCUSSION

We report herein our finding of a consistent association between the SNP rs912428 and OA of the knee. This association was discovered through a large-scale association study in which we screened close to 26,000 SNP markers located within 10 kb of ~14,000 annotated genes, combined with replication of suggested associations in 2 additional case-control samples. The SNP rs912428 is located in intron 1 of *LRCHI* (leucine-rich repeats and calponin homology containing 1), a novel gene with unknown function located on chromosome 13q14. Leucine-rich repeats are 20–29-residue sequence motifs that are present in many proteins that participate in protein-protein interactions and have different functions and cellular locations. The calponin homology domain is an actin-binding domain, which may be present as a single copy or in tandem repeats (which increases binding affinity). The calponin homology domain is found in cytoskeletal and signal transduction proteins, including actin-binding proteins such as spectrin, α -actinin, dystrophin, utrophin, and fimbrin, proteins essential for the regulation of cell shape (cortexillins), and signaling proteins such as the activators of Rho GTPases of the Vav family.

In the absence of data on the function of the gene product, we speculate that through interactions with the cellular cytoskeleton, *LRCHI* is involved in chondrocyte responses to pressure or tension. Disruption of this mechanism may contribute to the phenotype of primary OA. Compressive stress plays a role in chondrocyte metabolism and development (34,35). *LRCHI* may play a role in this signaling pathway, such that chondrocytes do not respond normally to compressive stress, which leads to the changes in cartilage composition and structure that are characteristic of OA. Similarly, perturbations in mechanotransduction signaling in osteoblasts and osteoclasts in the vicinity of the affected joint may contribute to aberrant bone remodeling, resulting in osteophyte formation. Our analysis of a smaller subsample of individuals with hand and hip OA suggested that a similar association might exist between *LRCHI* and hip OA, and to an even lesser extent, with hand OA.

In terms of odds ratios, the stronger association with OA in weight-bearing joints lends some indirect support to the hypothesis of an interaction between genetic variation in *LRCHI* and joint mechanics.

An association with an intronic SNP provides little evidence for a change in the amount or function of the protein that could explain the association. Association fine-mapping of the region surrounding the marker SNP indicated that the region of association was confined to intron 1 of *LRCHI*, which makes it unlikely that the observed association would be due to a nonsynonymous and disruptive coding change in linkage disequilibrium with our marker SNP. Therefore, it is more likely that the effect is mediated by a change in RNA splicing, editing, or expression. No other intronic SNP or haplotype combination had a stronger association with the trait than the initial marker SNP.

Some features and possible limitations of the present study merit particular consideration. First, as shown in Table 1, there was a significant difference in the average weight of the OA cases and controls in the discovery sample. This difference was recognized but accepted in favor of achieving a study design in which all cases and controls underwent knee radiography. This might have introduced a potential bias toward alleles associated with obesity rather than OA; however, the findings presented here were replicated in 2 independent OA sample sets, in which the weight difference between cases and controls was not significant. Second, only the sample used for the initial study had controls that underwent knee radiography. That means that an unknown proportion of the controls in the UK and the Newfoundland replication samples could have radiographic findings that would be consistent with a diagnosis of OA. Asymptomatic radiographic changes indicative of knee OA are not uncommon in the elderly. This would have the effect of attenuating the strength of the observed association in the replication samples.

A possible limitation is the lack of normal male control subjects. The UK replication sample consisted of both male and female OA cases that were compared with all-female controls. Moreover, we did not observe a sex-dependent allele frequency difference for the *LRCHI* marker SNP, which suggests that some gene effects may be relevant for both sexes; this is consistent with the recent findings reported by Kizawa et al (16). It is also possible that this gene may influence the progression of disease as much as prevalent OA changes (36).

In conclusion, the present study shows a remarkably consistent association between a SNP in *LRCHI* in 3 populations that were ascertained in different ways,

had different sex distributions, and were from different geographic locations. This indicates that *LRCHI* may play a central role in the pathogenesis of knee OA. The identification of a calponin homology domain protein as a potential key player suggests the involvement of a novel group of proteins in the development of OA and might thereby open novel therapeutic opportunities. However, additional experimental studies are needed to elucidate how *LRCHI* influences the risk of the disease.

ACKNOWLEDGMENTS

We would like to thank the subjects and staff from the following population and OA projects: the Chingford Study, the TwinsUK study, the Nottingham sibling study, and the St. John's, Newfoundland, OA population. We also thank the genotyping team at Sequenom for their contributions. The Arthritis Research Campaign supports the Chingford Study and the TwinsUK musculoskeletal database. TwinsUK is also funded by the Wellcome Trust.

REFERENCES

- Felson DT. Osteoarthritis. *Rheum Dis Clin North Am* 1990;16:499–512.
- Spector TD, Macgregor AJ. Risk factors for osteoarthritis [review]. *Osteoarthritis Cartilage* 2004;12 Suppl A:S39–44.
- Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. *BMJ* 1996;312:940–3.
- Kaprio J, Kujala UM, Peltonen L, Koskenvuo M. Genetic liability to osteoarthritis may be greater in women than men. *BMJ* 1996;313:232.
- MacGregor AJ, Spector TD. Twins and the genetic architecture of osteoarthritis. *Rheumatology (Oxford)* 1999;38:583–8.
- Meulenbelt I, Bijkerk C, Breedveld FC, Slagboom PE. Genetic linkage analysis of 14 candidate gene loci in a family with autosomal dominant osteoarthritis without dysplasia. *J Med Genet* 1997;34:1024–7.
- Ingvarsson T, Stefansson SE, Gulcher JR, Jonsson HH, Jonsson H, Frigge ML, et al. A large Icelandic family with early osteoarthritis of the hip associated with a susceptibility locus on chromosome 16p. *Arthritis Rheum* 2001;44:2548–55.
- Wright GD, Hughes AE, Regan M, Doherty M. Association of two loci on chromosome 2q with nodal osteoarthritis. *Ann Rheum Dis* 1996;55:317–9.
- Leppavuori J, Kujala U, Kinnunen J, Kaprio J, Nissila M, Heliovaara M, et al. Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: evidence for a locus on 2q. *Am J Hum Genet* 1999;65:1060–7.
- Chapman K, Mustafa Z, Irvan C, Carr AJ, Clipsham K, Smith A, et al. Osteoarthritis-susceptibility locus on chromosome 11q, detected by linkage. *Am J Hum Genet* 1999;65:167–74.
- Demissie S, Cupples LA, Myers R, Aliabadi P, Levy D, Felson DT, et al. Genome scan for quantity of hand osteoarthritis: the Framingham Study. *Arthritis Rheum* 2002;46:946–52.
- Stefansson SE, Jonsson H, Ingvarsson T, Manolescu I, Jonsson HH, Olafsdottir G, et al. Genomewide scan for hand osteoarthritis: a novel mutation in *matrilin-3*. *Am J Hum Genet* 2003;72:1448–59.
- Hunter DJ, Demissie S, Cupples LA, Aliabadi P, Felson DT. A genome scan for joint-specific hand osteoarthritis susceptibility: the Framingham Study. *Arthritis Rheum* 2004;50:2489–96.
- Forster T, Chapman K, Marcelline L, Mustafa Z, Southam L, Loughlin J. Finer linkage mapping of primary osteoarthritis susceptibility loci on chromosomes 4 and 16 in families with affected women. *Arthritis Rheum* 2004;50:98–102.
- Loughlin J. Genetic epidemiology of primary osteoarthritis. *Curr Opin Rheumatol* 2001;13:111–6.
- Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, et al. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. *Nat Genet* 2005;37:138–44.
- Risch NJ. Searching for genetic determinants in the new millennium. *Nature* 2000;405:847–56.
- Neame RL, Muir K, Doherty S, Doherty M. Genetic risk of knee osteoarthritis: a sibling study. *Ann Rheum Dis* 2004;63:1022–7.
- Hunter DJ, Hart D, Sneider H, Bettica P, Swaminathan R, Spector TD. Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. *Rheumatology (Oxford)* 2003;42:1311–6.
- Hart DJ, Spector TD. Cigarette smoking and risk of osteoarthritis in women in the general population: the Chingford Study. *Ann Rheum Dis* 1993;52:93–6.
- Nelson MR, Marnellos G, Kammerer S, Hoyal CR, Shi MM, Cantor CR, et al. Large-scale validation of single nucleotide polymorphisms in gene regions. *Genome Res* 2004;14:1664–8.
- Bansal A, van den Boom D, Kammerer S, Honisch C, Adam G, Cantor CR, et al. Association testing by DNA pooling: an effective initial screen. *Proc Natl Acad Sci U S A* 2002;99:16871–4.
- Masuda K, Miyabayashi T, Meachum SH, Eurell TE. Proliferation of canine intervertebral disk chondrocytes in three-dimensional alginate microsphere culture. *J Vet Med Sci* 2002;64:79–82.
- Kammerer S, Burns-Hamuro LL, Ma Y, Hamon SC, Canaves JM, Shi MM, et al. Amino acid variant in the kinase binding domain of dual-specific A kinase-anchoring protein 2: a disease susceptibility polymorphism. *Proc Natl Acad Sci U S A* 2003;100:4066–71.
- Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, et al. High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci U S A* 2001;98:581–4.
- Mohlke KL, Erdos MR, Scott LJ, Fingerlin TE, Jackson AU, Silander K, et al. High-throughput screening for evidence of association by using mass spectrometry genotyping on DNA pools. *Proc Natl Acad Sci U S A* 2002;99:16928–33.
- Kammerer S, Roth RB, Reneland R, Marnellos G, Hoyal CR, Markward NJ, et al. Large-scale association study identifies ICAM gene region as breast and prostate cancer susceptibility locus. *Cancer Res* 2004;64:8906–10.
- The International HapMap Consortium. The international HapMap project. *Nature* 2003;426:789–96.
- Lee DA, Reisler T, Bader DL. Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared with conventional monolayer techniques. *Acta Orthop Scand* 2003;74:6–15.
- Chubinskaya S, Huch K, Mikecz K, Cs-Szabo G, Hasty KA, Kuettner KE, et al. Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1 β in human cartilage from knee and ankle joints. *Lab Invest* 1996;74:232–40.
- Im HJ, Pacione C, Chubinskaya S, van Wijnen AJ, Sun Y, Loeser RF. Inhibitory effects of insulin-like growth factor-1 and osteo-

- genic protein-1 fibronectin fragment- and interleukin-1 β -stimulated matrix metalloproteinase-13 expression in human chondrocytes. *J Biol Chem* 2003;278:25386–94.
32. Gebauer M, Saas J, Sohler F, Haag J, Soder S, Pieper M, et al. Comparison of the chondrosarcoma cell line SW1353 with primary human adult articular chondrocytes with regard to their gene expression profile and reactivity to IL-1 β . *Osteoarthritis Cartilage* 2005. E-pub ahead of print.
 33. Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1 β . *Arthritis Res* 2001;3:381–8.
 34. Hunter CJ, Imler SM, Malaviya P, Nerem RM, Levenston ME. Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels. *Biomaterials* 2002;23:1249–59.
 35. Fitzgerald JB, Jin M, Dean D, Wood DJ, Zheng MH, Grodzinsky AJ. Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic AMP. *J Biol Chem* 2004;279:19502–11.
 36. Valdes AM, Hart DJ, Jones KA, Surdulescu G, Swarbrick P, Doyle DV, et al. Association study of candidate genes for the prevalence and progression of knee osteoarthritis. *Arthritis Rheum* 2004;50:2497–507.