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Lack of Support for the Presence of an Osteoarthritis Susceptibility Locus on Chromosome 6p

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Objective. To replicate, in a Northern Irish population, the previously reported association between a locus on chromosome 6 and hip osteoarthritis (OA).

Methods. Patients with hip OA were identified from a registry of patients who had undergone total hip replacement surgery over an 8-year period at a single large orthopedic unit in Northern Ireland. Patients identified as index cases were contacted by mail and asked to reply only if another family member also had undergone total hip replacement surgery. Using this approach, we identified 288 sibling pairs concordant for primary hip OA. DNA was extracted from peripheral blood, and microsatellite markers were amplified by polymerase chain reaction and subsequently genotyped.

Results. No evidence of linkage to this region was demonstrated by either 2-point analysis or multipoint analysis of 17 microsatellites.

Conclusion. The reported association between a locus on chromosome 6 and hip OA could not be confirmed in this population. Different methods of ascertainment and phenotyping of OA may contribute to the current inability to replicate genetic associations for hip OA.

Osteoarthritis (OA) is a common, complex disorder that shows heterogeneity with respect to the pattern of joint involvement, number of joints affected, age at onset, and clinical outcome. Several extrinsic and intrinsic risk factors that may differ between joint sites have

been identified (1). A strong genetic component for development of hip OA is evident from the increased concordance for radiographic hip OA seen in monozygotic twins compared with dizygotic twins (2) and from the increased risk of radiographic hip OA in siblings of patients who have undergone total hip replacement (THR) surgery for primary hip OA (3). The high estimated heritability observed in such studies (>50%) helps justify the search for site-specific genes that predispose to OA (4).

Chapman and colleagues (the Loughlin group) studied sibling pairs with large-joint (hip, knee) OA requiring joint replacement; subjects were identified from several centers within the UK (5). Their genome-wide linkage analysis in 194 pedigrees revealed a region suggestive of linkage on chromosome 6p, with a maximum multipoint logarithm of odds (LOD) score of 2.9. In a subsequent analysis, these investigators genotyped chromosome 6 to a higher density in an expanded cohort of 378 THR pedigrees. Finer linkage analysis of this 11.4-cM region with stratification for sex and site resulted in a maximum multipoint LOD score of 4.6 at marker D6S1573 in 166 female-only pedigrees (6). This finding represents the strongest evidence to date for a region that may harbor an OA susceptibility gene. Two attractive candidate genes that reside in this region are *COL9A1*, which encodes a minor cartilage collagen that may act to stabilize cartilage, and *BMP5*, which encodes a protease belonging to the transforming growth factor β family. However, single-nucleotide polymorphism analysis of these genes failed to show an association (7).

The aim of the present study was to perform linkage analysis on this region of chromosome 6 in a separate cohort of Northern Irish pedigrees with primary hip OA.

PATIENTS AND METHODS

Subjects. This study was approved by the local research ethics committee. Subjects in the Northern Irish sibling pair

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Table 1. Novel primer sequences used in multipoint analysis of chromosome 6p

Marker	Forward primer (5'→3')	Reverse primer (5'→3')	Heterozygosity, %
IL17	GACTTACCCAACTGGAATGTCC	AGTGCCGAGAAGTGCCATTGTC	66
IL17F	TCAGTTTGTGGTACTTTGTTATGG	TTGCTACATTGTTTATGTCATGG	63
FBOX9	GGTGAAATACTAAGGAGGTAGG	CTCGTGGAATTTACATTCTAGG	78
BMP5	GATGAGATTGCCTAAGCATGACG	TGTTACACAGACATAGGACCTGC	80
2326k(ca) _n	GTGTGAAAAGTGATGGGGAAAAGG	AGTGTTAGCTCAGTTGGTTAAGC	76
2521k(gt) _n	CAAACCTCAGCAGTTCTTTAGAGG	GTTACATTTCCACTACTTAGAGGC	78
2767k(ca) _n	GAGCTGTAGTAAGCCATGATGG	GTTGAAATCCTGCCTGGAACCTC	80
COL9A1	AGGGTGCCTTATTTCTTCTCC	AAGGGTTTCGGTTTTGTTGGGC	71

hip OA cohort were identified from the records of Musgrave Park Hospital and included patients who had undergone THR surgery for primary hip arthritis between 1996 and 2003. This hospital has the largest orthopedic unit in Western Europe. Index patients were contacted by mail and were asked to reply only if they had a first-degree relative who had also undergone THR surgery. Families containing at least 1 affected sibling pair concordant for hip OA were thus identified. Patients who had previously donated DNA samples for the Loughlin group (Oxford cohort) study were excluded, along with any patient with a history of inflammatory arthritis, hip trauma, or congenital hip abnormality. The method of exclusion according to results of radiography is described below. A control group of 10 Northern Irish adults was chosen at random and was used to estimate allele frequencies of the markers studied. Based on standard formulae, we estimated that if we assumed a genotype relative risk (γ) of 4.0, our hip OA cohort had 80% power at a 5% significance level.

Phenotypic characterization. All THR patients underwent clinical assessment to enable accurate site-specific OA phenotyping according to American College of Rheumatology (ACR) criteria and to enable further exclusion on a clinical basis (8). Patients with secondary causes of hip OA were excluded based on the assessment of preoperative radiographs, including measurement of the acetabular depth (9) and the center edge angle (10). Hip dysplasia was defined as an acetabular depth of <9 mm and/or a center edge angle of <25° (11). The minimum joint space width, representing the minimum space between the articular surface of the femoral head and the acetabular roof, was measured by a single observer (GKM) using Vernier calipers that were accurate to 0.02 mm. The degree of reproducibility for radiographic scoring was assessed using the weighted kappa test with 50 random pelvic radiographs that were graded 4 weeks apart.

Genetic analysis. Peripheral blood was obtained from participants, and genomic DNA was extracted using the Pure-gene DNA Isolation Kit (Flowgen, Nottingham, UK). Nine microsatellite markers for chromosome 6 from the ABI LMS-MD10 v2.5 kit (Applied Biosystems, Warrington, UK) were amplified using multiplex polymerase chain reaction (PCR) (Qiagen, Crawley, UK). The primer sequences for 8 additional markers that were developed in-house and were used in the analysis are shown in Table 1. Hardy-Weinberg equilibrium was established for all of the markers studied. PCR cycling conditions were as follows: 15 minutes at 95°C, then 30 cycles at 94°C for 30 seconds, 60°C for 90 seconds, and 72°C for 60 seconds, followed by 60°C for 30 minutes.

Allelic genotyping was performed using GeneScan and Genotyper software (Applied Biosystems). Multipoint linkage

analysis was subsequently performed with the GeneHunter-Plus program (<http://hgmp.mrc.ac.uk/Registered/Option/genehunter-plus.html>) (12), using allele frequencies found in our control population. The allele frequencies were compared between the control group and a random sample of 40 affected siblings (the first member of each sibling pair in the first 40 pedigrees), using Wilcoxon's signed rank test. *P* values less than 0.05 were considered significant.

RESULTS

A total of 3,505 patients who had undergone THR surgery were contacted by mail. Four hundred eighty-two replies were obtained, representing a positive response rate of 13.7% for self-reported THR in family members. Thirty-four index patients were excluded due to concomitant inflammatory arthritis (*n* = 17), congenital hip abnormality (*n* = 15), and hip trauma (*n* = 2). The remaining group of 288 sibling pairs comprised 180 women and 112 men from 109 THR pedigrees. The mean acetabular depth in men was 12.4 mm (95% confidence interval [95% CI] 11.9–12.7) and in women was 12.6 mm (95% CI 12.2–12.9). The mean center edge angle in men was 35.6° (95% CI 35.1–36.0) and in women was 34.7° (95% CI 34.4–34.9). The mean minimum joint space width in men was 2.44 mm (95% CI 2.16–2.88) and in women was 2.34 mm (95% CI 2.02–2.48). The kappa values for intraobserver reproducibility of measurements of the center edge angle, acetabular depth, and minimum joint space width were 0.85, 0.90, and 0.87, respectively. The specific OA phenotypes of the cohort are shown in Table 2.

Table 2. Subjects fulfilling ACR criteria for hip OA, according to phenotype*

Phenotype	No. in group	% fulfilling criteria
Hip only	162	56
Hip and knee	63	21.5
Hip and hand	22	7.5
Hip, knee, and hand	45	15

* ACR = American College of Rheumatology; OA = osteoarthritis.

Table 3. Distribution of alleles in affected sibling pairs and controls

Microsatellite	Chromosome 6p map position, Mb	Multipoint LOD score*		Allele frequency, P†
		All pedigrees	Females only	
D6S276	38.0	-2.16	-1.57	0.34
D6S1549	46.6	-2.11	-1.55	0.17
D6S282	48.2	-1.68	-1.40	0.24
IL17	52.0	-1.50	-1.38	0.32
IL17F	52.1	-1.48	-1.31	0.19
FBOX9	52.8	-1.42	-1.23	0.25
D6S1573	53.7	-1.34	-1.19	0.41
D6S294	55.1	-1.16	-1.02	0.37
BMP5	55.7	-1.12	-0.94	0.27
D6S1276	55.8	-0.91	-0.63	0.33
D6S257	56.0	-0.60	-0.35	0.24
D6S223	56.2	-0.75	-0.48	0.24
2326k(ca) _n	56.4	-0.92	-0.54	0.35
2521k(gt) _n	56.6	-1.01	-0.87	0.42
2767k(ca) _n	56.8	-1.25	-1.01	0.38
D6S1557	70.8	-2.34	-1.92	0.36
COL9A1	70.9	-2.87	-2.06	0.17

* LOD = logarithm of odds.

† By Wilcoxon's signed rank test.

Both 2-point (data not shown) and multipoint LOD scores at each marker locus remained negative throughout all intervals between markers, indicating that pathogenetic loci for OA are very unlikely to exist between the markers. These scores remained negative after substratification for female-only sibling pairs. There was no significant difference between the distribution of alleles in affected sibling pairs and controls (Table 3). Thus, there was no evidence to support linkage of an OA susceptibility locus to this region on chromosome 6p.

DISCUSSION

This study failed to demonstrate evidence for an OA susceptibility locus on chromosome 6p in Northern Irish families concordant for primary hip OA requiring THR surgery. Our data showed neither linkage nor a trend toward it before or after stratification for female-only families.

This study is the first to attempt to confirm the presence of an OA susceptibility locus that was previously identified by genome-wide screening. Replication of promising data is an important step toward identification of genes that predispose to complex disorders and avoids the need for rigorous statistical correction due to multiple testing. We typed many of the most influential markers from chromosome 6p used by Loughlin et al and undertook a comprehensive investigation of the region. We added several new markers in the vicinity of

OA candidate genes, including *IL17*, which has been shown to stimulate collagenase 3 activity in OA (13), and *FBOX9*, which is a member of the ubiquitin ligase family that is thought to influence synovial proliferation in animal models of arthritis (14). Our data also provide further evidence against *COL9A1* and *BMP5* as major OA susceptibility genes (7).

Several factors may have contributed to the discordant results between our study and that of Loughlin et al (6). First, our cohort was assembled from sequential patients undergoing THR surgery for defined clinical and radiographic OA in a single large orthopedic center serving a defined population. All subjects satisfied the ACR criteria for site-specific OA, and we were careful to exclude patients with dysplasia or other arthropathy. In contrast, the Oxford cohort was gathered in a less systematic manner from several centers throughout the UK, and radiographic details of the cohort have not been published. Therefore, the generalizability of the findings from each study may differ. The inclusion of subjects with hip dysplasia is unlikely to explain the promising multipoint LOD score obtained by Loughlin's group. Those investigators initially studied 297 OA families and obtained a modest maximum multipoint LOD score of 1.0, which was increased to 2.9 only after a subanalysis of 194 families containing sibling pairs concordant for THR. Increasing the number of THR families to 378 led to a slight reduction in significance, but further subanalysis of female-only THR families

(166 sibling pairs) produced the higher 2-point LOD score of 4.6. Although it was encouraging that both the initial and subsequent subsets of female-only THR families showed a similar linkage trend, a score obtained following extensive stratification must be interpreted with caution. Our data involving 288 sibpairs in 109 THR families, and a subset of 54 sibling pairs in 32 female-only THR families, showed negative multipoint LOD scores throughout the region of interest on chromosome 6p. Replication is of particular importance when positive data have been generated after subanalysis, and our failure to support the linkage must cast doubt on the presence of this OA susceptibility locus.

Failure to replicate linkage or association in studies of complex diseases is not uncommon and can occur because of either Type I or Type II errors (15). The current study and that by Loughlin et al appear to be comparable in terms of studying THR patients drawn from the UK Caucasian population. However, because we used careful clinical and radiographic criteria in participants enrolled over a defined time period from a single large center, we believe that a Type I error in the Oxford study (6) is more probable than a Type II error in the data that we present. In OA and other diseases of late onset, parental DNA is rarely available for study. Affected sibling pair analyses then become profoundly dependent on the marker allele frequencies used in the analysis, with positive results being generated anomalously if a frequency that is too low is applied for a common allele. We assessed marker allele frequencies with care. Software programs for multipoint analysis take no account of linkage disequilibrium, and slightly positive data can become inflated by analysis of multiple adjacent markers.

There are several caveats to our study. First, all of our participants had clinical OA that was sufficiently severe to warrant THR surgery. A study that included individuals with radiographic OA but a less severe clinical phenotype may yield different results. Second, we examined families who are resident in Northern Ireland, and it is possible, although unlikely, that these families may differ, in terms of their genetic predisposition to OA, from the Oxford cohort that was assembled from several centers elsewhere in the UK, including Northern Ireland.

The requirement to unravel the complex pathogenesis of OA gains importance as the prevalence of OA increases. Genes that may affect OA susceptibility are difficult to predict given the complexity of the tissues

that comprise a joint and the multiple systemic and extrinsic factors that may interact to influence phenotypic expression. This makes the hunt for genetic factors all the more challenging. Use of genome-wide screens of higher marker density with rigorous followup of candidate loci may provide the best strategy. The important starting point, however, is agreement with respect to the characterization of the phenotype that is recognized as common OA.

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