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Stratification Analysis of an Osteoarthritis Genome Screen—Suggestive Linkage to Chromosomes 4, 6, and 16

To the Editor:

We have previously carried out a two-stage genomewide linkage screen for osteoarthritis (MIM 165720) susceptibility loci, using an affected-sibling-pair approach (Chapman et al. 1999). In stage 1 of this screen, we tested 272 microsatellite markers in 297 families, each of which contained at least one pair of siblings who had undergone hip-, knee-, or hip and knee–replacement surgery for primary osteoarthritis. Loci that demonstrated evidence for linkage at nominal $P = .05$ were then taken through to stage 2, in which they were tested against a further 184 families. Sixteen markers within nine genomic regions from stage 1 had evidence of linkage, at $P = .05$. When the data for stages 1 and 2 were combined, the P value decreased for 3 of the 16 loci (D2S202, D11S907, and D11S903) and was constant for a 4th (D11S901). We subsequently concentrated our analysis on the chromosome regions to which these markers map. To test these linkages further, we genotyped additional markers and obtained maximum multipoint LOD scores (MLSs) of 1.2 for chromosome 2 and 3.1 for chromosome 11.

Because there is evidence, from epidemiological, twin, and segregation studies, that the genetic contribution to osteoarthritis differs between the sexes and between different joint groups (Lindberg 1986; Cooper et al. 1994; Kaprio et al. 1996; Chitnavis et al. 1997; Felson et al. 1998), we stratified our chromosomes 2 and 11 linkage data according to sex and site of osteoarthritis (hip or knee). This stratification indicated that the suggestion of linkage to chromosome 2 was principally accounted for by affected sibling pairs with hip osteoarthritis (MLS 2.2), whereas the suggestion of linkage to chromosome 11 was restricted to affected female pairs (MLS 2.8). Because this analysis highlighted substantial differences between the strata tested, we have now reanalyzed stage 1 of our genome screen, for the remaining 20 autosomes, to determine whether any regions harbor susceptibility loci that are obscured in the unstratified data set. We stratified our stage 1 data into the same six strata tested in our analysis of chromosomes 2 and 11: affected females only (132 families), affected males only (60 families), hips only (194 families), knees only (34 families), female hip (85 families), and male hip (44 families). (A more detailed breakdown of these families can be found in the study by Chapman et al. [1999].) We did not stratify for female knee or male knee, because the number of families was too small (16 and 4, respectively) to allow reliable inference of linkage. Multipoint linkage analysis was performed on the stratified data by means of the ASPEX program.

Ten of the 20 autosomes have one or more multipoint peaks with uncorrected MLS ≥ 1.0 for one or more of the six strata tested (table 1). The highest MLS is 3.9, for chromosome 4q in the female-hip strata, followed by 2.9, for chromosome 6 in the hip-only strata, and 2.1, for chromosome 16 in the female-hip strata. When we adjust MLS values to correct for the seven models tested (one unstratified analysis and six stratified analyses), by deducting $\log_7 = 0.8$ from the original values (Kidd and Ott 1984), chromosome 4 has an MLS value of 3.1, chromosome 6 has an MLS value of 2.1, and chromosome 16 has an MLS value of 1.3. The uncorrected multipoint plots of these three chromosomes are shown in figure 1.

The suggestion of linkage on chromosome 4 is centered on 4q12–4q21.2 and is restricted to female pairs with hip disease. Roby et al. (1999) have recently reported linkage of chromosome 4q to severe early-onset hip osteoarthritis in a large pedigree of Dutch origin. This locus maps to the telomeric end of 4q (4q35), placing it >50 cM distal to the linkage that we have observed. It is therefore unlikely that the two linkages have detected the same locus.

More than 50 cM of chromosome 6 has an uncorrected MLS ≥ 2.0 in the hip-only stratum, between markers D6S257 and D6S262. This region of chromosome 6 contains a strong candidate gene for osteoarthritis, COL9A1 (6q12–6q13). This gene maps within the 11-cM interval between D6S257 and D6S286 and encodes the $\alpha 1$ chain of type IX collagen. This collagen is a quantitatively minor cartilage collagen that decorates the type II collagen fibril and that interacts with extrafibrillar macromolecules (Ayad et al. 1994). Two transgenic mouse models have demonstrated that mutations in the equivalent mouse gene can result in an osteoarthritis phenotype. In the first model, a truncated form of the gene resulted in mice with a mild osteochondrodysplasia phenotype and secondary osteoarthritis (Nakata et al. 1993). In the second model, a knockout mouse had no congenital abnormality but developed a severe osteoarthritis that was comparable, in timing and pathology, to human primary osteoarthritis (Fässler et al. 1994). A more detailed analysis of this second model revealed that the synthesis of the $\alpha 1$ polypeptide chain was necessary for type IX collagen assembly (Hagg et al. 1997).

Chromosome 16 does not contain any known genes that can be considered as strong candidates for osteoarthritis susceptibility. As more genes are mapped, candidate loci on this chromosome may become apparent.

Overall, the stratification of our genome screen has revealed additional chromosomal regions that may har-

Table 1

Stratified MLSs

Chromosome	MLS (Corrected)[a]	Cytogenetic Position	Flanking Markers	Stratum
1	1.3 (<1.0)	1q31–1q44	D1S238, D1S103	Female only
3	1.5 (<1.0)	3p25–3p21	D3S1263, D3S1289	Female hip
3	1.5 (<1.0)	3p25–3p21	D3S1263, D3S1289	Female only
3	1.0 (<1.0)	3p21–3p14	D3S1289, D3S1285	Hip only
4	3.9 (3.1)	4q12–4q21.2	D4S398, D4S250	Female hip
4	1.7 (<1.0)	4q12–4q21.2	D4S398, D4S250	Female only
5	1.3 (<1.0)	5p13.3–5q11.1	D5S419, D5S407	Female only
6	2.9 (2.1)	6p21.1–6q22.1	D6S1610, D6S314	Hip only
6	1.8 (1.0)	6p23–6p21.3	D6S422, D6S265	Female hip
6	1.3 (<1.0)	6p21.3–6q15	D6S291, D6S286	Female hip
6	1.2 (<1.0)	6q13–6q22.1	D6S462, D6S314	Male hip
6	1.1 (<1.0)	6p23–6p21.3	D6S422, D6S265	Female only
7	1.5 (<1.0)	7q11.23–7q21.2	D7S502, D7S524	Hip only
7	1.3 (<1.0)	7q22.1–7q32	D7S2502, D7S684	Hip only
8	<1.0 (—)	—	—	—
9	<1.0 (—)	—	—	—
10	<1.0 (—)	—	—	—
12	1.3 (<1.0)	12p12.1–12q11	D12S358, D12S87	Female only
12	1.4 (<1.0)	12q13.3–12q23	D12S43, D12S338	Female hip
12	1.3 (<1.0)	12q13.3–12q23	D12S43, D12S338	Female only
13	<1.0 (—)	—	—	—
14	1.2 (<1.0)	14q24.3–14q32.2	D14S74, D14S51	Male hip
14	1.1 (<1.0)	14q24.3–14q32.2	D14S74, D14S51	Male only
15	<1.0 (—)	—	—	—
16	2.1 (1.3)	16p13.1–16q12.1	D16S407, D16S261	Female hip
16	1.7 (<1.0)	16p13.1–16q12.1	D16S407, D16S261	Female only
16	1.1 (<1.0)	16p13.1–16q12.1	D16S407, D16S261	Hip only
16	2.0 (1.2)	16q21–16q23	D16S265, D16S289	Female only
17	<1.0 (—)	—	—	—
18	1.1 (<1.0)	18p11.32–18p11.1	D18S63, D18S53	Female hip
19	<1.0 (—)	—	—	—
20	<1.0 (—)	—	—	—
21	<1.0 (—)	—	—	—
22	<1.0 (—)	—	—	—

[a] $\log_7 = 0.8$ deducted from the original MLS values.

bor susceptibility loci for osteoarthritis. Stratification increases the level of genetic homogeneity and can therefore assist in the mapping of loci for complex traits. Our analysis highlights the potential utility of this approach for osteoarthritis.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

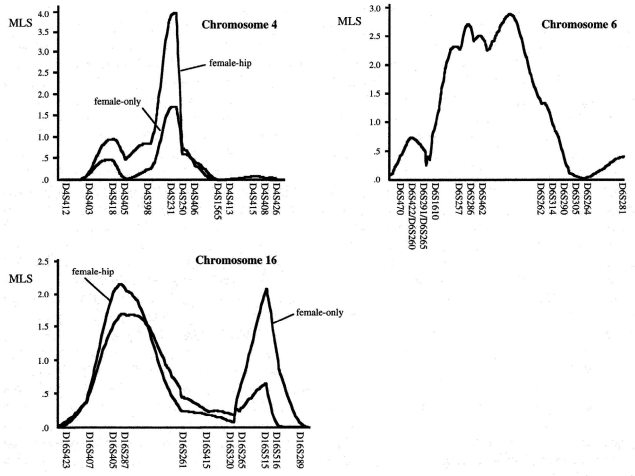

Figure 1 Multipoint analysis. *A*, Chromosome 4, female hip ($n = 85$ families) and female only ($n = 132$ families). *B*, Chromosome 6, hip only ($n = 194$ families). *C*, Chromosome 16, female hip ($n = 85$ families) and female only ($n = 132$ families).

ASPEX directory, ftp://lahmed.stanford.edu/pub/aspex (for ASPEX software)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for osteoarthritis [MIM 165720])

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