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Lumbar disc disease shows linkage to chromosome 19 overlapping with a QTL for hand OA

F M K Williams,¹ B S Kato,¹ G Livshits,² P N Sambrook,³ T D Spector,¹ A J MacGregor⁴

ABSTRACT

Objective: Cervical and lumbar degenerative disc disease (CDD and LDD, respectively) form part of the spine osteoarthritis (OA) phenotype and are known to be influenced by genetic factors. A genome-wide linkage analysis was performed to identify new chromosomal regions of interest.

Methods: Dizygotic healthy female twin volunteers (n = 348) from the TwinsUK register who had magnetic resonance imaging scans 10 years ago coded for degenerative disease, were identified. Multipoint genome-wide linkage analysis was conducted using 737 highly polymorphic markers of approximate spacing 10 cM.

Results: The mean age of the twins was 52 years. Significant linkage peaks (log of the odds (LOD) >3) were identified for LDD at three chromosomal regions. These included chromosome 1 (position 285 cM), chromosome 5 (position 175 cM) and chromosome 19 (position 80 cM). The peak on chromosome 19 had LOD = 4.06, and the empirical p = 6.7×10^{-4} confirmed reliability of the linkage signal. It lies close to a linkage peak previously obtained by our group for hand OA.

Conclusions: This genome-wide linkage study of CDD and LDD shows evidence of linkage for LDD on chromosome 19. The region of interest is likely to harbour genes that are common to LDD and hand OA.

Degenerative disease of the spine is common in the general adult population, with prevalence estimates for lumbar disc disease (LDD) as high as 56%.¹ Prevalence of degenerative disease increases with age in both sexes. Environmental factors make a contribution to the pathology; smoking and heavy lifting, for example, are recognised risk factors for LDD. Yet such factors, either singly or in combination, have not been shown to confer a large risk. Family² and twin³ studies, however, give high estimates of heritability—over 70% for both LDD and cervical disc disease (CDD). A number of candidate genes have been investigated in LDD, including the vitamin D receptor gene, the collagen I, IX and XI genes (reviewed in Battie and Videman⁴) and, more recently, genes encoding inflammatory mediators,⁵ but in only a few cases have the genes been replicated convincingly. Less is known of the epidemiology of cervical disc disease (CDD), but genetic factors have been shown to contribute, and similar pathological processes are likely to be involved. To date, there have been no studies of individual genes in CDD. The strong evidence for genetic factors influencing degenerative disease led us to investigate this further using a genome-wide approach.

Recent genome-wide association data demonstrate that previous linkage scans have been a

reliable way to detect loci contributing to complex genetic traits.⁶ The aim of the present study was to look for genetic linkage to magnetic resonance imaging (MRI)-evaluated lumbar and cervical degenerative disease through a genome-wide linkage scan. We studied MR images from dizygotic twins from the TwinsUK registry that had been obtained during the earlier studies, which demonstrated significant heritability estimates for the two traits, CDD and LDD.³ These MR scans had been coded for degenerative change, and stored DNA samples were used to perform a genome-wide linkage scan. DZ twins offer particular advantages for the study of an age-related phenotype: not only are twins in a pair matched for age, but they are more closely matched for environmental effects than conventional sib-pairs.

METHODS

Healthy adult female twin subjects had been recruited from the TwinsUK cohort (<http://www.twinsuk.ac.uk>) for the previous study of heritability.³ In the present study, zygosity was confirmed by genotyping. The twins were neither selected nor excluded by history of back pain or disc disease. None had a history of spinal fractures or spine surgery. Ethics committee approval had been obtained, and the twins gave informed consent. MRI was performed in the subjects using a Siemens (Munich, Germany) 1.0-T superconducting magnet. Serial sagittal images of the cervical, thoraco-lumbar junction and lumbar spine (T9-L5) were obtained using a fast spin echo sequence of time to recovery/time to echo 5000–4500/112 ms, with a slice thickness of 4 mm. To minimise diurnal variation in disc height, all MRI scans were performed more than 1 h after subjects arose in the morning, with no exercise or supine rest allowed between rising and the scan. Members of each twin pair were scanned at the same appointment and on the same machine. The MRI films had been scored for features of disc disease on 2 occasions within 14 days by 2 radiologists and a rheumatologist, using a standardised atlas employing a 4-point grading scale for each of the following features: disc height, disc bulge, disc signal change, and anterior osteophytes. The mean intra-observer kappa was 0.66 for CDD and LDD, and an inter-observer kappa of 0.50 and 0.60, respectively. A cumulative degeneration score was constructed from the sum of scores of degenerative changes at each level for CDD and LDD, as previously reported.³

DNA was isolated from venous blood by a standard technique. Genotyping included 737 highly polymorphic markers, using standard

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fluorescence-based genotyping methodologies from the ABI Prism linkage mapping set (Applied Biosystems; performed by Gemini Genomics Plc) and ordered according to the combined linkage-physical map of the human genome.⁷ Spacing between markers was, on average, less than 10 cM. The estimated genotyping error was <1%. Analysis was carried out in Stata 9 using the continuous cumulative degeneration score. Multipoint genome-wide linkage analysis was conducted using generalised linear modelling based on optimal Haseman and Elston methods⁸ in which the square of the sibling difference in OA phenotype, adjusted for age, is regressed on the estimated proportion of alleles identical by descent (IBD). An empirical p value was estimated using a permutation approach in which 1000 permutations of the dataset were performed for each log of the odds (LOD) score, keeping IBD and family structure intact.

RESULTS

The mean age (range) of the DZ twins (n = 348) was 52 (45–72) years. The mean (standard deviation) height of twins was 162.0 (5.9) cm and mean weight 65.5 (11.4) kg. Questionnaire answers revealed that 76% of women were postmenopausal, 46% smoked regularly, either currently or previously (more than 10 cigarettes per day), and 13% had regularly consumed >10 units of alcohol per week currently or previously. Also, by questionnaire, the frequency of severe pain of duration >1 month at any stage in the subject's lifetime was 18% for low back and 10% for neck.

No significant peaks of linkage were obtained for CDD. For LDD, peaks with LOD>3 were identified at chromosomes 1, 5 and 19 (fig 1). The peaks on chromosomes 1 and 5 had LOD >6 and empirical p = 3.7×10^{-4} and p = 3.3×10^{-4} , respectively. The peak on chromosome 19 had LOD = 4.06 and empirical p = 6.7×10^{-4} , providing good evidence of linkage of LDD to this region. The marker that was closest to the peak was D19S902 at 76 cM (summary of findings, table 1).

Conclusions

Genetic factors account for over 70% of the variation in expression of CDD and LDD in normal healthy twin volunteers.¹ This analysis shows convincing evidence of LDD linkage at three chromosomal sites but no evidence of linkage for CDD.

This is the first linkage study to examine the genetic basis of MRI-defined degenerative disease in an unselected sample. Our findings contrast with those of a recently published family linkage study of symptomatic lumbar disc disease⁹ which demonstrated significant linkage to chromosome 21q. This Finnish study included a substantially different spectrum of disease compared with the present study: probands were ascertained through their presentation with sciatica, and family members were assessed through questionnaire ascertainment of symptoms and in most cases MRI scans. The reported linkage may reflect, therefore, several end-stage processes leading to disc protrusion and pain. Although in the present sample there was an association between reported lumbar and neck pain,¹⁰ the majority of twins had no back pain symptoms. Thus, the phenotype under study here reflects very early disease in subjects who are largely asymptomatic and may shed light on the genes involved in anatomical and pathological changes which take place early in the degenerative process.

The regions identified in the present study do not contain the candidate genes hitherto investigated in LDD. Of particular interest is our finding of a peak on chromosome 19, a region

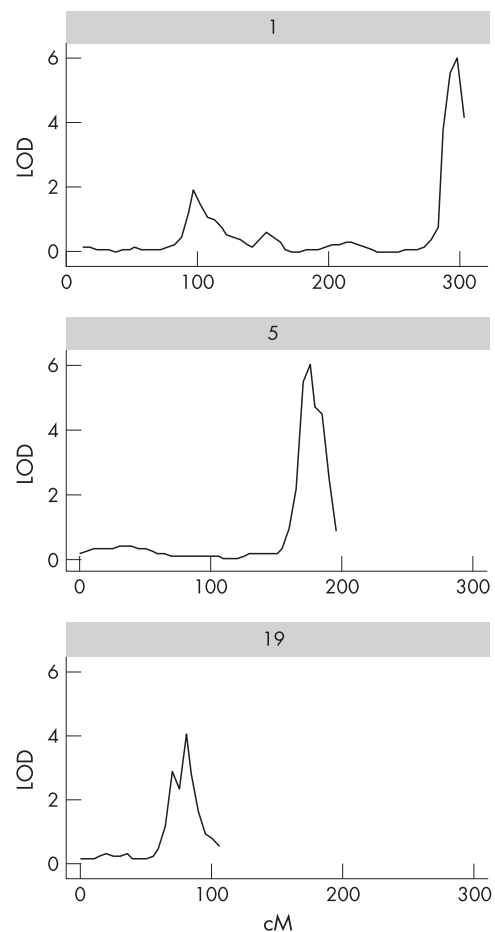


Figure 1 Linkage scan for degenerative disease score for lumbar spine on chromosomes 1, 5 and 19 multipoint linkage analysis results of age-adjusted degenerative change score for lumbar spine. cM, centiMorgan distance from p telomere.

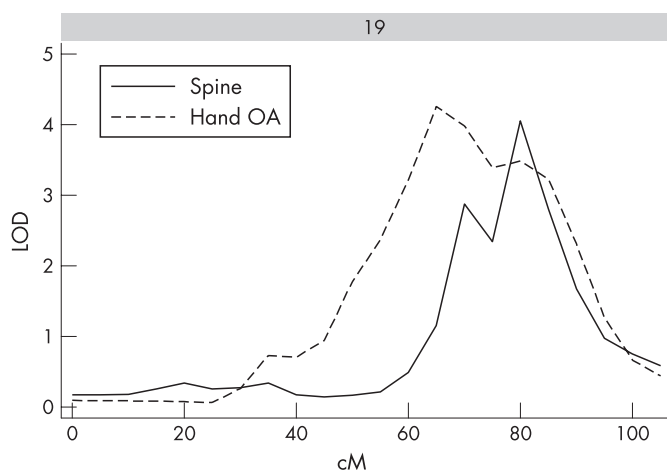
estimated to contain ~300 genes. A linkage peak at a similar position on chromosome 19 has been identified with hand OA in TwinsUK volunteers (fig 2).¹¹ The hand linkage study (n = 1028 DZ twins)¹¹ included 260 twins having MRI spine reported on in the present study. The hand phenotype used a composite score derived from assessment of distal- and proximal interphalangeal joints and carpometacarpal joints. Further support for these results comes from a similarly positioned peak on chromosome 19 in hand OA reported by the Framingham group.¹² It would appear that this region on chromosome 19 merits further investigation and is likely to contain genes of some importance in LDD and hand OA.

No peaks of interest were identified for CDD. This may be a reflection of relatively low statistical power, with CDD less prevalent than LDD in this sample. In addition, cervical discs are smaller than lumbar discs, so the resolution of the MRI images is not as good, and abnormalities may be less easy to identify accurately. The phenotypic correlation between degeneration scores in the lumbar and cervical spine in these data was 0.5, and the extent to which specific genes are expressed at these two sites may well differ. The site-specific variation in the reported linkages and associations for OA suggest considerable heterogeneity of the contribution of specific genes to the degenerative process.

The single-sex twin pair design has advantages over studies of ordinary sib-pairs, in that it eliminates intra-pair age differences

Table 1 Details of the most significant linkage peaks identified for lumbar disc disease

Chromosome	Peak LOD score	Flanking markers (± 1 LOD)	1 LOD support interval
1	6.05	D1S2842 and D1S2836	1q43.7–1q44.5
5	6.05	D5S422 and D5S429	5q34–5q35.1
19	4.06	D19S902 and D19S571	19q13.32–19q13.41

**Figure 2** Multipoint linkage for lumbar disc disease and hand osteoarthritis on chromosome 19 linkage peaks for lumbar disc disease (solid line) and hand osteoarthritis (broken line) on chromosome 19, with both phenotypes adjusted for age. cM, centiMorgan distance from p telomere.

and diminishes effects of the individual-specific random environment. Also, it is unaffected by the potential sex-specific metabolic pathways and genetic influences which are known to influence OA and are likely to affect disc degeneration. The expression of LDD in women is thought to lag behind men by approximately 10 years, but further differences in degenerative disc disease between the sexes have not, to our knowledge, been reported. Clearly, the results of this study pertain to women and cannot necessarily be extrapolated to men—it will be of interest to see whether findings are replicated in cohorts of male subjects.

Additional strengths of the present approach include the large sample size and the use of well-defined MRI phenotypes. MRI is regarded as the most sensitive imaging modality for detecting disc disease,¹⁵ and we obtained a very high intra-observer consistency of assessment.³ The same twin sample had been used to demonstrate that both phenotypes had a significant genetic component, and the phenotype distributions deviated from normality less than other OA traits. There is, to our knowledge, no evidence that degenerative disease of the spine is different in twins, and we have shown that twins and singletons are, in fact, similar for a number of disease and lifestyle traits including peripheral joint OA.¹⁴ While this analysis is based on cross-sectional data of degenerative disease, it may be that change over time has a greater influence on symptoms. To address this, longitudinal studies of the twin cohort using follow-up MRI scans are under way.

Degenerative disc disease overlaps with spinal OA and shares a number of features with peripheral OA. First, the pathology appears similar with loss of joint space/disc narrowing, endplate sclerosis and osteophyte formation. Second, they may have

genes in common (the vitamin D receptor gene, and the COX2 gene at the knee and the matrilin 3 gene in the hand). Finally, their rates of progression are not independent.¹⁵ Taken together, this evidence suggests that genetic factors predisposing to OA are likely, to some extent, to be shared between sites. The results presented here support this and suggest that the similarities between the spine and peripheral joint pathologies are founded, at least in part, on shared genetic factors.

This is the first linkage study of LDD in healthy volunteer subjects. The work throws the spotlight on an area of chromosome 19 as potentially harbouring genes responsible for determining the mechanisms that influence the degenerative process in both the spine and the hand. None of the peaks obtained in this linkage study contain the previous candidate genes, so it seems highly likely that the region contains novel candidate genes for degenerative change.

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Competing interests: None.

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