

CONCISE REPORTS

Association of two loci on chromosome 2q with nodal osteoarthritis

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Abstract

Objective—To search for genetic association between microsatellite marker loci and sibling pairs with nodal osteoarthritis (NOA).

Methods—Using the affected sibling pair method of analysis, genomic DNA from 66 sib pairs with NOA was analysed for association with highly polymorphic microsatellite marker loci. The microsatellite markers were amplified using polymerase chain reaction and typed on polyacrylamide gels.

Results—A significant association ($p < 0.05$) was identified between NOA and two loci on the short arm of chromosome 2 (2q 23–35). Candidate genes for osteoarthritis in this region include: fibronectin, a glycoprotein present in the extracellular matrix of normal cartilage; the $\alpha 2$ chain of collagen type V, a major constituent of bone; and the interleukin-8 receptor, important in the regulation of neutrophil activation and chemotaxis.

Conclusions—The chromosomal region 2q 23–35 requires further detailed study in NOA. Confirmation of these findings in large independent data sets and further analysis of candidate genes in this region will be important in unravelling the molecular basis for this common disease.

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Osteoarthritis (OA) is the most common condition to affect synovial joints; however, it is an ill understood condition, with a spectrum of clinical presentations and outcomes.¹ There is increasing acceptance that osteoarthritis may represent, not one specific disorder, but rather a series of disease subsets that lead to similar clinical and pathological alterations. The best recognised of these subsets is nodal generalised osteoarthritis (NOA), which is characterised by polyarticular interphalangeal and thumb base OA, Heberden's node formation, a preponderance in women, and early inflammatory onset.² It is in this subset of OA that a familial predisposition is best recognised. There are relatively few genetic studies of the subset, though the possibility of an association with the HLA system has been suggested.^{3 4}

A certain uniformity of cartilage response in OA suggests that some common pathway at the molecular or cellular level leads to the pro-

gressive degeneration of articular cartilage. The major constituents of articular cartilage include collagen types II, VI, IX, X, and XI, chondroitin sulphate proteoglycan (aggrecan), link proteins, and hyaluronic acid. Type II collagen, encoded by a gene on the long arm of chromosome 12, is the major collagenous protein of cartilage, and recent genetic studies in OA have concentrated on the role of this gene in the pathogenesis of OA.

Two Finnish families with multiarticular familial OA, and one family with mild OA and associated chondrodysplasia, have demonstrated linkage between the disease and type II collagen markers.^{5 6} Association analysis of nearly 100 OA and control individuals has shown a statistically significant clustering of a type II collagen marker haplotype in OA patients.⁷ Recently, however, no linkage or association was found between generalised OA and markers for type II collagen, cartilage link protein, or cartilage matrix protein, using sib pair analysis.⁸

As part of a wider genome search using microsatellite markers and the affected sib pair method of analysis, we analysed microsatellite polymorphisms from chromosome 2q in 66 affected sib pairs with NOA. The short arm of chromosome 2 (2q) contains a candidate gene for OA—the $\alpha 3$ chain of collagen type VI (COL6A3). This structural component of cartilage has been assigned to 2q 36–37.

Sib pairs are expected to share, on average, 50% of their genes as identical by descent from their parents. If affected sib pairs share a significantly increased proportion of genes at a particular marker locus, it suggests the region containing that marker is associated with disease susceptibility.

Patients and methods

Approval for the study was obtained from the local Research Ethics Committee. Consecutive patients with symptomatic NOA attending the OA clinic in the Rheumatology Unit in Nottingham were questioned concerning a family history of NOA. NOA was defined as the presence of polyarticular interphalangeal OA with Heberden's (with or without Bouchard's) nodes affecting at least two rays of each hand (in the absence of overt trauma), with or without large joint involvement. OA was confirmed radiographically in probands and defined as the presence of definite joint

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Heterozygosity and t statistics for microsatellite markers on chromosome 2q in sib pairs with nodal generalised osteoarthritis

Marker	Heterozygosity	Test statistic (t)	p
D2S111	0.81	1.25	
GCG	0.82	1.72	< 0.05
D2S326	0.86	1.74	< 0.05
D2S103	0.82	-0.16	
D2S118	0.79	-3.21	
D2S155	0.77	0.76	
D2S128	0.79	-0.66	
D2S126	0.82	1.64	< 0.05
D2S396	0.83	0.13	
D2S407	0.80	-0.52	
D2S125	0.82	-1.51	
D2S206	0.80	0.74	

space narrowing with evidence of bone response (osteophytes with or without sclerosis) on a standardised screen (hands, pelvis, standing anteroposterior knees, and lateral 30° flexion knees). Patients who reported live, geographically accessible affected siblings were invited to participate in the study and asked for permission to contact their siblings. Siblings were seen either in hospital or in their homes. Disease status was confirmed clinically according to hand involvement as defined above.

GENETIC MARKERS

Genomic DNA was extracted from peripheral blood leucocytes. Microsatellite marker loci (table) were amplified using polymerase chain reaction and typed on polyacrylamide gels by standard methods.⁹

STATISTICAL ANALYSIS

The affected sib pair method of linkage analysis was used to compute a *t* statistic that tested whether affected relatives shared alleles at the marker locus more often than would occur by chance. This *t* statistic based on allele sharing does not involve parental genotypes, and is weighted to permit the excessive sharing of rare alleles to outweigh the sharing of common alleles (the intermediate weighting function $\sqrt{1/p}$ was used, where *p* denotes the frequency of shared marker alleles).^{10 11} The analysis makes no assumptions about mode of inheritance or genotype penetrance, and is useful for disorders of late onset in which parental genotypes may be unavailable. This effectiveness, however, is offset by the fact that the *t* statistic is influenced by the numbers analysed and is therefore not a direct measure of the strength of linkage.

The computer program APM (Weeks and Lange) was used for analysis.^{10 11} The *t* statistic for large data sets (those with more than 20 families) has an approximately normal distribution and is interpreted in a one tailed test.

Results

Forty four families with two or more affected siblings were identified. Thirty three families had two and 11 families had three accessible affected siblings. A total of 99 individuals were studied (14 men and 85 women); 71 of them

had large joint OA and, of those, 65 had knee OA. The mean age was 65 years (range 44 to 84 years) and symptom duration was 17 years (range 1 to 48 years). There were 60.3% woman-woman pairs, 34.5% woman-man pairs, and 5.2% man-man pairs.

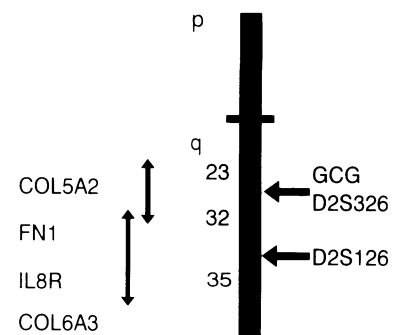
The table lists the heterozygosity values and one sided *p* value for the test statistic $\sqrt{1/p}$ for the microsatellite markers analysed in each of the 66 sib pairs. There was no association with markers flanking COL6A3 (D2S125 and D2S206); however, markers GCG and D2S326 on chromosome 2q 23-32 and marker D2S126 on 2q 33-35 gave an excess of sharing of alleles identical by descent (*p* < 0.05). We found no association of NOA with the HLA marker D6S260. No association was found between the $\alpha 1$ chain of collagen VI (COL6A1) marker, D21S171, or the human proteoglycan link protein marker, CRTLI.

Discussion

The results demonstrate an association between NOA and two regions on chromosome 2, 2q 23-32 and 2q 33-35. These regions contain three known genes that could be involved in the pathogenesis of OA. The markers GCG and D2S326 map to chromosomal location 2q 23-32, as does the gene for the $\alpha 2$ chain of collagen type V (COL5A2). The marker D2S126 maps to 2q 33-35, as do the genes for the $\alpha 1$ chain of fibronectin (FN1) and the interleukin-8 receptor (IL8R) (figure).

Experimental work suggests that repetitive high stresses resulting from impulsive loading provide a mechanism for joint degeneration that may explain the preferential involvement of the distal interphalangeal joints in NOA.^{12 13} Recent reports suggest that increased bone mass, especially of subchondral bone, may result in increased mechanical stress on cartilage during joint loading, and that this stiff bone transmits greater force to overlying cartilage, making it more vulnerable.¹⁴ Mutations in COL5A2, a major constituent of bone, could therefore be implicated in the pathogenesis of NOA.

Fibronectin is a multifunctional glycoprotein present in low levels in the extracellular matrix of normal cartilage. In OA, fibronectin content is markedly increased in the altered matrix



Stylised diagram of chromosome 2, showing relative position of microsatellite markers (GCG, D2S326, D2S126) and putative candidate genes (COL5A2, FN1, IL8R, COL6A3) (see text for further details)

because of an increased synthesis by the chondrocytes and accumulation in the extracellular matrix. At least a proportion of the fibronectins synthesised in degenerated cartilage are composed of isoforms more sensitive to proteolytic cleavage that are absent from normal cartilage.¹⁵ Whether fibronectin acts as a deleterious or repair agent in OA is unknown.

The IL8R gene has been assigned to 2q 35. Interleukin-8 (IL-8), a cytokine produced by monocytes, T cells, and fibroblasts, is involved with neutrophil activation and chemotaxis. As there is documented evidence for an inflammatory component in NOA,² abnormalities in the IL8R gene could influence the inflammatory process if genetically susceptible individuals were to suffer an environmental insult. There may of course be other, as yet unidentified, genes in this region which could be associated with NOA.

In our study group, no association was found between the microsatellite D6S260 that lies 10 cM from the HLA system and NOA, suggesting that the HLA system does not have a significant effect on the pathogenesis of NOA.

In monogenic disease, a p value < 0.001 is required to confirm linkage. This criterion may be viewed as too strict for complex disease, as the disorder may be the result of interaction between many different loci, each with a variable degree of effect on the disease. No genetic region can be completely excluded unless all cases of the disease can be accounted for by all currently known genes. Therefore, a significant association at a locus cannot be ignored.

Our results suggest that the chromosomal region 2q 23–35 requires further detailed study

in patients with NOA. Confirmation of our findings in large independent data sets, and analysis of candidate genes in this region for mutations in patients with NOA will be important in unravelling the molecular basis for this common disease.

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