Linkage analysis of chromosome 2q in osteoarthritis


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Abstract

Background. In independent linkage studies chromosome 2q11-q24 and chromosome 2q23–35 have previously been implicated as regions potentially harbouring susceptibility loci for osteoarthritis (OA).

Objective. To test chromosome 2q for linkage to idiopathic osteoarthritis.

Methods. Using a cohort of 481 OA families that each contained at least one affected sibling pair with severe end-stage disease (ascertained by hip or knee joint replacement surgery), we conducted a linkage analysis of chromosome 2q using 16 polymorphic microsatellite markers at an average spacing of one marker every 8.5 cM.

Results. Our results provide suggestive evidence for a locus at 2q31 with a maximum multipoint logarithm of the odds score (MLS) of 1.22 which increased to 2.19 in those families concordant for hip-only disease (n=311). This suggestive linkage was greater in male-hip families (MLS = 1.57, n = 71) than in female-hip families (MLS = 0.71, n = 132).

Conclusions. Chromosome 2q is likely to contain at least one susceptibility locus for OA.

Idiopathic osteoarthritis (OA) is a debilitating joint disease involving focal cartilage loss [1]. Evidence has accumulated that OA has a major genetic component with twin studies estimating that the heritability of radiographic OA of the hand, knee and hip ranges from 36 to 68% [2]. First-degree relatives of individuals who have had hip or knee replacement for OA have been estimated to have up to a 2.3-fold increased risk of developing the disease [3]. These and similar findings [4, 5] have prompted researchers to attempt the systematic identification of OA susceptibility loci. Two groups have previously reported linkage of OA to chromosome 2q in families containing affected sibling pairs [6, 7]. These studies consisted of a relatively small number of families (44 and 27) and the linkages reported showed only slight overlap, with one located at 2q23-q35 and the other at 2q11-q24. As part of a two-stage genome screen for OA susceptibility loci using affected sibling pairs [8], we noted one marker on chromosome 2q (D2S202, 2q31) that demonstrated suggestive evidence of linkage in a first stage of 297 families, with the evidence in favour increasing when an additional 184 families were genotyped. In light of these findings we decided to analyse this chromosome in more detail.

Patients and methods

Affected sibling pairs
Families with at least two siblings who had undergone one or more replacements of the total hip, or of the total knee, or both, for primary idiopathic OA, were recruited. A detailed description of the families has been published previously [8].

Markers and genotyping
The microsatellite markers used to provide coverage of chromosome 2q were obtained from the Genome Database (http://www.hgmp.mrc.ac.uk/gdb/gdbtop.html) or from the ABI Prism Linkage Mapping Set (Version 2, PE Applied Biosystems, http://www.perkin-eler.com/ab). The markers were amplified with either the forward or the reverse primer in a polymerase chain reaction (PCR) pair fluorescently labelled. The amplification products were electrophoresed through 6% acrylamide using an Applied Biosystems 377 Automated
These 16 markers were then genotyped in an additional 184 families. In this second stage, D2S202 had an increased evidence for linkage at this marker. As for D2S202, increasing the number of families had therefore increased the evidence for linkage at this marker. Although D2S202 was the only marker on chromosome 2 that had a P-value < 0.05 in stage 1, one other chromosome 2 marker had a P-value < 0.10 in this stage: D2S72 (P = 0.07). This marker is approximately 2 cM distal to D2S202. When D2S72 was genotyped in stage 2 there was further evidence for linkage with a P-value of 0.024. When screens 1 and 2 were combined a P-value of 0.0086 (LOD = 1.23) was obtained for D2S72. As for D2S202, increasing the number of families had increased the evidence for linkage at this marker. The LOD scores for D2S202 and D2S72 were robust to misspecification of allele frequencies (data not shown).

**Linkage and stratification analysis**

Error checking of the data, single-point linkage analysis (using the ANALYZE package), multipoint linkage analysis (using the ASPEX program) and stratification [by sex, by joint replaced (hip or knee) and by sex combined with joint replaced] were performed as described previously [8].

**Results**

**Genome screen**

As mentioned in the Introduction, we have performed a genome-wide screen for OA susceptibility loci [8]. The linkage analysis strategy was to genotype a sparse map of 272 microsatellite markers in a first stage containing 297 of our 481 families. Any marker that had a nominal P-value ≤ 0.05 in stage 1 would then be examined in the remaining 184 families (stage 2). The aim of this strategy was to take through to stage 2 only those markers that demonstrated reasonable evidence of linkage [10]. In stage 2 we were not necessarily expecting to repeat any positive linkage results of stage 1 but instead were looking for further evidence of linkage, even if only moderate: only if a marker’s P-value for stages 1 and 2 combined was less than or equal to the P-value for stage 1 would this support linkage at that marker. Sixteen markers from stage 1 had nominal P ≤ 0.05, including marker D2S202 from chromosome 2q [P = 0.036, logarithm of the odds (LOD) = 0.70]. These 16 markers were then genotyped in an additional 184 families. In this second stage D2S202 had a P-value of 0.07 (LOD = 0.49). When the data for stages 1 and 2 were combined and compared with stage 1 only, the combined P-value had decreased and the LOD score had increased for D2S202 [P = 0.009 (LOD = 1.21) for the combined stages vs 0.036 (LOD = 0.70) for stage 1 only]. Increasing the number of families had therefore increased the evidence for linkage at this marker. Although D2S202 was the only marker on chromosome 2 that had a P-value ≤ 0.05 in stage 1, one other chromosome 2 marker had a P-value ≤ 0.10 in this stage: D2S72 (P = 0.07). This marker is approximately 2 cM distal to D2S202. When D2S72 was genotyped in stage 2 there was further evidence for linkage with a P-value of 0.024. When screens 1 and 2 were combined a P-value of 0.0086 (LOD = 1.23) was obtained for D2S72. As for D2S202, increasing the number of families had increased the evidence for linkage at this marker. The LOD scores for D2S202 and D2S72 were robust to misspecification of allele frequencies (data not shown).

**Finer mapping of 2q**

The evidence of suggestive linkage for two adjacent markers at P < 0.01, combined with the previous reports of linkage of OA to 2q, prompted us to genotype the stage 2 families for seven other chromosome 2q markers from our original marker set that flanked D2S202 and D2S72. In addition we genotyped seven new chromosome 2q markers for all 481 families. Multipoint analysis gave a maximum multipoint LOD score (MLS) of 1.22 near to marker D2S72 (Fig. 1).

As pointed out above, evidence for linkage of OA to 2q has been reported. Wright et al. [6] reported a linkage analysis of 12 markers in 44 families. Their 12 markers encompass over 65 cM of 2q and three were linked at P ≤ 0.05: GCG, D2S326 and D2S126. These three markers flank our suggestive linkage at D2S202 and D2S72: GCG and D2S326 are at least 20 cM proximal whilst D2S126 is approximately 25 cM distal. D2S326 was the only one of the 12 markers reported in Wright et al. [6] that was also used in our study. In our 481 families this marker had a LOD score of 0.51 (P = 0.062). Although not significant at P ≤ 0.05 this result is tending towards significance and may represent an independent confirmation of the linkage reported by Wright et al. [6]. Leppavuori et al. [7] reported linkage of OA to 2q in 27 families with a parametric pairwise LOD score of 2.34 at the IL1R1 locus (2q12-q13). This is at least 70 cM proximal to our suggestive linkage peak at D2S72, which maps to 2q31. One of our chromosome 2 markers maps to 2q12-q14.2: D2S160. In our 481 families this marker had a LOD score of 0.67 (P = 0.04). This result may also represent an independent confirmation of the linkage reported by Leppavuori et al. [7].

Overall, our single-point and multipoint analyses provide suggestive evidence for linkage of OA to chromosome 2q. This is the third report indicating that 2q may harbour an OA susceptibility gene.

**Stratification**

Epidemiological, segregation and twin pair studies have suggested that the genetic contribution to OA differs...
between males and females [1, 4, 11]. Furthermore, differences in the heritability values between joint groups have been reported [3, 12, 13]. Overall, these studies have contributed to the hypothesis that there may be genetic heterogeneity of OA between the sexes and between different joint groups. We therefore stratified our results for chromosome 2q by six strata: those families that were affected females-only (196 families), affected males-only (102 families), hips-only (male and/or female) (311 families), knees-only (male and/or female) (54 families), affected females-only who had undergone hip replacement but not knee replacement (female-hip pairs) (132 families) and affected males-only who had undergone hip replacement but not knee replacement (male-hip pairs) (71 families). We did not stratify for female-knee or male-knee as the number of families was too low (21 and eight, respectively) to allow reliable inference of linkage.

The following MLS values were obtained for the six strata tested: 0.55 for females-only between D2S2330 and D2S326, and 0.96 for males-only between D2S117 and D2S202 (Fig. 2A and Table 1); 2.19 for hips-only between D2S117 and D2S202, and <0.50 for knees-only near to D2S364 (Fig. 2B), 0.71 for female-hips between D2S325 and D2S157, and 1.57 for male-hips between D2S117 and D2S202 (Fig. 2C). These results suggest that hips-only pairs are the major contributing strata to the suggestive linkage on 2q. There were much greater numbers of hips-only pairs than knees-only pairs (311 vs 54) and this could account for our inability to detect linkage in the knees-only strata. However, when the unstratified multipoint plot and the hips-only multipoint plots are compared (Figs 1 and 2B), the hips-only

**Table 1. Stratified maximum MLS**

<table>
<thead>
<tr>
<th>Strata</th>
<th>MLS</th>
<th>Corrected MLS</th>
<th>Between markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females-only</td>
<td>0.55</td>
<td>0.00</td>
<td>D2S2330 and D2S326</td>
</tr>
<tr>
<td>Males-only</td>
<td>0.96</td>
<td>0.18</td>
<td>D2S117 and D2S202</td>
</tr>
<tr>
<td>Hips-only</td>
<td>2.19</td>
<td>1.41</td>
<td>D2S117 and D2S202</td>
</tr>
<tr>
<td>Knees-only</td>
<td>&lt;0.50</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>Female-hips</td>
<td>0.71</td>
<td>0.00</td>
<td>D2S325 and D2S157</td>
</tr>
<tr>
<td>Male-hips</td>
<td>1.57</td>
<td>0.79</td>
<td>D2S117 and D2S202</td>
</tr>
</tbody>
</table>

*Log 6 = 0.78 deducted from the original MLS values to account for the six stratification tests performed [14].

![Multipoint analysis of chromosome 2q with the data stratified.](image-url)

Fig. 2. Multipoint analysis of chromosome 2q with the data stratified. (A) Females-only (n = 196 families) and males-only (n = 102). (B) Hips-only (n = 311) and knees-only (n = 54). (C) Female-hips (n = 132) and male-hips (n = 71).
stratum is more significant across the chromosome. This suggests that the linkage in the unstratified families is being obscured, presumably due to the presence of knees-only pairs, or hip and knee pairs. Of our 481 families, 65 pairs were of mixed joint replacement status (one sibling had undergone hip replacement only, the second sibling knee replacement only). These 65 mixed joint replacement pairs were not one of the original six strata tested. The highest MLS in these 65 pairs was 0.41 between D2S335 and D2S326; there was no peak between D2S117 and D2S202 (data not shown). These results suggest that the linkage on 2q is principally accounted for by hip disease.

The MLS in male-hips was 1.57 whereas the MLS in female-hips was 0.71 (Fig. 2C). There was a greater number of female-hip pairs than male-hip pairs (132 vs 71) so a lack of power in the female-hips cannot account for the apparent stronger linkage in the male-hips.

In conclusion, this stratification analysis indicates that the suggestive linkage to 2q is principally accounted for by hip-only disease and that this hip-only linkage may be more pronounced in males than females.

Nodal OA

The families used by Wright et al. [6] and Leppavuori et al. [7] were ascertained by OA of the hand. Of our 481 families, 46 affected pairs (from 46 families) were concordant for the presence of three or more Heberden’s nodes. When these families were analysed, none of the 16 chromosome 2 markers were significant at \( P \leq 0.05 \), with the lowest \( P \)-value being 0.13 for D2S72. The MLS was 0.19 between D2S139 and D2S160 (data not shown). Our results do not therefore support a restriction of the suggestive 2q locus to hand OA.

Discussion

Using affected sibling pair analysis we have obtained evidence for suggestive linkage of OA to chromosome 2q with a MLS of 1.22. There are three factors that make our analysis of 2q noteworthy: (i) markers D2S202 and D2S72 both had \( P \)-values \( \leq 0.07 \) in each of the two stages that constituted our original genome screen; (ii) 2q has been implicated as potentially harbouring OA susceptibility loci in other studies; and (iii) stratification analysis of our data highlighted differences in the six strata tested and generated a MLS of 2.19 in the hip-only stratum.

The factor that two adjacent markers supported moderate linkage in both stages suggests that our result for 2q is robust: increasing the number of families genotyped increased the evidence for linkage at these markers.

For genes that have only moderate effects, independent confirmation is essential to confirm true as opposed to false positive susceptibility status. When our data are considered in the context of the reports of Wright et al. [6] and Leppavuori et al. [7], there are reasonable grounds for assuming that 2q may contain an OA susceptibility locus.

Since there is evidence to suggest that the heritability of OA varies between the sexes and between different joint groups, these strata merited independent analysis. Stratification of our 2q data highlighted a substantial difference between families that have hip OA and those that have knee OA. These results, if confirmed in additional studies, could provide biological clues as to the nature of the suggestive susceptibility on 2q which might assist in the choosing of candidate genes.

The average density of the 2q markers used is one marker every 8.5 cM but these markers are not equally distributed. The largest gap is 23 cM, between D2S139 and D2S160. A finer linkage map of this chromosome is therefore important to quantify the nature of the susceptibility on this chromosome more accurately and to determine whether the troughs that are clearly evident in the multipoint analyses are real or simply reflect lower marker density, a lack of power to detect linkage in these regions, or both. If real, these may indicate that this chromosome harbours more than one OA susceptibility locus.

As pointed out by Leppavuori et al. [7], 2q12-q22 contains several interleukin and interleukin-associated genes. The expression of certain interleukin genes, including IL-1β, is altered in OA joint tissue [15]. Furthermore, interleukins regulate a number of enzymes that degrade the cartilage extracellular matrix, including metalloproteinases [16]. The interleukin cluster is therefore a logical candidate for OA susceptibility.

Although OA is primarily characterized by the degeneration of articular cartilage, one finding that is commonly observed is an increase in the density and mass of the subchondral bone below the articulating cartilage [17]. This has led to the suggestion that increased bone mass precedes any gross changes and that cartilage loss is secondary to this change. Under this assumption, genes that are determinants of bone integrity or bone mass can be considered candidates for OA susceptibility loci. Type V collagen is a minor constituent of bone where it forms heterotypic fibrils with the major bone collagen, type I. The type V collagen gene COL5A2 maps within 1 cM of D2S152. Although this section of 2q is part of a trough between two of our linkage grounds for assuming that 2q may contain an OA susceptibility locus.

The parathyroid hormone receptor 2 (PTHrR2) gene is located approximately 6 cM distal to D2S202/D2S72 at 2q33 [18]. PTHrR2 is predominantly expressed in the brain where it is thought to bind parathyroid hormone [19]. Parathyroid hormone is a regulator of calcium and phosphate homeostasis and is therefore important for bone integrity. PTHrR2 should therefore also be considered as a candidate.

Overall, the results presented here, together with those reported previously, indicate that an OA susceptibility locus may reside on chromosome 2q. With an MLS of 2.19 in our hip pairs, this linkage should be described as suggestive at this stage. More detailed linkage analysis with additional markers and additional families, combined with association analysis, will define this locus further.
Acknowledgements

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References