Short reports

Linkage mapping of a large Colombian family segregating for X linked retinoschisis: refinement of the chromosomal location

Barkur S Shastry, James F Hejtmancik, Alvaro Rodriguez, Francisco Rodriguez, Marta L Tamayo

Abstract

Juvenile X linked retinoschisis (RS) is a bilateral vitreoretinal dystrophy that develops early in life. Previous linkage studies have localised the RS gene to Xp22.1-p22.3 between DXS207 and AFM 291WF5, which represents a genetic distance of approximately 3.7 cM. In an effort to facilitate the eventual cloning of the RS gene, we have analysed a large Colombian family, using 10 microsatellite markers that have been mapped to the region Xp22.1-p22.3. A total of 93 members, including 19 affected and eight unaffected males, two affected females, and six obligate carrier females were analysed. Close linkage was observed between the disease locus and DXS999 (Z_{max}=2.27, \theta_{max}=0.05), DXS987 (Z_{max}=2.61, \theta_{max}=0.1), DXS443 (Z_{max}=4.23, \theta_{max}=0.1), and DXS274 (Z_{max}=3.49, \theta_{max}=0.05) markers. Recombination with the RS locus was found for all marker loci except DXS197, DXS43, and DXS1195. These results place the RS locus within an interval of approximately 2 cM between the flanking markers DXS1053 and DXS999, approximately 1.7 cM closer than the previously reported boundary. The results also further confirm the lack of genetic heterogeneity of RS. (J Med Genet 1997;34:504-506)

Keywords: retinoschisis; X linked; linkage

Juvenile retinoschisis (RS) is a recessively inherited X linked eye disorder which results in poor visual acuity. The disorder develops early in life and is characterised by variable acuity, vitreous veil, cartwheel, splitting of nerve fibre layer, and vitreous degeneration.\(^4\)\(^5\) It has a high degree of penetrance and most affected subjects are males. The majority of female carriers have normal vision and cannot be identified by clinical signs alone,\(^4\) although a small proportion of heterozygous females can be detected by abnormal rod-cone interactions.\(^4\)\(^6\) The progression of the disease is from mild to moderately severe and can, if complicated by retinal detachment and vitreous haemorrhage, ultimately lead to blindness. Although foveal schisis is the most common abnormality, there is a wide phenotypic variation and the condition often appears without any family history. The biochemical defects underlying RS are unknown, but histopathological\(^7\) and electrophysiological studies have suggested the involvement of Muller cells.

Previous linkage studies have localised the RS gene to the short arm of the X chromosome with close linkage to the markers DXS207 and DXS274.\(^8\)\(^9\) We previously reported\(^1\) linkage studies on four families of different geographical origins using microsatellite markers; no evidence for locus heterogeneity was found for RS. To facilitate the eventual cloning of the RS gene we have studied a new Colombian family using 10 microsatellite markers located close to the RS gene.

Members of all families were given complete ophthalmological examinations by two of us (A and F Rodriguez). We collected 20 ml venous blood from several affected and unaffected subjects and high molecular weight DNA was extracted. All oligonucleotide primers were synthesised commercially with the primer sequences obtained from published articles. Genomic DNA from several affected and unaffected family members was amplified by the polymerase chain reaction (PCR). The reaction conditions were 30 cycles of 1.5 minutes at 94°C, one minute at 60°C, two minutes at 72°C in a buffer containing 100 ng genomic DNA, thermostable Taq polymerase (2-4 units), 50 \mu M each of four deoxynucleotides, 1 \muCi of \(\alpha\)-\(^32\)P dATP, 10 pmol each of the primer, 1.5 mmol/l MgCl\(_2\), and 10 mmol/l tris-HCl, pH 8.3. The PCR products were separated by electrophoresis on a 9% acrylamide gel and visualised by autoradiography. Two point linkage analysis was carried out using the LINKAGE program package.\(^10\) For all analyses, penetrance used for the disease allele is 0.999 and the gene frequency is 0.0002.\(^11\)
The pedigree used for the linkage analysis contains 27 affected males, four affected females, and 23 obligate carriers with five consanguineous and several multiple marriages (family is not shown). A total of 93 members, including 19 affected and eight unaffected males, two affected females, and six obligate carrier females were analysed. The segregation of PCR alleles identified with 10 microsatellite markers is shown below these subjects (fig 1; the rest of the subjects are not shown in the figure). Obligate recombinations between retinoschisis and DXS987 are seen in three unaffected subjects: IV.14, V.1, and V.4. DXS207 and DXS1053 also show obligate recombination with retinoschisis in two of these unaffected subjects, V.1 and V.4. Markers DXS197, DXS43, and DXS1195 show a single allele for all people tested. In spite of this, they show small positive lod scores owing to the preponderance of affected subjects inheriting this allele from mothers whose DNA was not available for testing. DXS999 shows an obligate recombination with retinoschisis in affected subject IV.23. DXS443 shows obligate recombination in unaffected subjects IV.14 and V.4, while DXS365 shows obligate recombination in V.4 and DXS274 shows an obligate recombination event in IV.14. Both affected females, IV.8 and V.8, are the offspring of affected fathers, one in a consanguineous mating. Both are homozygous for all markers throughout the region, consistent with homozygosity for the retinoschisis mutation.

Results of two point linkage analysis for the disease locus and the 10 markers are presented in table 1. Four markers gave significant lod scores. A lod score of 2.27 at recombination fraction 0.05 was obtained with the marker DXS999, while DXS443 yielded a value of 4.23 at a recombination fraction of 0.10. DXS274 gave a maximum lod score of 3.49 at a recombination fraction of 0.05 and DXS987 gave a maximum lod score of 2.61 at a recombination fraction of 0.10. Recombination with the RS locus was found for all marker loci except DXS197, DXS43, and DXS1195. Based on the recombination events observed and taking into account the most recent CEPH

Table 1 Pairwise linkage results

<table>
<thead>
<tr>
<th>Marker</th>
<th>0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Zmax</th>
<th>Omega_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS987</td>
<td>-2.08</td>
<td>0.56</td>
<td>2.17</td>
<td>2.61</td>
<td>2.51</td>
<td>1.91</td>
<td>1.04</td>
<td>2.67</td>
<td>0.13</td>
</tr>
<tr>
<td>DXS207</td>
<td>-1.37</td>
<td>0.37</td>
<td>1.43</td>
<td>1.71</td>
<td>1.61</td>
<td>1.19</td>
<td>0.63</td>
<td>1.73</td>
<td>0.126</td>
</tr>
<tr>
<td>DXS1053</td>
<td>-2.62</td>
<td>-0.84</td>
<td>0.27</td>
<td>0.61</td>
<td>0.71</td>
<td>0.56</td>
<td>0.31</td>
<td>0.72</td>
<td>0.17</td>
</tr>
<tr>
<td>DXS197</td>
<td>0.76</td>
<td>0.74</td>
<td>0.66</td>
<td>0.56</td>
<td>0.38</td>
<td>0.22</td>
<td>0.10</td>
<td>0.76</td>
<td>0</td>
</tr>
<tr>
<td>DXS43</td>
<td>1.35</td>
<td>1.32</td>
<td>1.20</td>
<td>1.04</td>
<td>0.72</td>
<td>0.44</td>
<td>0.19</td>
<td>1.35</td>
<td>0</td>
</tr>
<tr>
<td>DXS1195</td>
<td>1.13</td>
<td>1.10</td>
<td>0.98</td>
<td>0.85</td>
<td>0.58</td>
<td>0.35</td>
<td>0.15</td>
<td>1.13</td>
<td>0</td>
</tr>
<tr>
<td>DXS999</td>
<td>1.03</td>
<td>1.86</td>
<td>2.27</td>
<td>2.26</td>
<td>1.90</td>
<td>0.74</td>
<td>0.22</td>
<td>2.29</td>
<td>0.07</td>
</tr>
<tr>
<td>DXS443</td>
<td>1.35</td>
<td>3.32</td>
<td>4.21</td>
<td>4.23</td>
<td>3.58</td>
<td>2.57</td>
<td>1.33</td>
<td>4.28</td>
<td>0.07</td>
</tr>
<tr>
<td>DXS365</td>
<td>0.26</td>
<td>1.25</td>
<td>1.74</td>
<td>1.79</td>
<td>1.54</td>
<td>1.09</td>
<td>0.56</td>
<td>1.79</td>
<td>0.10</td>
</tr>
<tr>
<td>DXS274</td>
<td>2.17</td>
<td>3.13</td>
<td>3.49</td>
<td>3.37</td>
<td>2.76</td>
<td>1.94</td>
<td>1.01</td>
<td>3.49</td>
<td>0.05</td>
</tr>
</tbody>
</table>
family data and map distance, the most likely location for RS is (DXS987, DXS207, DXS1053, DXS43, DXS1195)-RS-DXS999-DXS443-DXS365-DXS274. Since the genetic distance between markers DXS207 and DXS999 has been estimated as 2 cM, these results place the RS locus to within an interval of approximately 2 cM, about 1.7 cM closer than the previously reported boundary of the candidate region for the disease. RS belongs to the still poorly defined group of vitreoretinal dystrophies with no known cure. The disease locus has been previously mapped to the short arm of the X chromosome. In the present study we have reduced the position of the RS locus to an interval of approximately 2 cM, about 1.7 cM closer than the previous boundary. Identification of recombinant events between RS and the markers which were studied places the RS gene between markers DXS1053 and DXS999. These results improve the feasibility of cloning the RS gene. In addition, the closely linked flanking markers (DXS443 is most informative in this family and detected homozygosity in two affected females) will be of significant value in the molecular genetic diagnosis of the RS carriers and homozygotes, since carriers of this disease cannot be identified by clinical means alone. Results of the study will also help in counselling patients regarding their genetic risks for development of the disorder. The data presented above also support the proposition that RS is genetically a homogenous disease. Future studies on cloning and characterisation of a candidate gene may provide valuable insight into the mechanisms underlying the pathogenesis of RS.

Note added in proof

While this paper was in press, an article by George et al (J Med Genet 1996;33:919-22) reported that RS is located between DXS43 and DXS999, which is consistent with the present report.

We thank the members of the Colombian family who donated blood samples for this study. We would also like to thank Drs F J Giblin and J Bernal for critically reading the manuscript. Our appreciation also to G Keyeuex, C Duran, C Rodas and R Gomez for laboratory work and other members from Fundacion Oftalmologica Nacional and Instituto de Genetica Humana in Bogota, Colombia for their valuable field work. The above work was supported in part by a grant from the National Eye Institute (EY05230); in Colombia by a grant from Colciencias (cod-6207-04-322-95).