Genetic mapping of X-linked ocular albinism: linkage analysis in British families

S J Charles, A T Moore, J R W Yates

Abstract
Genetic linkage studies were performed in 16 British families affected by X-linked ocular albinism (XLOA) using RFLPs from the Xp22.3 region. Linkage was confirmed between the XLOA locus (OAI) and the loci DXS143 (dic56; Zmax = 15.90 at \( \theta = 0.0 \), confidence interval (CI) 0-0.035), DXS85 (782; Zmax = 15.67 at \( \theta = 0.04 \), CI = 0.007-0.11), and DXS237 (GMGX9; Zmax = 12.65 at \( \theta = 0.08 \), CI = 0.03-0.17). Multipoint linkage analysis placed OAI between DXS85 (782) and DXS237 (GMGX9) with odds exceeding 10^4 to 1 to give the map DXS85-OAI-DXS143-DXS237-XG-Xpter. OAI lies close to DXS143 (dic56) but in the absence of recombinants the order of these loci could not be determined.

X-linked ocular albinism (XLOA) is a cause of reduced visual acuity and nystagmus in males and is associated with refractive errors, strabismus, iris translucency, fundus hypopigmentation, and foveal hypoplasia. The effects of the mutant gene are not confined to the eyes: visual evoked potentials show evidence of abnormal neuronal crossing at the optic chiasm and, although skin and hair pigmentation is normal, skin histology shows abnormal giant melanosomes termed macromelanosomes in all affected males.

The vision of carrier females is normal, but ophthalmic examination shows a characteristic 'mud splattered' fundus appearance. Carriers may occasionally have a normal ophthalmic examination and several carriers are described with reduced visual acuity and nystagmus similar to an affected male. Since we have previously reported that 74% of obligate carriers have iris translucency and 84% have macromelanosomes on skin biopsy, but the most reliable sign of the carrier state is the characteristic 'mud splattered' fundus appearance with hyperpigmented streaks in the periphery seen in 92% of obligate carriers.

The XLOA gene locus (OAI) was first localised to distal Xp by linkage to the Xg blood group. Tight linkage has been reported to DXS85 (782), and other studies have shown linkage to markers in Xp22.3: DXS237, DXS278, DXS143, DXS452. A family has been reported with X-linked ichthyosis and XLOA, associated with a 3 Mb deletion encompassing the steroid sulphatase gene and DXS237, but not DXS143. This would be compatible with placing OAI distal to DXS143, but not with another report placing OAI between DXS143 and DXS85. The precise positioning of OAI has therefore remained controversial.

In this study we report linkage analysis on 16 British families affected by XLOA using the DNA markers DXS237 (GMGX9), DXS143 (dic56), and DXS85 (782).

Patients and methods

Patients
Sixteen multigeneration British families affected by XLOA were systematically assessed by an ophthalmologist (SJ). Within each kindred all available family members had a full ophthalmic examination, including assessment of best corrected visual acuity, ocular movements, colour vision (Ishihara and City University plates), slit lamp examination to assess iris translucency, and ophthalmoscopy. Where possible a skin biopsy was performed in each adult.

Primary diagnostic criteria for affected males were reduced visual acuity, iris translucency, hypopigmented fundi, macromelanosomes on skin biopsy, and a family history consistent with X-linked inheritance. In all families at least one male met these criteria. Obligate carriers were diagnosed on the basis of having an affected father, or having an affected son plus an affected brother or other maternal male relative. Females at risk of having inherited the XLOA gene were only diagnosed as carriers and included in linkage analysis if the 'mud-splattered' fundus appearance with peripheral linear hyperpigmented streaks was present, with or without iris translucency. At risk females with an entirely normal ophthalmic examination were included as unaffected in the linkage analysis, but if ophthalmoscopy showed mild retinal pigment epithelial abnormalities they were excluded from the analysis. A total of 210 subjects was examined including 55 affected males, 48 obligate heterozygotes, and 60 'at risk' females. Venous blood samples were obtained from 192 subjects for DNA extraction and Xg blood grouping.

Molecular studies
DNA was tested for three RFLPs in the Xp22.3 region using the DNA probes

### Table 1 DNA probes used in the localisation of OAI

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Enzyme</th>
<th>Alleles (kb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS237</td>
<td>GMGX9</td>
<td>HindII</td>
<td>4.0</td>
<td>18</td>
</tr>
<tr>
<td>DXS143</td>
<td>dic56</td>
<td>BclI</td>
<td>8.9</td>
<td>19</td>
</tr>
<tr>
<td>DXS85</td>
<td>782</td>
<td>EcoRI</td>
<td>14.0</td>
<td>20</td>
</tr>
</tbody>
</table>

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Table 2  Two point linkage data between OAI and DXS237, DXS143, DXS85, and the XG blood group locus.

<table>
<thead>
<tr>
<th>Location</th>
<th>Recombination fraction</th>
<th>Zmax</th>
<th>Ommax</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>XG</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXS237</td>
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<td></td>
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<tr>
<td>DXS143</td>
<td>0.05</td>
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<td>DXS85</td>
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<td>DXS85</td>
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<td>DXS85</td>
<td>0.00</td>
<td>1.58</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

GMGX9, dic56, and 782 (table 1). DNA extraction from blood (fresh and frozen) and Southern analysis were performed by standard techniques. Probes were labelled with 32P and hybridised to Southern blots at 45°C in the presence of 50% (by volume) formamide, 5 × SSC, 4 × Denhardt's, 10% (by weight) dextran sulphate, and 0.1 mg ml⁻¹ salmon sperm DNA. Filters were washed to a final stringency of 0.5 to 1.0 × SSC at 65°C and exposed to x ray film for one to seven days at -70°C. Blood samples (EDTA) were also typed for Xg blood group (Dr Patricia Tippett, MRC Blood Group Unit, London).

GENETIC LINKAGE ANALYSIS

Linkage analysis was performed using two point (LIPE25) and multipoint (LINKAGE23) programs. OAI gene frequency was estimated at 0.0001. Confidence limits were determined as the values of the recombinant fractions at lod scores one unit below the maximum. For multipoint linkage analysis the marker order and map distances described by Yates et al26 were used and the map was constructed using the LINKMAP program of the LINKAGE package.

Results

The results of the two point linkage analysis are shown in table 2. DXS143 is closely linked to OAI with a maximum lod score of 15.90 at zero recombination (confidence interval 0-0.035). Twelve families were informative for DXS143. The peak lod score for DXS237 was 12.65 at 0.08 recombination (CI 0.03-0.17) and for DXS85 the lod score was 15.67 at recombination fraction 0.04 (CI 0.007-0.11).

One family (CAM 4220) had a rare allele with GMGX9/HindIII, giving 2.5 kb/1.6 kb fragments and showing mendelian inheritance and a recombinant with the disease. The smaller fragment could easily be distinguished from the usual 1.5 kb fragment of the 2.5 kb/1.5 kb allele.

There were eight subjects contributing recombinants with DXS237 or DXS85. These comprised six affected males, one normal male, and one ‘at risk’ female with definite clinical signs of the carrier state.

Multipoint linkage analysis showed that the most likely location for OAI is in the DXS85-DXS237 interval (figure) to give the order DXS85-(DXS143,OAI)-DXS237-XG-Xpter. A location between DXS85 and DXS237 was at least 1.2 × 105 times more likely than in adjacent intervals. The peak multipoint lod score was 26.9 with OAI placed 1.2 cM proximal to DXS143 (dic56), but this score was not significantly higher than for positions distal to this marker. OAI lies close to DXS143 but in the absence of recombinants the order of these loci could not be determined.

Discussion

This study confirms the localisation of the OAI locus to the Xp22.3 region. Previous studies have suggested that DXS85 (782) might be the most closely linked DNA marker with a cumulative lod score of 7.80 at zero recombination.23 This study places OAI distal to DXS85 at a recombination fraction of 0.04 with a maximum lod score of 15.67 and indicates that OAI may lie closer to DXS143 (dic56). No recombinants were found between OAI and DXS143. Analysis of recombinants in this study confirms localisation of OAI proximal to DXS237 and distal to DXS85, but absence of recombinants with DXS143 does not allow further localisation about this locus. No family had more than one subject recombinant for the markers DXS237 or DXS85. There is therefore no evidence of genetic heterogeneity of XLOA in the British population.

Recombinants between DXS143 and OAI have been reported but give conflicting information about the order. Bergen et al26 described a normal male who was recombinant for DXS143 but non-recombinant for DXS237, placing OAI distal to DXS143. Schur et al26 reported an affected male who was recombinant for DXS143 and distal markers, placing OAI proximal to DXS143. Assuming correct diagnosis of XLOA in each study, these discrepancies are hard to explain.
Normal males are unlikely to be misdiagnosed as having the condition and there are no reported cases of non-penetration. Affected males may have visual acuity as good as 6/9 without nystagmus, but foveal hypoplasia and iris transcluency should not escape formal ophthalmic examination, as was reported in both studies. The recombinant of Bergen et al\textsuperscript{13} was uninformative for the locus proximal to DXS143 so that a double crossover event cannot be excluded. Alternative explanations would be locus heterogeneity or a DNA typing error.

This study indicates that OAI is closely linked to the DXS143 locus and multipoint analysis slightly favours the order XG-DXS237-DXS143-OAI1-DXS85 over the order XG-DXS237-OAI1-DXS143-DXS85, but the difference is not significant. Further linkage data are required to order OAI precisely with respect to DXS143 and other loci in this region.

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