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

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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; $Z_{\max} = 15.90$ at $\theta = 0.0$, confidence interval (CI) 0.0-0.035), *DXS85* (782; $Z_{\max} = 15.67$ at $\theta = 0.04$, CI=0.007-0.11), and *DXS237* (GMGX9; $Z_{\max} = 12.65$ at $\theta = 0.08$, CI=0.03-0.17). Multipoint linkage analysis placed *OAI* between *DXS85* (782) and *DXS237* (GMGX9) with odds exceeding 10⁴:1 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 males¹ and is associated with refractive errors, strabismus, iris translucency, fundus hypopigmentation, and foveal hypoplasia.² The effects of the mutant gene are not confined to the eyes: visual evoked potentials show evidence of abnormal neuronal crossing at the optic chiasm³ and, although skin and hair pigmentation is normal, skin histology shows abnormal giant melanin granules termed macromelanosomes^{4,5} in all affected males.⁶

The vision of carrier females is normal, but ophthalmic examination shows a characteristic 'mud splattered' fundus appearance.⁷ Carriers may occasionally have a normal ophthalmic examination and several carriers are described with reduced visual acuity and nystagmus similar to an affected male.⁸⁻¹⁰ 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.⁶

The XLOA gene locus (*OAI*) was first localised to distal Xp by linkage to the Xg blood group.^{11,12} Tight linkage has been reported to *DXS85* (782),^{13,14} and other studies^{15,16} 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*.¹⁷ This would be compatible with placing *OAI* distal to *DXS143*,¹⁵ but not with another report placing *OAI* between *DXS143* and

DXS85.¹⁶ 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 (SJC). 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*.

Locus	Probe	Enzyme	Alleles (kb)	Reference
<i>DXS237</i>	GMGX9	HindIII	4.0	18
			2.5, 1.5	
<i>DXS143</i>	dic56	BclI	8.9	19
			7.4	
<i>DXS85</i>	782	EcoRI	14.0	20
			7.0	

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

	Recombination fraction						Zmax	θmax	Confidence interval
	0.00	0.01	0.05	0.10	0.20	0.30			
<i>XG</i>	−∞	−6.33	−1.24	0.51	1.54	1.44	1.58	0.24	
<i>DXS237</i>	−∞	8.78	12.29	12.60	10.86	7.86	12.65	0.08	0.03–0.17
<i>DXS143</i>	15.90	15.63	14.53	13.12	10.10	6.88	15.90	0.00	0.0–0.035
<i>DXS85</i>	−∞	14.89	15.61	14.73	11.85	8.27	15.67	0.04	0.007–0.11

GMGX9, dic56, and 782 (table 1). DNA extraction from blood (fresh and frozen) and Southern analysis were performed by standard techniques.²¹ 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 (LIPED²²) 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 al*²⁴ 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).

Multipoint linkage analysis with *OAI* and *XG*, *DXS237*(*GMGX9*), *DXS143*(*dic56*), and *DXS85*(*782*).

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 × 10⁴ 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.²⁵ 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 al*¹⁵ described a normal male who was recombinant for *DXS143* but non-recombinant for *DXS237*, placing *OAI* distal to *DXS143*. Schnur *et al*¹⁶ 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-penetrance. Affected males may have visual acuity as good as 6/9 without nystagmus, but foveal hypoplasia and iris translucency should not escape formal ophthalmic examination, as was reported in both studies. The recombinant of Bergen *et al*¹⁵ 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-OAI-DXS85* over the order *XG-DXS237-OAI-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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