ORIGINAL ARTICLE

A locus for isolated cataract on human Xp

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Purpose: To genetically map the gene causing isolated X linked cataract in a large European pedigree.
Methods: Using the patient registers at Birmingham Women's Hospital, UK, we identified and examined 23 members of a four generation family with nuclear cataract. Four of six affected males also had complex congenital heart disease. Pedigree data were collated and leucocyte DNA extracted from venous blood. Linkage analysis by PCR based microsatellite marker genotyping was used to identify the disease locus and mutations within candidate genes screened by direct sequencing.
Results: The disease locus was genetically refined to chromosome Xp22, within a 3 cM linkage interval flanked by markers DXS9902 and DXS999 (Zmax=3.64 at θ=0 for marker DXS8036).
Conclusions: This is the first report of a locus for isolated inherited cataract on the X chromosome. The disease interval lies within the Nance-Horan locus suggesting allelic heterogeneity. The apparent association with congenital cardiac anomalies suggests a possible new oculocardiac syndrome.

Congenital cataract is the most common treatable cause of childhood blindness in the western world.[1] In certain instances, cataracts may be inherited, frequently as an isolated bilateral autosomal dominant condition (ADC). ADC is phenotypically highly heterogeneous[2] reflecting a complex underlying genotype[3]; 15 independent loci are now known and mutations identified in the genes encoding the lens specific crystallins,[4] connexins,[5] aquaporin,[6] and beaded filament protein, *BFSP2*.[7]

The existence of X linked non-syndromic congenital cataract has been debated. A number of pedigrees have been reported, though in many other modes of inheritance appear more likely. The recognition of chromosomal deletions of varying size in this region of the X chromosome and the resulting phenotypes observed suggest that a cataract locus may reside within the region Xp22.3-p21.1.[8] It has been proposed, however, that X linked cataract is either synonymous or closely related to the Nance-Horan syndrome (NHS) locus, mapped to Xp.

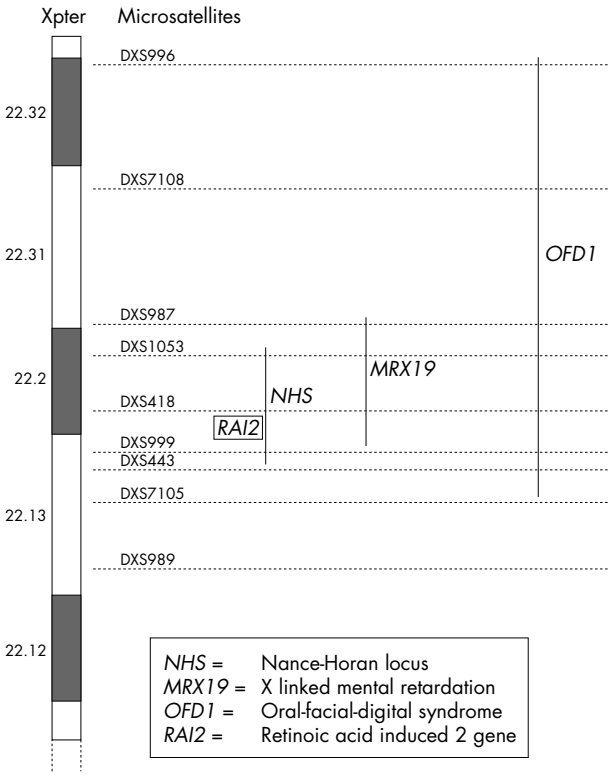

Figure 1 Chromosome Xp22 annotated with disease intervals coinciding with the Nance-Horan disease interval.

NHS (OMIM 302350)[9] is a rare X linked disease characterised by severe congenital cataract in hemizygous males with (1) microcornea or microphthalmia, (2) distinctive dental anomalies (supernumerary incisors, crown shaped permanent teeth), (3) evocative features, (4) anteverted pinnae of the ears, and (5) mental retardation in some. Cataracts are fully penetrant in heterozygous females and are confined to the posterior Y sutures.[10–12] Obligate carriers also have widely spaced, cone or screwdriver shaped teeth.[13] The variable phenotype has led some to suggest that NHS is a contiguous gene syndrome, but there is little genetic evidence to support this view.[14]

Linkage studies have refined the NHS disease locus to a 3.5 cM interval on Xp22.2,[15 16] a region syntenic with the mouse cataract disease locus *Xcat*.[17] The gene responsible has not been identified. Recently, *RAI2*, the retinoic acid induced gene 2, has been excluded.[15] Fig 1 shows the relevant region of the X chromosome with disease loci that coincide with the NHS disease interval. Several diseases with certain similar features have been mapped to intervals that coincide (oral-facial-digital syndrome, OMIM 311200; non-specific X linked mental retardation 19, OMIM 300114), raising the possibility that they are indeed allelic.

In this article, we report the genetic mapping of a large, four generation pedigree with isolated non-syndromic cataract to the short arm of chromosome X.

MATERIAL AND METHODS

Phenotyping

The Birmingham Women's Hospital Clinical Genetics Service database, Birmingham, West Midlands, UK provided details of

Abbreviations: ADC, autosomal dominant cataract; NHS, Nance-Horan syndrome; VSD, ventriculoseptal defect

Table 1 Age and best corrected visual acuity for affected subjects in family 1

			Right			Left		
Affected subject	Sex	Age in years	VA	Lens status	Age operated	VA	Lens status	Age operated
II.1	Female	72	6/6	Pseudophakic	68 years	6/6	Pseudophakic	68 years
III.3	Female	48	6/6	NLO		6/6	NLO	
III.4	Female	46	6/9	NLO		6/6	NLO	
III.5	Female	40	6/6	NLO		6/6	NLO	
III.7	Female	75	6/9	NLO		6/9	NLO	
IV.1	Male	23	NPL	Aphakic	3 months	CF	Aphakic	3 months
IV.2	Female	28	6/6	NLO		6/6	NLO	
IV.3	Male	21	HM	Aphakic	6 weeks			
IV.4	Male	17	CF	Aphakic	2 months	6/12	Aphakic	2 months
IV.5	Male	20	6/36	Pseudophakic	1 year	6/24	Pseudophakic	1 year
IV.6	Female	34	6/6	NLO		6/6	NLO	
V.1	Male	8	6/12	Aphakic	At birth	6/60	At birth	At birth
V.4	Male	14	HM	Aphakic	At birth	6/18	Pseudophakic	At birth

NLO=nuclear lens opacities.

Table 2 Lod scores for linkage between the X linked cataract locus and polymorphic markers spanning the interval Xp22.32-21.13 ordered telomere to centromere. Lod scores are calculated modelling for X linked recessive transmission. Disease gene frequency 0.0001

	Lod score (Z) at recombination (θ) of										
Maker	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
DXSGATA124B04	–∞	2.25	2.16	1.93	1.64	1.33	1.01	0.70	0.42	0.19	0
DXS1223	–∞	1.57	1.62	1.54	1.41	1.23	1.03	0.81	0.56	0.30	0
DXS7103	2.53	2.30	2.06	1.81	1.54	1.26	0.98	0.68	0.40	0.15	0
DXS7108	–∞	2.80	2.72	2.50	2.20	1.87	1.49	1.10	0.70	0.32	0
DXS85	0.13	0.10	0.07	0.05	0.03	0.01	0.00	–0.01	–0.01	–0.01	0
DXS1043	3.75	3.38	2.99	2.58	2.16	1.72	1.26	0.81	0.42	0.15	0
DXS1224	–∞	–0.80	–0.54	–0.35	–0.30	–0.23	–0.17	–0.12	–0.08	–0.04	0
DXS9902/DXSGATA175D03	–∞	3.14	3.04	2.78	2.45	2.08	1.67	1.22	0.77	0.35	0
DXS1195	0.49	0.43	0.36	0.30	0.25	0.20	0.15	0.11	0.07	0.03	0
DXS8036	3.64	3.29	2.93	2.54	2.15	1.73	1.30	0.87	0.47	0.17	0
DXS999	–∞	1.95	1.94	1.79	1.58	1.34	1.08	0.81	0.54	0.27	0
DXS7593	–∞	2.80	2.70	2.46	2.16	1.82	1.45	1.08	0.70	0.34	0

a large four generation family with isolated inherited congenital cataract. Members provided a full history and underwent a full clinical assessment by appropriate physicians.

Genotyping

Genomic DNA was extracted from EDTA sequestered blood samples taken with informed consent and local ethical approval using the Nucleon II DNA extraction kit (Scotlab Bioscience). PCR based microsatellite marker genotyping using the Genethon microsatellite markers[18] at 5-10 cM intervals was performed as described previously.[19]

Linkage analysis

Data were collated using the Cyrillic pedigree management software (version 2.1.3; Cherwell Scientific Publishing Ltd, The Magdalen Centre, Oxford Science Park, Oxford OX4 4GA). Two point lod scores were calculated using the MLINK programs.[20] Although the disease appeared fully penetrant in heterozygous females, linkage analysis was modelled as an X linked recessive disorder with a gene frequency of 0.0001 assumed for the cataract locus.

RESULTS

The pedigree

Careful clinical examination showed that all affected males had required cataract extraction in the first few months of life with a uniformly poor outcome. This finding contrasted markedly with affected females who had very mild central nuclear opacities requiring no treatment until typically the sixth decade (table 1). Such observations raised the possibility that inheritance was X linked with full penetrance in heterozygotes. Unfortunately, none of the affected males had children and it was thus impossible to confirm the absence of male to male transmission. The complete pedigree is shown in fig 2.

The phenotype

In support of X linked inheritance, the appearance of the cataract was distinct from any ADC phenotype seen previously.[2] The only phakic members of the family were female and, in each, cataracts were very slowly progressive, fan shaped, and nuclear in distribution (fig 3). There was no evidence of the features of NHS in any affected males or obligate carriers. Interestingly, however, four of the six affected males had a ventriculoseptal defect (VSD) and other cardiac developmental anomalies. No other family members gave a history of cardiac anomalies.

Linkage analysis

After excluding linkage to a number of markers on the X chromosome, we obtained significantly positive lod scores for marker DXS9902/GATA175D03. Indeed, only one recombinant (IV.1) is observed with this marker. Further linkage analysis provided strong evidence that the disease locus indeed lay centromeric to DXS9902, most likely residing between this

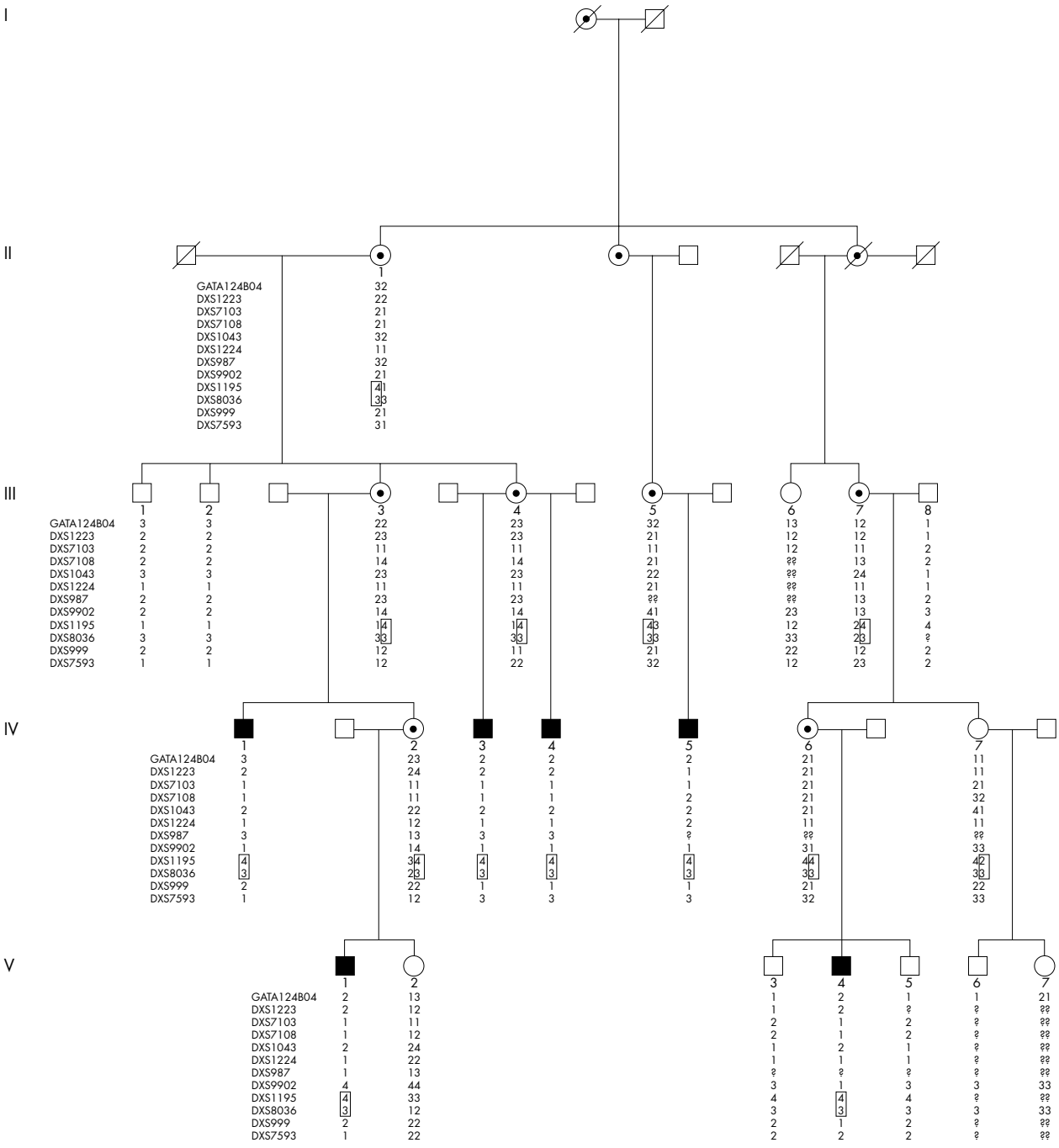

Figure 2 Family pedigree showing segregation of Xp22 microsatellite markers, listed in descending order from the telomere. Severely affected subjects are designated by filled symbols. Heterozygous carrier females with lens opacities are indicated by circles with central dots. The disease haplotype is boxed. ? indicates undetermined allele.

marker and DXS999 (at which IV.1 is no longer a recombinant but a crossover is observed with his maternal grandmother, III.3). Markers within this locus confirmed linkage of the family to the chromosomal region Xp22.13 ($Z=3.64$ at $\theta=0$ for marker DXS8036). Since the condition appears fully penetrant in heterozygous females, lod scores at this marker were also calculated modelling for dominant disease giving $Z=4.60$ at $\theta=0$ for marker DXS8036.

No recombinants are observed at DXS7103 and DXS1224, though critically these markers are uninformative for IV.5, who is a recombinant with all adjacent microsatellites. As double recombination events are most unlikely over such small map distances, this subject is most probably a recombinant at DXS7103 and DXS1224 excluding linkage to this interval.

Haplotype analysis of the abridged family for markers within the Xp22 region is shown in fig 2 and lod scores in table 2.

DISCUSSION

This is the first description of a family with isolated, non-syndromic, X linked cataract. The existence of familial congenital cataract inherited in this way has been debated. The only possibly convincing X linked pedigree previously described is that by Krill *et al*.[21] In this family, hemizygous males had sutural cataracts. The differential diagnosis of X

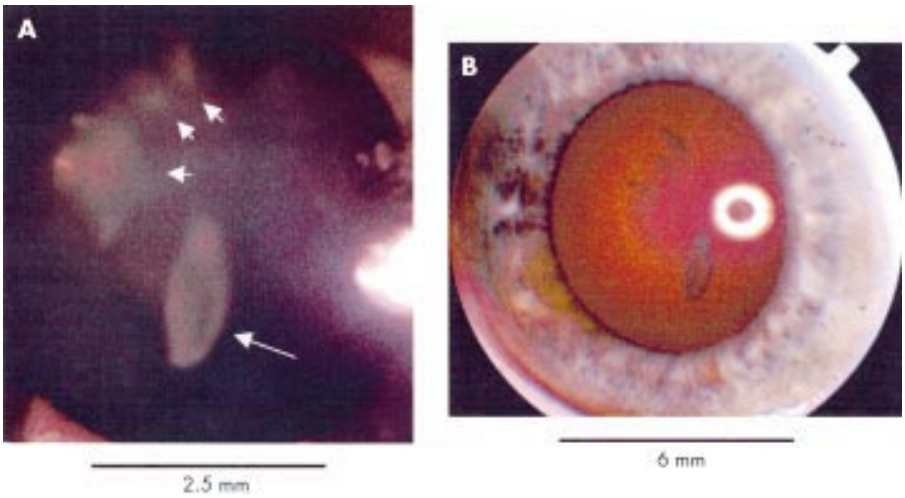

Figure 3 The X linked cataract phenotype. (A) Slit lamp view (diffuse illumination) of heterozygous 50 year old female showing fan of nuclear lens opacification (arrowed). (B) Same eye (lower magnification), lens in retroillumination. The same pattern of opacification was observed in all heterozygous females. All hemizygous males had required cataract extraction in the first few weeks of life.

linked cataract includes the syndromes of Nance-Horan, Lenz, and Lowe. It has been previously suggested that X linked isolated cataract may indeed be synonymous with Nance-Horan syndrome.[8]

In our family, X linked inheritance (with complete penetrance in heterozygous females) was suggested by affected subjects in successive generations, consistently severely affected males (requiring cataract surgery in the first months of life), contrasting markedly with asymptomatic or mildly visually disabled carrier females. Unfortunately, there were no male offspring born to affected males. Further support was lent by the cataract phenotype that consisted of a sea fan of nuclear opacity in affected females and total opacity in hemizygous males, a combination of appearances not seen in autosomal dominant cataract.

Complex congenital cardiac anomalies were also noted in four of the six affected males and were not present in any unaffected subjects. The possibility that these abnormalities segregate with cataract formation in our family may prove instructive in identifying candidate genes. Several syndromes have been documented where congenital cataract and cardiac anomalies form a part. Arrhythmogenic right ventricular dysplasia associated with anterior polar cataract has been tentatively mapped to 14q[22 23] and the association of cataract, microphthalmia, septal heart defects, and deafness has been reported as a dominantly inherited syndrome.[24 25] The oculofacio-cardio-dental (OFCD) syndrome comprises cataract, microphthalmia, facial abnormalities, cardiac defect (atrial septal defect and VSD), and dental abnormalities.[26 27] Interestingly, the condition appears to be X linked (lethal in hemizygous males), raising the possibility that a less deleterious mutation in the same gene might account for the spectrum of anomalies seen in our family.

To test the inheritance hypothesis, linkage analysis was performed across the X chromosome using the Genethon 5-10 cM microsatellite marker set. Linkage to markers at Xp22.2 was detected and the disease interval refined to lie between DXS9902 and DXS999 ($Z_{max}=3.64$ at $\theta=0$ for marker DXS8036). The interval (*CXN*, congenital X linked nuclear cataract locus), which is less than 2.5 cM is encompassed by the Nance-Horan locus (DXS1053-DXS443).[15 16] This most likely suggests that allelic heterogeneity within the same gene can result in either isolated cataract or cataract associated with other systemic anomalies and thus refines the disease locus. Alternatively, in accord with the Warburg hypothesis and with the recognition that a microdeletion of Xp22.3 results in ocular anomalies (microphthalmia, sclerocornea) and cardiac anomalies associated with linear skin defects,[28] a lens gene and one or more other genes may reside within the disease interval.

Although the *CXN* locus is gene rich, there is no obvious cataract candidate gene. The retinoic acid induced -2 (*RAI2*) gene, previously considered a good candidate for Nance-Horan syndrome by Walpole *et al*,[15] lies outside the *CXN* disease interval.

This is the first report of a family with isolated cataract mapping to one of the sex chromosomes. Linkage to a refined region of the Nance-Horan locus in all likelihood reflects allelic heterogeneity and, given the possible segregation of cardiac anomalies with cataract in our family, it will be fascinating to explain the underlying genotype-phenotype correlation.

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