X linked exudative vitreoretinopathy: clinical features and genetic linkage analysis

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Accepted for publication 28 October 1992

Figure 1 Full family pedigree. Individuals used for DNA linkage analysis are indicated by an asterisk (*).

Abstract

A four generation family in which familial exudative vitreoretinopathy is inherited as an X linked condition is described. Essentially the condition is one of abnormal vascularisation and signs at birth are those of a retinopathy superficially resembling retinopathy of prematurity, retinal folds, or, in advanced cases, enophthalmos or even phthisis. Prognosis depends on the progression of the retinal changes. The family members, including seven affected males and five obligate carrier females, have been typed for 20 DNA markers, and linkage analysis suggests a gene locus either at Xq21.3 or at Xp11. As the latter region includes the locus for the gene for Norrie disease, it is possible that this and X linked vitreoretinopathy are allelic. We can further speculate that the differences in severity of the clinical manifestations are dependent only upon the timing of the insult.

(*Br J Ophthalmol* 1993; 77: 168-170)

Familial exudative vitreoretinopathy (FEVR) is usually inherited as an autosomal dominant condition, with an equal sex ratio, and transmission from one generation to the next.¹⁻⁶ However one of the pedigrees reported by Criswick and Schepens in 1969 is compatible with X linkage,⁷ and families have been reported which may be compatible with the disorder we describe here.^{8,9} In 1979 Dudgeon described a family in which two brothers had FEVR and two male second cousins, linked through the maternal line, had congenital retinal folds.¹⁰ He postulated

that the two conditions could have a common pathogenetic mechanism, and he noted that the intervening females had no abnormal clinical findings. Four further affected males have been born to this family and we have had the opportunity to make further clinical assessments and to carry out linkage analysis as a first step in the identification of the disease gene.

Materials and methods

CLINICAL FINDINGS

Fourteen family members in four generations of the family first described by Dudgeon,¹⁰ including seven affected males and five obligate carrier females, were assessed clinically and provided blood samples for DNA analysis. The pedigree is shown in Figure 1.

DNA AND LINKAGE ANALYSIS

A volume of 10-20 ml of venous blood from family members was collected in potassium ethylenediaminetetra-acetic acid tubes. DNA was extracted using an Applied Biosystems 430A nucleic acid extractor.

Inheritance of the disease locus together with known polymorphic loci was followed using restriction fragment length polymorphisms and (AC)_n repeat polymorphisms. If tightly linked the two loci (that is, the disease and the particular polymorphic marker) will cosegregate during meiosis. If not tightly linked recombination events are observed between the loci.

Two point linkage analysis was performed using the computer program LIPED¹¹ with the data management program LINKSYS.¹² Females in this family who are not obligate carriers were excluded from the linkage analysis. The LIPED program calculates the overall likelihood that the two loci are linked, and the overall likelihood that they are unlinked. The logarithm of the ratio of these likelihoods is the lod score. Positive lod scores favour linkage, negative lod scores are evidence against linkage. The accepted threshold is that of +3.0 for significant evidence of linkage in an autosomal disorder. However in an X linked condition +2.0 is considered significant given that the disease locus is necessarily on the X chromosome.

DNA probes were generously provided by Dr J-L Mandel (DXS52), Dr Ian Craig (DXS255), or were obtained from the ATCC (American Type Culture Collection). PCR (polymerase chain reaction) primers for (AC)_n repeat analysis were a generous gift of Dr Kay Davies (MAO, DXS228), or were synthesised on an Applied Biosystems oligonucleotide synthesiser.

Figure 2 X chromosome showing localisation of the 12 informative DNA probes.

Table 1 Two point lod scores for the disease locus versus the 12 informative DNA marker loci

Marker loci	Recombination fraction							Z_{max}	α	Θ
	0.00	0.001	0.05	0.10	0.20	0.30	0.40			
DXYS20 (Xp22.32)	—∞	−8.68	−2.06	−1.02	−0.85	0.11	0.15	0.15	0.40	0.40
DXS164 (Xp21.2)	—∞	−9.58	−2.93	−1.86	−0.91	−0.45	−0.17	−0.17	0.40	0.40
DXS7 (Xp11.4-p11.3)	0.30	0.30	0.26	0.21	0.13	0.07	0.02	0.30	0.00	0.00
DXS228 (Xp11.4-p11.3)	1.81	1.80	1.63	1.44	1.07	0.70	0.34	1.81	0.00	0.00
MAO (Xp11.4-p11.4)	0.94	0.94	0.85	0.75	0.56	0.37	0.18	0.94	0.00	0.00
DXS255 (Xp11.22)	—∞	−2.37	0.78	1.12	1.16	0.91	0.51	1.21	0.15	0.15
DXS453 (Xq11.2-q12)	—∞	0.61	2.04	2.05	1.75	1.27	0.67	2.06	0.08	0.08
DXYS1 (Xq11.2-q12)	—∞	−0.60	0.86	0.91	0.69	0.36	0.09	0.92	0.11	0.11
DXYS1 (Xq21.31)	2.11	2.10	1.94	1.77	1.40	0.98	0.51	2.11	0.00	0.00
DXS456 (Xq21.1-q23)	—∞	−5.94	−1.01	−0.28	0.24	0.35	0.25	0.36	0.32	0.32
DX454 (Xq21.1-q23)	—∞	−3.86	−0.60	−0.14	0.17	0.22	0.15	0.22	0.30	0.30
DXS52 (Xq28)	—∞	−3.31	−0.08	0.15	0.36	0.53	0.32	0.54	0.32	0.32

For Southern blot analysis, DNA was digested with the appropriate restriction enzyme, electrophoresed through agarose gels, blotted onto nylon membranes, hybridised to 32 P-labelled DNA probes and the polymorphic bands detected by autoradiography, as described elsewhere.

(AC)_n repeat analysis was carried out under standard conditions. Briefly, one PCR primer was 5'- 32 P-labelled using γ - 32 P-ATP and polynucleotide kinase, 35 cycles of amplification were performed, and the labelled products were visualised by autoradiography of denaturing polyacrylamide gels. In toto, probes for 20 marker loci, spanning the X chromosome, were studied of which 12 proved informative. The localisation of the informative probes is shown in Figure 2.

Results

CLINICAL ASSESSMENT

The four affected males of generation IV have been described previously¹⁰; the three new affected males belong to generation V. None of the intervening females shows any signs of disease, and IV.8 had normal fluorescein angiography. There is no suggestion of incontinentia pigmenti in any family member.

IV.6, born 27 March 1960, was noted to have a

phthisical right eye at 13 years, and a retinal fold in the left eye extending across the posterior pole towards a patch of chorioretinal scarring in the temporal periphery. At the age of 18 he developed a retinal detachment in the left eye with a profuse subretinal yellow exudate. Visual acuity in the left eye was 4/60 and cryotherapy was performed to prevent a further reduction of vision.

IV.10, born 24 November 1963, presented at the age of 14 years with visual failure. Both eyes contained fibrous tissue in the temporal retinal regions and white retinal areas, with and without pressure. Peripheral cystoid degeneration was noted in each eye. Cryotherapy was performed on both eyes, but he later developed a retinal tear in the right, with subsequent detachment. Further treatment was successful but he remains partially sighted with acuities of 3/60 and 6/36.

IV.12, born 17 April 1970, has been partially sighted from birth and has had no episodes of further visual deterioration. He has nystagmus, microphthalmos, and retinal folds in each eye.

IV.13, born 15 October 1971, at 5 months was diagnosed like his brother to have retinal folds and a fibrous tissue mass in the left temporal retina, with 'snowflake' opacities in the vitreous. Symptoms were stationary until the age of 15 when he developed a retinal tear in the left eye and total retinal detachment. This was treated with vitrectomy and silicone implants, but he subsequently developed a cataract and has little residual vision in that eye.

Two brothers, V.1, born 17 May 1978 and V.2, born 5 October 1982, were examined and diagnosed soon after birth because of the family history. Their retinas resembled retinopathy of prematurity, with avascular temporal peripheries, and vascular shunts at the junction between the vascularised and avascular areas. After treatment with cryotherapy, the ocular status has been stable and their family consider the children to have good vision, although they are myopic.

V.5, born 22 November 1988, was also examined soon after birth because of the family history. He has a detachment of the right retina, and a traction detachment of the left temporal retina which also included the macula. He was treated with cryotherapy. He has defective acuity and nystagmus, but is developing well.

The pedigree is illustrated in Figure 1.

Figure 3 Part of generations IV and V showing typings for the DNA markers for the loci DXS228, MAO, DXS255, DXS1, DXS453, and DXYS1.

LINKAGE ANALYSIS

The family was typed for 20 markers spanning

the X chromosome. Twelve of these markers proved informative. Two point linkage data for the disease locus versus the 12 markers are presented in Table 1.

Recombination was observed between the disease gene and the markers DXYS20, DXS164, DXS255, DXS1, DXS453, DXS456, DXS454, and DXS52. No recombination was observed with the markers DXS7 (Xp11.4-p11.3), DXS228 (Xp11.4-p11.3), MAO (Xp11.4-p11.3), and DXYS1 (Xq21.31).

A maximum lod score of 2.11 at a recombination fraction of zero were obtained with DXYS1, while DXS228 gave a maximum lod score of 1.81 at zero recombination fraction (see Table 1).

Direct counting of the recombinant meioses was carried out in an attempt to position the disease gene. Individuals V.2 (affected) and V.3 (normal) have inherited the same DXS255-DXS1-DXS453 haplotype from their mother (see Fig 3), excluding the disease gene from this interval.

Discussion

The clinical features of FEVR include retinal folds, fibrovascular masses, white intraretinal deposits, subretinal exudates, and retinal detachment.^{2,5,10,13,14} Van Nouhuys observed that exudates only occurred in 16 of 170 eyes (9.4%) with dominant FEVR and that typical retinal folds were only found in 14 (8.2%).^{2,3} These features are considered to be secondary to maldevelopment of the retinal vascular system.^{2,15,16}

The differential diagnosis includes retinal folds owing to other causes, incontinentia pigmenti, retinopathy of prematurity, and Norrie disease. Enophthalmos and phthisis are simply manifestations of end stage disease but can lead to diagnostic confusion with Norrie disease.

FEVR is characterised by a variable clinical picture, remaining subclinical in some, but with onset of symptoms in early life or adulthood in others.^{1,2,4,5} Clinical examination may be normal in about one fifth of obligatory carriers¹ but Ober *et al* on the basis of three families, considered that adult gene carriers could always be recognised by fluorescein angiography.⁴

This family with vitreoretinopathy differs clinically from FEVR because obvious signs are present at birth in affected males, and because adult female gene carriers show no evidence of disease even on fluorescein angiography. The pedigree is typical of X linked inheritance. The normal sex ratio in FEVR suggests that the X linked condition is rare and indeed only three previously reported families are consistent with X linkage.⁷⁻⁹

Linkage analysis suggests that one of two regions is a likely location of the gene responsible for this disorder, one in Xq21.3, in proximity to DXYS1 or one in Xp11, in proximity to MAO-DXS228. Ultimately fine localisation may provide a tightly linked marker for use in genetic counselling, carrier detection, and prenatal diagnosis.

It is interesting to note that the gene for Norrie disease is also located at Xp11. We can speculate therefore that Norrie disease and X linked

FEVR may be produced by different mutations in the same gene.

In Norrie disease neuroectodermal migration is affected, resulting in maldevelopment of the inner retina with absence of ganglion cells. The retinal vascular system, as a consequence, does not develop normally, producing a picture resembling retinopathy of prematurity or what has often previously been labelled persistent primary hypoplastic vitreous. It has been postulated by Hittner and Kretzer that this represents the end of an insult acting as early as 7 weeks' gestation.¹⁷ In contrast these latter workers consider that the maldevelopment in FEVR occurs later (between 29 and 40 weeks' gestation) and that at this age, with all retinal neural elements laid down, it affects vascularisation that is secondary to, and stimulated by, photoreceptor differentiation. It is possible, therefore, to conceive that these two conditions, Norrie disease and XLVR, both producing defective vascularisation, differ only in the timing of their insults; and that such differences in timing affect the severity and extent of their associated congenital malformations.

We first thank the family for being extremely helpful in providing blood samples, and we thank their general practitioners who kindly took the blood in Scotland and posted it back to Birmingham: Drs Bryson, Waterson, McClusker and Begg.

A number of ophthalmologists have been involved with the care of these patients and have provided information on their clinical states, and we thank Mr C J Tallents and Mr J D Scott for information on IV.13, and Mr Z J Gregor and Mr A I Macfarlane for information on V.5; we also thank Mr Z J Gregor for information on IV.8.

We gratefully acknowledge the financial support of the Wellcome Trust and the British Retinitis Pigmentosa Society.

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