Prenatal exclusion of Norrie's disease

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Abstract

We report on the use of DNA marker probes and linkage analysis to exclude Norrie's disease in the male fetus of a high risk carrier. There are no clinical markers in females carrying the Norrie's disease gene; thus DNA linkage analysis is an essential technique in the management of families 'at-risk' for this severe ophthalmic disease. The principles of DNA linkage are discussed.

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Norrie's disease (ND, McKusick catalogue number 31060) is an X-linked recessive form of blindness. The main features of ND, as described by Warburg,¹ are:

(1) bilateral retinal dysplasia and detachment evident at birth or in early infancy, with subsequent cataract formation, opacification of the cornea, and optic atrophy. Chronic hypotony is usual, following a period of secondary glaucoma and ocular pain;

(2) progressive mental deterioration is common, with onset usually in early childhood and psychotic behavioural patterns developing later;

(3) variable hearing loss.

Other features include growth retardation and wasting, a narrow nasal bridge, large ears, hypogonadism, or undescended testes.²

The dense white or yellow-white retrolental mass due to detached, dysplastic retina results in leukocoria and requires urgent clinical investigation and possible enucleation in order to exclude retinoblastoma (see Fig 1). Eventual phthisis bulbi has led to ND being mis-diagnosed as congenital microphthalmia.³ No biochemical,

ocular, or systemic abnormality has been reported in carrier females.

Pathology

The primary pathological process in ND is an abnormal retinal embryogenesis and retinal dysplasia.⁴ Thus the primary tissue defect is neuroectodermal in origin. Histological examination reveals a dysplastic retina with rosettes of poorly differentiated photoreceptors. The rosettes are encased in cords of proliferating embryonic cuboidal epithelium. Secondary changes comprise neuroglial proliferation from the retina into the early vitreous, retinal detachment, and haemorrhage. With fibrous contraction a retrolental mass forms in the hypotonous, microphthalmic eye.

Linkage studies

Gene loci are linked if they are physically close on a chromosome. Their separation by meiotic 'crossing-over' (recombination) is therefore of low probability. A single cross-over event between two homologous chromosomes is illustrated in Figure 2. Linkage analysis of a human pedigree uses observed recombination events between the disease gene and marker loci to calculate the proportion of recombinant individuals (the recombination fraction) and LOD score for the disease gene and a chosen marker. An LOD score is the Logarithm (base₁₀) of an Odds ratio:

$$\frac{\text{odds the two loci are linked with recombination fraction } \theta}{\text{odds the two loci are unlinked at recombination fraction } 0.5}$$

From the calculated 'genetic' distances between the disease gene and the accurately located markers, a map can be constructed giving the most probable location of the disease gene. Conversely, the most tightly linked probe markers can be used to track the gene through the generations of a human pedigree. Two DNA probe markers used in this study are radioactively-labelled short lengths of single-stranded DNA (oligonucleotides) which will hybridise to unique nucleotide sequences in single-stranded X chromosome DNA. The creation of restriction fragment length polymorphisms (RFLPs) and their identification with such DNA probes is shown in Figure 3.

A combination of DNA linkage studies and deletion mapping has assigned the ND locus to Xp11.3, a region near the centromere on the short arm of the X chromosome. In 1985, Gal *et al*⁵ and Bleeker-Wagemakers *et al*⁶ detected close linkage between ND and locus DXS7 (DNA marker L1.28). Recombination between these two loci was reported by Katayama *et al*⁷ and Ngo *et al*⁸ and a maximum LOD score of 7.46 at 1% recombination implied that the ND

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Figure 1 Right eye of proband II6 showing central corneal oedema, shallow anterior chamber, atrophic iris, miotic pupil, and cataract.

Figure 2 Diagram of a single cross-over between the arms of paired homologous chromatids in metaphase of meiosis I. An average of 55 cross-over events occur in male meiosis; more in the female.

gene locus may be approximately 1 million nucleotide base pairs (1Mb) away from the DXS7/L1-28 marker site.

Further DNA markers for ND are listed in Figure 4; they are all located on the proximal short arm of the X chromosome. Recent accounts of relevant molecular genetic techniques in the ophthalmic literature have been provided by Jay and Inglehearn,⁹ Esakowitz *et al.*,¹⁰ and by Redmond *et al.*¹¹

Material

The Northern Ireland family in this report was originally described by Johnston *et al.*¹² There are three affected males as shown in the partial pedigree (Fig 5).

The proband (II6) was born in 1970. In the first month of life he was noted to have right microphthalmos with a hazy cornea, a shallow anterior chamber, and elongated ciliary processes. A yellow-white retrolental mass and ectropion uveae developed. The initial diagnosis

Figure 3 Diagram illustrating the process of DNA digestion with restriction enzymes and subsequent electrophoresis to separate the polymorphic fragments, Southern blotting and scoring of the RFLPs.

was persistent hyperplastic primary vitreous, but by the age of 1 year he had developed bilateral corneal oedema with miosed pupils, atrophic, vascularised irides, and cataracts. Mental retardation was noted.

The proband's cousin (II9) was traced by Johnston *et al.*⁹ This boy's left microphthalmic eye had been previously enucleated as retinoblastoma could not be excluded. Histological examination of the globe confirmed the diagnosis of ND.

The possible ND carrier II2 became pregnant and required urgent genetic counselling and prenatal investigation with fetal sexing and DNA linkage analysis of amniotic fluid cells.

Methods

DNA from 13 members of the family was extracted from venous blood leucocytes by the method of Jeanpierre¹³ and the sample concentrations were standardised to approximately 500 µg/ml. The restriction enzymes *TaqI* and *EcoRI* were used to digest DNA aliquots and the fragments were separated by electrophoresis in 0.8% agarose gels and transferred to Hybond N nylon membranes (Amersham) by vacuum blotting.

The *TaqI* 'blot' was hybridised with the probe L1-28. The RFLPs identified by *TaqI*/L1-28 comprise a 2-allele system, the fragments (alleles 1 and 2) being 12 and 9 kilobases in size with general population frequencies of 68% and 32% respectively. The *EcoRI* 'blot' was hybridised with the multi-allelic probe, M27β. With this probe females have a 95% chance of being heterozygous. Males, with only one X chromosome, are hemizygous and show only one allele.

The polymerase chain reaction (PCR) was used with primers for the DXS426 locus.¹⁴ This

Figure 4 Schematic diagram of the proximal short arm of the X chromosome (Xp). The Norrie disease gene is represented by a solid block and the relative location order of DNA markers (linked to ND) is shown. Recombination values (Theta) represent genetic 'distances' between the loci measured in centiMorgans (1 cM = 1% recombination = 1 Mb).

Figure 5 Partial pedigree: DNA linkage analysis results for individual III1 are prenatal, and predicted the high likelihood of an unaffected male being born. Affected males are shown as solid squares; I3 is deceased. Females I5 and I7 are marked as obligate carriers as they have affected sons.

locus is a stretch of DNA in which cytosine/adenine dinucleotides are repeated a variable number of times and the locus is thus polymorphic. The repeated cycles of the reaction 'amplify' the polymorphic chains of DNA to a concentration far greater than that of irrelevant DNA. After gel electrophoresis, radioactive labelling is unnecessary as the amplified polymorphic bands fluoresce when the gel is stained with ethidium bromide under UV light.

The results for the three polymorphisms are shown in Figure 5, with the disease associated haplotype shown in bold type. The computer program MLINK v5.03³ has been used to calculate disease and carrier status risks for key individuals using the recombination values given in Figure 4.

Discussion

The affected male II6 from whom DNA analysis is available, has the haplotype [2,2,2] for the three markers used. No unaffected male shows this haplotype and it is likely that the disease gene is linked to this haplotype. The sisters, I5 and I7, both obligate carriers for the ND gene, share the same genotype. Carrier risk estimation calculated by MLINK v5.03 for the female I2 is 93.4%.

The daughters of I2 (II2 and II3) have had DNA analysis using the same markers. II2 has inherited the [2,2,2] haplotype from her mother, whereas II3 has inherited [2,2,4], showing a crossover with the marker farthest from the ND locus (M27B). This does not alter the carrier risk estimation which was computed as 91.5% for

both daughters. II2, at high risk of being a carrier, became pregnant. An early amniocentesis¹⁶ was carried out at 10 weeks' gestation. Fluorescence Y chromatin analysis revealed that the fetus was male. Amniocytes were cultured for 2 weeks and DNA was extracted for prenatal diagnosis using the multi-allelic markers M27B and DXS426.

The fetal haplotype was [-,1,3] for these markers (see Fig 5). Thus it was likely that III1 had not inherited the ND gene. The risk of being affected was computed as 9% with MLINK v. 5.03. By comparison, risk estimation based on the pedigree alone indicates a 46% likelihood of an affected child (III1). The parents were advised of the risk estimation and the pregnancy continued to term. A healthy male baby was delivered.

Linkage analysis using multi-allelic probes has a high probability of being informative in individual families, and provides more accurate genetic counselling. The application of molecular biology techniques, as demonstrated by this case, will help to reduce the incidence of severe inherited ophthalmic disease.

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