A fetus with an X;1 balanced reciprocal translocation and eye disease

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Abstract
A 19 week female fetus is described with a de novo X;1 reciprocal balanced translocation, with the breakpoint on the X chromosome at Xp11.4, and eye pathology consistent with the early stages of Norrie disease. The fetus seems to be an example of a female manifesting an X linked recessive disease, and it was shown that the normal X chromosome was completely inactivated in all cells examined. Norrie disease has been mapped to Xp11.3, and fluorescence in situ hybridisation studies showed that the Norrie disease gene had not obviously been disrupted. Mutation screening by SSCP analysis showed no aberrant fragments of the coding region of the gene. Several eye disease genes map to the same region of the X chromosome, but are excluded on grounds of pathology. One possibility is that this fetus has a Norrie-like eye disease caused by the mutation of another gene located at Xp11.4. If this is so, there are implications for prenatal diagnosis.


Genes for a number of eye diseases are located on the X chromosome and several, such as Norrie disease, retinitis pigmentosa type 2, cone dystrophy, congenital night blindness, and Aland Island eye syndrome, map to the same region of the short arm. One of these, Norrie disease, has been well characterised.

In Norrie disease, blindness is often present from birth, and a proportion of patients later develop mental retardation, psychotic behaviour, and sensorineural deafness. Linkage analysis and deletion mapping led to the gene being localised to the region of Xp11.3.5,6 A candidate gene was isolated by positional cloning, within which mutations have been identified in affected, but not in normal, people.6,7 Despite this, chromosomal studies on a family with affected subjects and an X chromosome rearrangement suggested that the gene was located around Xp11.4.4 The gene product has significant homologies to a number of extracellular proteins,9 and three dimensional modelling shows a similarity to transforming growth factor β.10

We describe a fetus found at prenatal diagnosis to have a de novo balanced X;1 reciprocal translocation in whom, following termination of pregnancy at 19 weeks, the eyes were clearly abnormal and appeared to show pathological changes consistent with the early stages of Norrie disease.

Case report
This was the sixth pregnancy of a healthy white couple aged 37 and 41 years. The previous children, five boys and one girl, are well, including a boy, one of twins and now 13 years old, who had surgery during infancy for transposition of the great vessels. The family history did not indicate anything significant except for a niece on the paternal side who had multiple abnormalities and died. No precise diagnosis had been made but a chromosome abnormality was suspected. For this reason, and on grounds of maternal age, chorionic villus sampling was performed in the 11th week of pregnancy. The chromosome complement from both direct and cultured preparations showed a female fetus with a balanced reciprocal translocation between the X chromosome and chromosome 1. In view of this unexpected finding, and because the tissue sample had been very small and obtained from a single site, an amniocentesis was subsequently performed. The chromosome abnormality was confirmed. Both parents had normal karyotypes, so the fetus appeared to have a de novo translocation: 46,X,t(X;1)(p11.4;p36.3). The parents were informed that, since the X chromosome was involved, there was some risk of a mental or physical abnormality in the child, and because the gene for Norrie disease has been mapped to the area of the X chromosome involved in the translocation, there was the possibility that this gene might have been disrupted. The parents requested termination of the pregnancy.

The female fetus was of a size and stage of development consistent with its gestational age of 19 weeks. There was mild ocular hypertelorism and small, low set ears. The right lung had only two lobes, but the fetus was otherwise grossly normal except for the eyes.

Pathology of the eyes
Both eyes were clearly abnormal (fig 1). On macroscopic examination, the right eye showed an organised retrolenticular fibrotic mass with the vessels being ensheathed in a denser mass of fibrous tissue filling the vitreous and causing focal detachment of the retina. The left eye was similar, but less severely affected. It showed a mass of vessels persisting in the vitreous forming a conical postlenticular mass, from which there had been small focal haemorrhages. The leash of vessels was associated with an
each showed the abnormal karyotype with the breakpoint on the X chromosome clearly at p11.4.

X inactivation studies were performed on fibroblast cells using 5-bromodeoxyuridine (BrdU) incorporation, followed by staining with Hoechst 33258 in the dark, then ultraviolet light exposure, and final staining with Leishman’s. Since this method uses a B pulse of BrdU, late replicating regions appear pale.

In all 43 metaphases studied, the normal X chromosome was the late replicating chromosome. This normal X was consistently pale throughout its length and so was considered to be completely inactivated. Neither of the translocated products showed any evidence of late replication. In particular, the derived X was always dark staining and so appeared to be the active X chromosome in every cell examined.

DNA probes and in situ hybridisation
Chromosomal fluorescence in situ hybridisation (FISH) analysis was performed to determine whether the translocation disrupted the Norrie disease gene. Purified DNA from three cosmid clones, M8, G8, and A10 which cover the entire Norrie disease gene region (kindly supplied by Dr Wolfgang Berger, Human Genetics Department, University Hospital, Nijmegen, The Netherlands), were labelled with biotin by nick translation (Bio Nick Labelling System, BRL Life Technologies, USA) and used individually as FISH probes on metaphase chromosomes.11 Hybridisation was carried out for 16 hours at 37°C, and signals were developed with fluorescein labelled avidin and biotinylated anti-avidin. The slides were mounted in antifading medium containing DAPI as counterstain, and viewed using a cooled CCD camera and image analysis system (Digital Scientific Ltd, UK). G banding was enhanced using the Smartcapture software.

Each of the three cosmids, A10, G8, and M8, hybridised to the normal X chromosome at Xp11.3. The three cosmids also hybridised to the translocated X proximal to the breakpoint. No hybridisation signals were seen on the derived chromosome 1 (fig 2). This would suggest that the Norrie gene is intact in this patient.

Mutation screening
DNA was prepared from cultured fibroblasts by standard methods and mutation screening by single strand conformation polymorphism (SSCP) analysis was kindly performed by Dorien Van de Pol (Human Genetics Department, University Hospital, Nijmegen, The Netherlands). No aberrant fragments for the coding part of the Norrie disease gene were detected, suggesting that there is no mutation in this region in this fetus, although it does not completely exclude the possibility of a mutation there, or of a mutation elsewhere in the gene outside the open reading frame.

Cytogenetic studies
Blood, umbilical cord, spleen, ovary, and skin from the fetus were cultured separately, and

Figure 1. Macrosopic appearance of a transverse section of (above) right eye and (below) left eye, showing an organised retrolenticular fibrotic mass more marked in the right eye than the left.

organising delicate fibrous membrane. Microscopically, the presence of a persistent primary hyperplastic vitreous in both eyes was confirmed, arising as a result of the prolonged presence of the hyaloid artery within the eyes and the subsequent development of a fibrovascular membrane. In the retinas there were some artefactual Lange’s folds, but no evidence of true retinal dysplasia. As appropriate for 19 weeks gestation, photoreceptor formation was not complete and active cell division and neuroblastic activity was present. There was thus no evidence of a primary neuroectodermal defect at this stage. It was considered that the postlenticular masses observed were capable of evolving into an organised vitreous which would have resulted in retinal traction and detachment. The findings thus appeared to be consistent with the early stages of Norrie disease.
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**Figure 2** Fluorescence in situ hybridisation with cosmids G8 showing a signal on the short arm of the normal X chromosome, and proximal to the breakpoint on the der(X) (solid arrow heads), but no signal on the derived chromosome 1.

**Discussion**

This fetus with a balanced X:1 reciprocal translocation and the breakpoint at Xp11.4 showed distinctive eye pathology which is consistent with reported examples of Norrie disease. There are two other published cases of Norrie disease arising as a result of a chromosomal rearrangement. In one, a pericentric inversion of the X chromosome led to the assignment of the gene locus to Xp11.4, while the other case, involving an X;10 balanced translocation, simply located it at Xp11. Thus at the outset it seemed that our fetus too had Norrie disease. In such cases, it is assumed that chromosome breakage occurs within, and disrupts, the normal gene.

However, linkage studies, usually in microdeletion cases, have all suggested that the Norrie disease gene is located at Xp11.3 rather than Xp11.4. In situ hybridisation studies in our case using three cosmids covering the entire Norrie gene confirmed its location at Xp11.3, and also showed that there was no obvious disruption of the Norrie disease gene in this fetus. In addition, SSCP analysis suggested that there is probably no mutation in the coding region of the Norrie disease gene. The gene is 27 kb long, and contains three exons. The open reading frame occurs in the last part of exon 2 and the first part of exon 3. The possibility is excluded that the breakpoint was within the most distal part of exon 3, because that region lies approximately 20 kb from the distal end of cosmid M8, and so a hybridisation signal would have been expected to be visible on the derived chromosome 1 in the FISH analysis. Adjacent to the untranscribed region of exon 1 is a 5' regulatory sequence. Since the 5' end of the gene is orientated towards the centromere, it does not seem likely in our case that, even if there was no disruption in the gene itself, this regulatory region has been affected by the translocation. Another possibility, that the translocation breakpoint exerts a position effect on the Norrie disease gene, cannot be excluded, for translocation breakpoints more than 100 kb from SOX9 and PAX6 are known to be associated with the respective disease phenotype. In the latter case, the breakpoint was 3' to the gene. Overall, however, there is no evidence for a translocation or large deletion of the Norrie disease gene itself in this fetus.

There are several possibilities. There could be a point or other small mutation in a region of the Norrie disease gene other than the coding sequence which has arisen de novo in addition to the translocation. However, this is considered unlikely. Another possibility is that the fetus has a different eye disorder, either a previously unrecognised "Norrie-like" disease or because one of the other eye disease genes known to be in the same region of the X chromosome, or a dominant gene on chromosome 1, has been disrupted by the translocation. Retinitis pigmentosa 2 is located at Xp11.4; however, it, and the other disorders which map to that region of Xp are excluded because their pathology in no way resembles that found in the fetus. There are no known eye disease genes in the region of chromosome 1 involved, but a retinoblastoma binding protein is possibly located there. The cloning of the breakpoints in this case could be a strategy to identify a gene for a Norrie-like syndrome.

It may be of relevance to note that it has recently been found that X linked familial exudative vitreoretinopathy, previously thought by linkage data to involve a gene close to that of Norrie disease, actually results from a mutation in a highly conserved region of the Norrie disease gene itself, suggesting allelic heterogeneity at the Norrie locus. Because of the lack of knowledge of early pathology, X linked familial exudative vitreoretinopathy cannot be excluded in this fetus.

The fact remains that despite the molecular genetic findings, the eyes of this fetus clearly show pathological features consistent with the presumed early stages of Norrie disease. However, because of its rarity, the early stages of the pathogenesis are not delineated. A previously reported 11 week fetus, diagnosed as affected by the use of genetic markers, had histologically normal eyes, leading to the suggestion that the primary abnormality of vascular proliferation probably occurs in relation to persistent hyperplastic primary vitreous body after 14 weeks. A second case from the third trimester suggests that by then overt retinal detachment can have occurred, for diagnosis was made in utero at 34...
weeks by sonography. The globes had appeared normal on the scan at 28 weeks but the detachments were total at 34 weeks. The findings in our case at 19 weeks were consistent with an intermediate stage, with the pathogenetic process initiated. There was persistent primary hyperplastic vitreous body but no macroscopic retinal detachment. The X inactivation studies showed that in all cells examined, as far as it is possible to ascertain, the normal X was completely inactivated. If this is reflected throughout the body, then the milder phenotype observed in the eyes compared with what is seen later in gestation and postnatally cannot be explained by incomplete X inactivation, and is a true statement of early pathogenesis.

Our case is also interesting because, like that of Ohba and Yamashita involving Norrie disease and an X;10 translocation, the fetus was female. These are rare examples of an X linked recessive gene being manifest in females. Usually this is because of what is termed “non-random X inactivation.” This has been confirmed in this fetus, where every cell examined has the normal X chromosome inactivated. However, it is important to recognise that the actual origin of this observation is probably not preferential inactivation of one of the X chromosomes. Rather, it is believed that at the outset, inactivation occurred randomly, but subsequently there was cellular selection in favour of those cells with the most complete genetic constitution. This is represented by the cells with the translocated X which bears the major part of an autosome, without which a person could not survive. Ultimately, in all cells, the normal X chromosome bearing the normal allele at the relevant locus is thus inactivated, and the disrupted allele (or other mutation if this is the case) on the active translocated chromosome is expressed.

Finally, since this case suggests the possibility that what is currently regarded as one severe, early onset eye disease may actually be two similar disorders caused by mutations at separate but relatively close loci, there are practical implications. It is important that this possibility is taken into account, particularly by those undertaking the prenatal diagnosis of Norrie disease by linkage analysis.

We are extremely grateful to Dr Wolfgang Berger for the gift of the cosmids and to Dorien Van de Pol for performing the SSCP studies.

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