

MUTATION IN BRIEF

Missense Mutation (Arg121Trp) in the Norrie Disease Gene Associated With X-Linked Exudative Vitreoretinopathy

Sigrid Fuchs*, Ulrich Kellner, Heike Wedemann, and Andreas Gal

Institut für Humangenetik, Medizinische Universität, D-23538 Lübeck (S.F., H.W., A.G.), Augenklinik und Poliklinik, Universitätsklinikum Benjamin Franklin der FU Berlin, D-12200 Berlin, Germany (U.K.), Fax: 49-40-4717-5138

Communicated by Peter Humphries

Familial exudative vitreoretinopathy (EVR) is an hereditary condition characterized by local peripheral vascular anomalies or absent peripheral vascularization of the retina (Criswick and Schepens, 1969). The ophthalmological features are very similar to those seen in retinopathy of prematurity. The clinical manifestation can be extremely variable and ranges from peripheral vitreous strands to retinal folds and traction. In the most severe cases, complete retinal detachment may lead to phthisis bulbi (shrinkage of the globe). In the majority of cases, EVR is inherited in an autosomal dominant manner (adEVR, McKusick No. 133780), but a few families with X-linked inheritance (XEVR, McKusick No. 305390) have also been reported (Godel et al., 1978; Plager et al., 1992; Fullwood et al., 1993). Norrie syndrome is an X-linked recessive disorder (for review, see Warburg, 1966), which shares some similarity with EVR in its ophthalmological features. However, the eye condition in Norrie disease appears to be at a more advanced stage at birth. Furthermore, Norrie disease is often associated with progressive sensorineural deafness and/or mental retardation, never observed in XEVR. The Norrie disease gene has recently been cloned (Berger et al., 1992a; Chen et al., 1992), and various mutations of the gene have been identified in many patients (Berger et al., 1992b; Meindl et al., 1992). Very recently, a missense mutation (L124F) in the Norrie disease gene has also been detected in a family with XEVR (Chen et al., 1993).

Here we present the results of a molecular genetic analysis of the Norrie disease gene in a 29-year-old male patient with X-linked EVR. Low visual acuity of the propositus has been noted since birth. When he was three years old, the right eye was enucleated because an intraocular tumor was

suspected after recurrent iritis. Histological examination revealed severe intraocular inflammation without any malignancy. Visual acuity on the left eye remained unchanged until adulthood. In the last few years a slight decrease of visual acuity was noted. At last examination, he was 29 years old. Visual acuity was 20/300 with medium myopia (-3.0 diopters) and accompanying nystagmus. In the left eye, the anterior segment was normal. The retina showed severe temporal dragging of the vessels, including the macula. In the area of the macula, a scar with peripheral hyperpigmentation and central depigmentation was seen. At last examination, no exudates were present. However, it cannot be excluded that areas of central and peripheral scars have been partly induced by retinal exudates. The peripheral retina showed pigmentation between the equator and the ora serrata on the temporal side. Nasally, equatorial degenerations were present.

Severely reduced visual acuity is known in several maternal male relatives. The mother and maternal grandmother of the propositus have normal visual acuity. Two sisters and a brother of the mother as well as their children (two sons, two daughters) have normal visual acuity, as have the two daughters of the propositus. However, an ophthalmoscopic examination of these family members has not yet been performed. In conclusion, the characteristic funduscopic picture, the low (but still retained) visual acuity, and the lack of extraocular manifestations together with the family history strongly suggest that the patient de-

Received July 15, 1994; accepted October 3, 1994.

*To whom reprint requests/correspondence should be addressed.



FIGURE 1. Detection of the C-to-T transition in codon 121 of the Norrie disease gene by *Msp*I restriction analysis. Fragment lengths are given in base pairs (bp) on the right side. M, molecular-weight standard (pBR322/*Msp*I). Lane 1, unaffected female control.

scribed in this communication has XEVR, rather than Norrie disease.

We analyzed genomic DNA of the patient by single-strand conformation polymorphism (SSCP) following polymerase chain reaction (PCR) amplification of the three exons of the Norrie disease gene (experimental conditions and primers are described in Berger et al., 1992b). The PCR product of exon III gave an aberrant SSCP pattern (not shown). Direct sequencing revealed a C-to-T transition of the first nucleotide (pos. 777 in Berger et al., 1992a) in codon 121 that predicted the replacement of arginine-121 by tryptophan (R121W) in the Norrie protein. This C-to-T substitution destroys one of the two *Msp*I restriction sites that are present in the wild-type sequence, giving rise to a novel fragment of 153 base pairs. Using *Msp*I restriction analysis, we were able to show that both the patient's mother and maternal grandmother carry one mutant allele (Fig. 1). No change in the *Msp*I restriction pattern has been detected in 60 X chromosomes of unrelated controls.

Little is known about why different mutations of the Norrie gene lead to different phenotypes. Molecular modeling suggests that the Norrie disease protein has a 3-D structure with a "cystine knot motif" similar to that of transforming growth factor- β (TGF- β). It has been speculated that the Norrie protein plays a regulatory role in the development of the neuroretina and central nervous system (Meitinger et al., 1993). In patients with Norrie disease, the majority of mutations are located in

exon III of the Norrie disease gene (Berger et al., 1992a; Meindl et al., 1992) and are thought to alter considerably the protein's secondary structure, and especially the conserved "cystine knot motif". The R121W mutation reported here is located in the C-terminal end of the Norrie protein. The amino acid substitution results in the replacement of a basic residue by a nonpolar one that predicts a change of the secondary structure and consequently may well disturb the function of the Norrie protein. The fact that the characteristic "cystine knot motif" does not seem to be affected directly may explain the less severe phenotype associated with the R121W mutation. However, a patient with Norrie disease and R121Q was recently reported in a large Spanish family (Fuentes et al., 1993). Obviously, different missense mutations of the same codon in the Norrie disease gene can cause either Norrie disease or XEVR, suggesting that additional factors may also modulate the final clinical manifestation.

So far a single missense mutation (L124F) in the Norrie disease gene has been reported in a family with XEVR (Chen et al., 1993). Therefore, R121W is the second amino acid substitution which is considered to be responsible for XEVR. Thus our data provide further evidence that Norrie disease and XEVR are allelic disorders.

ACKNOWLEDGMENTS

This work was financially supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

- Berger W, Meindl A, van de Pol TJR, Cremers FPM, Ropers HH, Doerner C, Monaco A, Bergen AAB, Lebo R, Warburg M, Zergollern L, Lorenz B, Gal A, Bleeker-Wagemakers EM, Meitinger T (1992a) Isolation of a candidate gene for Norrie disease by positional cloning. *Nature Genet* 1:199-203.
- Berger W, van de Pol D, Warburg M, Gal A, Bleeker-Wagemakers L, de Silva H, Meindl A, Meitinger T, Cremers F, Ropers HH (1992b) Mutations in the candidate gene for Norrie disease. *Hum Mol Genet* 7:461-465.
- Chen Z-Y, Hendriks RW, Jobling MA, Powell JF, Breakefield XO, Sims KB, Craig IW (1992) Isolation and characterization of a candidate gene for Norrie disease. *Nature Genet* 1:204-208.
- Chen Z-Y, Battinelli EM, Fielder A, Bunday S, Sims K, Breakefield XO, Craig IW (1993) A mutation in the Norrie disease gene (NDP) associated with X-linked familial exudative vitreoretinopathy. *Nature Genet* 5:180-183.
- Criswick VG, Schepens CL (1969) Familial exudative vitreoretinopathy. *Am J Ophthalmol* 68:578-594.
- Fuentes J-J, Volpini V, Fernandez-Toral F, Coto E, Estivill X (1993) Identification of two new missense mutations (K58N and R121Q) in the Norrie disease (ND) gene in two Spanish families. *Hum Mol Genet* 2:1953-1955.
- Fullwood P, Jones J, Bunday S, Dudgeon J, Fielder AR, Kilpatrick MW (1993) X-linked exudative vitreoretinopathy: Clinical

- features and genetic linkage analysis. *Br J Ophthalmol* 77:168–170.
- Godel V, Romano A, Stein R, Avinoam A, Goodman RM (1978) Primary retinal dysplasia transmitted as X-linked recessive disorder. *Am J Ophthalmol* 86:221–227.
- Meindl A, Berger W, Meitinger T, van de Pol D, Achatz H, Doerner C, Haasemann M, Hellebrand H, Gal A, Cremers F, Ropers HH (1992) Norrie disease is caused by mutations in an extracellular protein resembling C-terminal globular domain of mucins. *Nature Genet* 2:139–143.
- Meitinger T, Meindl A, Bork P, Rost B, Sander C, Haasemann M, Murken J (1993) Molecular modelling of the Norrie disease protein predicts a cysteine knot growth factor tertiary structure. *Nature Genet* 5:376–380.
- Plager DA, Orgel IK, Ellis FD, Hartzer M, Trese MT, Shastry BS (1992) X-linked recessive familial exudative vitreoretinopathy. *Am J Hum Genet* 114:145–148.
- Warburg M (1966) Norrie's disease: A congenital progressive oculo-acustico-cerebral degeneration. *Acta Ophthalmol* 89:1–147.