Ophthalmic Genetics – 1996, Vol. 17, No. 2 pp. 67-74
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Buren (The Netherlands) 1996

Accepted 23 March 1996

Ocular phenotypes associated with two mutations (R121W, C126X) in the Norrie disease gene

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Abstract *Purpose:* To describe the ocular phenotypes associated with 2 mutations in the Norrie disease gene including a manifesting carrier. *Methods*: Ophthalmological examinations were performed in 2 affected males and one manifesting carrier. Genomic DNA was analyzed by direct sequencing of the Norrie disease gene. *Results:* Family 1: A 29-year-old male had the right eye enucleated at the age of 3 years. His left eye showed severe temporal dragging of the retina and central scars. Visual acuity was 20/300. DNA analysis revealed a C-to-T transition of the first nucleotide in codon 121 predicting the replacement of arginine-121 by tryptophan (R121W). Both the mother and maternal grandmother carry the same mutation in heterozygous form. Family 2: A 3-month-old boy presented with severe temporal dragging of the retina on both eyes and subsequently developed retinal detachment. Visual acuity was limited to light perception. His mother's left eye was amaurotic and phthitic. Her right eye showed severe retinal dragging, visual acuity was reduced to 20/60. DNA analysis revealed a T-to-A transversion of the third nucleotide in codon 126 creating a stop codon (C126X). The mother and maternal grandmother were carriers. *Conclusion:* Mutations in the Norrie disease gene can lead to retinal malformations of variable severity both in hemizygous males and manifesting carriers.

Key words Norrie disease; X-linked exudative vitreoretinopathy; X-linked primary retinal dysplasia; manifesting carrier

Introduction The Norrie disease (ND) gene maps on the proximal short arm of the X chromosome (Xp11.4-p11.3) and has recently been cloned.[1,2] The tertiary structure of the ND gene product has been modelled and revealed similarity with a group of cysteine-rich proteins including transforming growth factor β, nerve growth factor, platelet-derived growth factor, and glial cell-derived neurotrophic factor.[3] These proteins have in common a cen-

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Acknowledgement: This study was financially supported by the Deutsche Forschungsgemeinschaft (Ga 210/5-4).

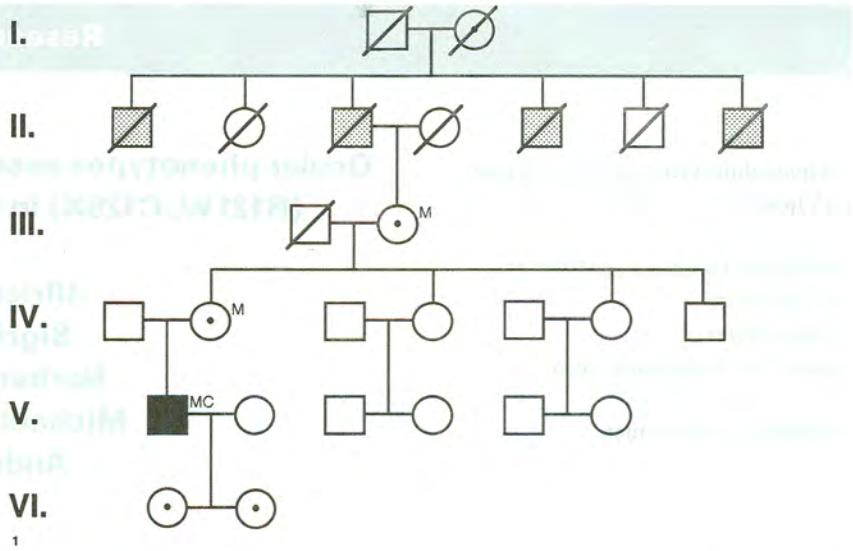

Fig. 1. Pedigree of family 1. Black symbol indicates patient, grey symbols probably affected family member. Dots indicate obligate carriers or those identified by molecular genetic analysis. M indicates that molecular genetic analysis was performed, C that clinical examination was performed.

tral 'cystine knot motif' consisting of three disulfide bridges each interconnecting two different cysteine residues. Some of these proteins play a regulatory role in the development of the central nervous system. It has been speculated that the ND gene product plays a regulatory role in the development of the retina as well as the central nervous system.[3]

Mutations in the ND gene are associated with three forms of retinal malformation: Norrie disease (McKusick #310600);[4-14] X-linked exudative vitreoretinopathy (XEVR; McKusick #305390);[15-19] and X-linked primary retinal dysplasia (XPRD; McKusick #312550).[10,20]

Norrie disease is characterized by retinal dysplasia at birth which progresses to bilateral retinal detachment and blindness during early infancy. Mental retardation and progressive hearing loss may develop in 25-50% of patients with Norrie disease.[21] In XEVR, the retina may be either detached or attached with peripheral avascular areas at birth. During the first two decades of life, progressive retinal malformation develops ranging from retinal folds to complete retinal detachment.[22-25] While amaurosis can be present, reduced visual function is retained in several patients. Mental retardation and hearing loss have not been described in XEVR. Ocular involvement in general is more severe in XEVR compared to autosomal dominant exudative vitreoretinopathy.[25,26] XPRD is characterized by congenital retinal folds with severely reduced visual acuity.[27] Mental retardation and hearing loss have not been observed.

The purpose of this study is to describe the ophthalmic findings associated with 2 mutations in the ND gene including a pedigree with a manifesting carrier.

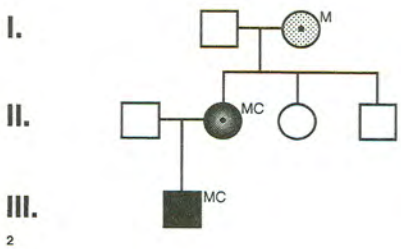

Fig. 2. Pedigree of family 2. Black symbol indicates patient, shaded symbol (II/2) manifesting carrier, grey symbol (I/2) probable manifesting carrier. M indicates that molecular genetic analysis was performed, C that clinical examination was performed.

Patients and methods Three patients, two males and a female, from two families were examined by routine ophthalmologic examinations (Figs 1, 2). Data of other family members as well as blood samples of patients and family members were obtained after informed consent was given. All examinations were performed according to the guidelines of the 'Declaration of Helsinki'.

Genomic DNA was analyzed by single strand conformation polymorphism

(SSCP) and direct sequencing following amplification of the 3 exons of the ND gene by polymerase chain reaction (PCR). The experimental conditions and primers were the same as described by Berger et al.[4]

Results

FAMILY 1 *Clinical evaluation* A 29-year-old male (V/1, Fig. 1) had been born with normal birth weight at estimated term. Low visual acuity was present since birth. At the age of 3 years, an intraocular tumor was suspected in the right eye because of retinal detachment and recurrent inflammation and the eye was enucleated. Histologic examination revealed severe intraocular inflammation without any malignancy. Further details of the histologic examination are not available. Visual acuity in the left eye remained unchanged until adulthood. In the last few years, a slight decrease of visual acuity was noted subjectively. An oscillating nystagmus was present. No signs of hearing loss or mental retardation were present.

At the age of 29 years, visual acuity was 20/300 (-3.0 D). The anterior segment was normal. The retina showed severe temporal dragging of the vessels including the macula (Fig. 3). At the posterior pole, a scar with peripheral hyperpigmentation and central depigmentation was seen, which may be related to previous exudative changes. The peripheral retina showed pigmentation between the equator and the ora serrata on the temporal side. Nasally, equatorial degenerations were present. No avascular areas or active exudation were seen.

The father of the propositus is unknown to him. The maternal grandmother of the propositus knew of severely reduced visual acuity in several deceased male family members, including her father (Fig. 1). However, further details on the course of the disease or results of an ophthalmologic examination are not available. The grandmother and mother of the propositus have normal visual acuity as do other living family members.

Molecular genetic evaluation The results of molecular genetic analysis in this family have been reported previously.[18] The PCR product of exon III gave an aberrant SSCP pattern. Direct sequencing revealed a C-to-T transition of the first nucleotide 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 MspI restriction sites present in the wild-type sequence. Using MspI restriction analysis, we could show that both the patient's mother and maternal grandmother carry one mutant allele (results not shown). No change in the MspI restriction pattern has been detected in 60 X chromosomes of unrelated controls.

FAMILY 2 *Clinical evaluation* The boy (III/1), Fig. 2) was born with a birth weight of 3350 g at estimated term. General pediatric examination revealed normal findings. At the age of 3 months, the parents detected a leukocoria on the right eye. There was no fixation of objects with both eyes. The only reaction to light was lid closure at bright illumination. No nystagmus was noted.

The child was examined under general anesthesia at the age of 3 months. The right eye (axial length: 17.6 mm) was smaller than the left one (19.3 mm) and showed corneal edema, flat anterior chamber, iris atrophy with posterior

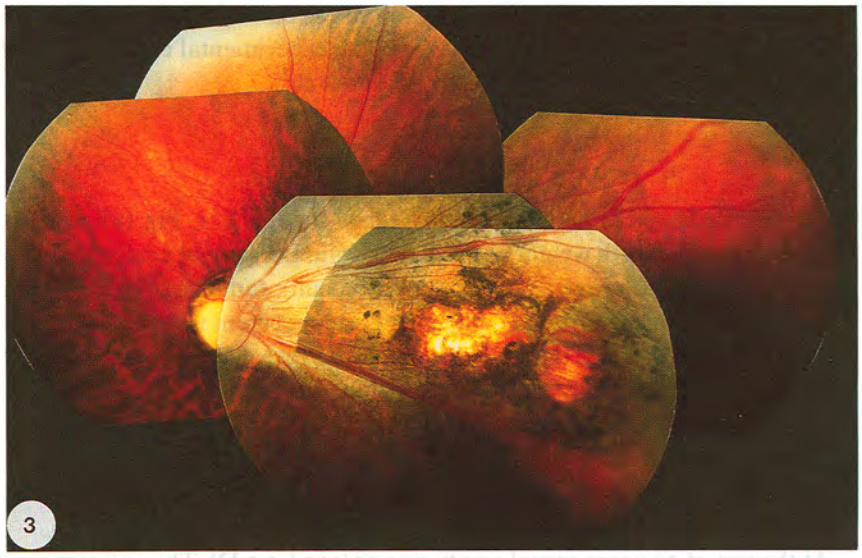

Fig. 3. Severe temporal dragging of retinal vessels and macular scars in the left eye of the 29-year-old male (V/1) of family 1. Visual acuity was 20/300. The right eye was enucleated.

synechiae, circumferential anterior traction on the ciliary processes, medium diffuse lens opacities, and dense opacities within the vitreous with no visibility of the retina. Ultrasonography revealed a temporal retinal fold. The left eye had clear cornea and lens, normal anterior chamber, and mild circumferential traction on the ciliary processes. There was a dense central vitreous opacity posterior to the lens. The origin of this vitreous opacity could not be defined, a long-standing vitreous hemorrhage or a partially persistent primary vitreous are possible explanations. The retina showed a severe traction to the temporal side with a retinal fold from the optic disc to the upper temporal periphery. In connection with the fold, few vitreous hemorrhages were seen near the retina. No calcification was seen in the eyes by ultrasonography or CT scan. The intraocular pressure was normal in both eyes. During the first year of life, progressive retinal detachment with anterior traction was followed by further flattening of the anterior chamber and secondary glaucoma. Lensectomy was performed at the age of 6 months on the right eye and at the age of 12 months on the left eye with successful control of secondary

Fig. 4. Severe temporal dragging of retinal vessels and peripheral scars in the right eye of the 31-year-old manifesting carrier (II/2) of family 2. Visual acuity was 20/60 in this eye. There was no fundus visibility in the amaurotic left eye.

glaucoma. No signs of hearing loss or mental retardation were noted.

The mother (II/2, Fig. 2) of the propositus had low vision in both eyes since childhood. She underwent retinal surgery on the right eye in her first year of life, but detailed information on the procedure is not available. The left eye became phthitic and amaurotic during childhood. At the age of 31 years, visual acuity was 20/60 in the right eye. In the right eye, severe temporal dragging of the retina including the macula was seen (Fig. 4). Several scars were present in the temporal periphery, which may be related to retinal surgery. There was no fundus visibility in the left eye due to lens opacities. Hearing and mental development were normal.

One eye of the maternal grandmother of the propositus was enucleated due to an unknown cause. The other eye was said to be normal, as was the vision in all other known family members (Fig. 2). Ophthalmoscopic examinations were not performed in any of the family members.

Molecular genetic evaluation The results of molecular genetic analysis in this family have been reported previously.[14] In the boy's DNA, a T-to-A transversion of the third nucleotide of codon 126 in the ND gene was detected. This sequence alteration creates a stop codon TGA (C126X), predicting a premature termination of protein synthesis. The resulting protein should lack the last 8 amino acids of the wild-type Norrie protein, including 3 conserved cysteine residues involved in the formation of the 'cystine knot motif', suggesting that the C126X mutation is responsible for the development of Norrie disease in the propositus. The T-to-A substitution destroys a MaeIII restriction site present at this position in the wild-type sequence. MaeIII restriction analysis of additional family members revealed that the patient's mother and maternal grandmother carried the same mutation (results not shown). No mutation of codon 126 was identified in 60 unrelated control X chromosomes.

Discussion The point mutations identified in both families are considered the primary cause for the development of retinal malformation. The mutations were not observed in 60 unrelated control X chromosomes. The R121W mutation identified in family 1 has recently been reported in two unrelated families with XEVR.[17,19] Two different mutations in the same codon were associated with XEVR,[16] XPRD,[10,17] or Norrie disease.[7] In family 2, the stop mutation C126X is present in the propositus and his mother, who is probably a manifesting carrier.

Which diagnosis can be attributed to our patients based on clinical findings? The attached retina with temporal dragging in the remaining eye, the retained visual acuity, and the lack of extraocular manifestations in the patient in family 1 suggest XEVR. The course of the disease in the other eye, however, was more progressive and enucleation was required in early infancy. In family 2, the young child was functionally blind at birth and progressive retinal detachment developed in both eyes within the first year of life. This course is typical for Norrie disease. Clinically, Norrie disease and XEVR appear to be different progressed stages of the same disease.

Clinical and molecular genetic findings in these two families as well as findings in similar families reported to date raise the question whether Norrie disease, XEVR, and XPRD are similar or dissimilar based on clinical findings. Several families were reported as having XEVR.[15,16,22-25] XEVR and XPRD[27] appear to be the same entity. Clinical findings in XPRD with congenital retinal

folds and retained visual acuity are similar to XEVR and the R121G mutation in the ND gene has been reported in one XPRD family[10] (additional details in Meindl et al.[17]). Meindl et al.[17] described clinical findings similar to XEVR as 'less severe course of Norrie disease'. The families described with XEVR, XPRD, and a 'less severe course of Norrie disease' have in common that neither mental retardation nor hearing loss were present. In each family, one or more affected patients retained some visual acuity; however, blindness at birth was present in other patients. Moreover, progressive retinal detachment and amaurosis within the first decades of life were observed in several patients. In contrast, other authors have described families with the clinical diagnosis of Norrie disease in which all affected patients were blind at birth but had no mental retardation or hearing loss.[7] One might be tempted to separate XEVR with retained visual acuity from Norrie disease with early blindness and with or without extraocular manifestation. This terminological differentiation, however, has not much relevance because it depends largely on family size and number of affected patients. Family counselling must mention that early blindness may occur in every affected patient. However, in families with a patient who retained vision, mental retardation and hearing loss are unlikely to develop.

In the absence of a gross structural abnormality of the X chromosome, nonrandom X inactivation is one of the most frequent causes for occurrence of X-linked phenotypes in females. In family 2, both the mother and grandmother of the propositus are heterozygous for the C126X mutation. The mother developed a retinal fold in one eye and a phthisis in the other one. In the maternal grandmother, one eye was enucleated probably for a similar reason. Two female cases have been reported to date with the clinical picture of Norrie disease. In a young girl, normal ophthalmoscopic findings at birth with development of retinal detachment at the age of 2 years were associated with a C69S mutation of the ND gene.[6,28] In a second female patient, an X-autosome translocation with breakpoint in Xp11.3-p11.4 was associated with full-blown Norrie disease.[29] Possibly, a third manifesting carrier has been reported, but no molecular genetic data are available.[30] In XPRD, which resembles XEVR, congenital retinal folds have been observed in females.[27] Consequently, manifesting females may be expected in all families with ND. Nevertheless, after reviewing the literature, it seems that the overall frequency of manifesting carriers is low. It is not known which factors are responsible for the mild disease manifestation in the two carriers in family 2. Skewed X inactivation may one explanation. In the mother's blood leukocytes, however, there was only a slight shift in the pattern of X inactivation, as has been obsered in another female carrier.[6]

In contrast to other retinal diseases (e.g. retinitis pigmentosa), in which molecular genetic analysis has revealed multiple mutations in several genes, mutations in the Norrie disease gene have been identified in three disorders, which before have been differentiated based on clinical findings. Due to the variability of clinical findings in Norrie disease, XEVR, and XPRD, it is difficult to distinguish between these diseases based on clinical data. The analysis of genotype-phenotype correlation suggests that point mutations in certain regions of the gene are frequently associated with isolated eye pathology

Codon	*Amaurosis*		*Deafness*	*Mental retardation*	*Reference*
	Yes	*No*			
Splice site (E1/I1)	+		+	+	14
Splice site (E2/I2)			–	+	4
L13R	+		+	+	11
S29X	ND				5
C39R	+		+		9
Y44C	+		–	+	5
S57X			+	+	4
K58N	+		?	+	7
V60Q	ND				5
L61F			+	–	4
L61P	ND		–	–	13
L62P	ND				10
A63D	ND		–	–	13
C65W	ND		?	?	13
C69S			+	+	6
R74C	?		+	–	4, 10
S75C			+	+	4
R90P	ND		–	–	4
C95R	+		–	–	12
C96Y			–	+	4, 5
K104Q		+	–	–	17
R109X	ND		+	+	13
C110R	+		+	–	10, 14
C110X			?	–	4
R121G	XEVR/XPRD				10, 16
R121Q	variable		–	–	7, 17
R121W	late		–	–	17-19, this paper
I123N	ND		+	+	13
L124F	late		–	–	15
C126X	+		–	–	14, this paper
C128X	+		–	+	8, 13

+: present in at least one patient; –: absent in all patients; ?: uncertain; E: Exon; I: Intron.
Retinal malformation was diagnosed but no specific details were given. ND: Norrie disease; XEVR: X-linked exudative vitreoretinopathy; XPRD: X-linked primary retinal dysplasia.

(Table 1), although exceptions have also been reported.[13] Further analysis of clinical and molecular genetic findings in more families is necessary to determine whether or not these three diseases are distinguishable. Therefore, it is important that molecular genetic reports on Norrie disease or XEVR also include basic clinical data to facilitate comparison of different patients and their respective clinical courses.

References

1 Berger W, Meindl A, Van de Pol TJR, et al. Isolation of a candidate gene for Norrie disease by positional cloning. Nat Genet 1992; 1: 199-203.

2 Chen Z-Y, Hendriks RW, Jobling MA, et al. Isolation and characterization of a candidate gene for Norrie disease. Nat Genet 1992; 1: 204-208.

3 Meitinger T, Meindl A, Bork P, et al. Molecular modelling of the Norrie disease protein predicts a cysteine knot growth factor tertiary structure. Nat Genet 1993; 5: 376-380.

4 Berger W, Van de Pol D, Warburg M, et al. Mutations in the candidate gene for Norrie disease. Hum Mol Genet 1992; 7: 461-465.

5 Meindl A, Berger W, Meitinger T, et al. Norrie disease is caused by mutations in a extracellular protein resembling C-terminal globular domain of mucins. Nat Genet 1992; 2: 139-143.

6 Chen Z-Y, Battinelli EM, Woodruff G, et al. Characterization of a mutation within the NDP gene in a family with a manifesting carrier. Hum Mol Genet 1993; 2; 1727-1729.

7 Fuentes JJ, Volpini V, Fernandez-Toral F, Coto E, Estivill X. Identification of two new missense mutations (K58N and R121Q) in the Norrie disease (ND) gene in two Spanish families. Hum Mol Genet 1993; 2: 1953-1955.

8 Wong F, Goldberg MF, Hao Y. Identification of a nonsense mutation at codon 128 of the Norrie disease gene in a male infant. Arch Ophthalmol 1993; 111: 1553-1557.

9 Joos KM, Kimura AE, Vandenburgh K, Bartley JA, Stone EM. Ocular findings associated with a Cys39Arg mutation in the Norrie disease gene. Arch Ophthalmol 1994; 112: 1574-1579.

10 Zhu D, Maumenee IH. Mutation analysis of the Norrie disease gene in eleven families. Invest Ophthalmol Vis Sci 1994; 35: 1265.

11 Fuchs S, Xu SY, Caballero M, et al. A missense point mutation (Leu13Arg) of the Norrie disease gene in a large Cuban kindred with Norrie disease. Hum Mol Genet 1994; 3: 655-656.

12 Isashiki Y, Ohba N, Yanagita T, et al. Mutations in the Norrie disease gene: a new mutation in a Japanese family. Br J Ophthalmol 1995; 79: 703-704.

13 Schuback DE, Chen ZI, Craig IW, Breakefield XO, Sims KB. Mutations in the Norrie disease gene. Hum Mutat 1995; 5: 285-292.

14 Fuchs S, Van de Pol D, Beudt U, et al. Three novel and two recurrent mutations of the Norrie disease gene in patients with Norrie syndrome. Hum Mutat 1996; in press.

15 Chen Z-Y, Battinelli EM, Fielder A, et al. A mutation in the Norrie disease gene (NDP) associated with X-linked familial exudative vitreoretinopathy. Nat Genet 1993; 5: 180-183.

16 Johnson K, Mintz-Hittner H, Perry YM, Ferrell RE. X-linked familial exudative vitreoretinopathy caused by an arginine to leucine substitution in exon 3 of the Norrie gene. Am J Hum Genet 1994; 55 (Suppl): A 1309.

17 Meindl A, Lorenz B, Achatz H, et al. Missense mutations in the NDP gene in patients with a less severe course of Norrie disease. Hum Mol Genet 1995; 4: 489-490.

18 Fuchs S, Kellner U, Wedemann H, Gal A. Missense mutation (Arg121Trp) in the Norrie disease gene associated with X-linked exudative vitreoretinopathy. Hum Mutat 1995; 6: 257-259.

19 Shastry BS, Hejtmancik JF, Plager DA, Hartzer MK, Trese MT. Linkage and candidate gene analysis of X-linked familial exudative vitreoretinopathy. Genomics 1995; 27: 341-344.

20 Ravia Y, Braier-Goldstein O, Bat-Miriam KM, et al. X-linked recessive primary retinal dysplasia is linked to the Norrie disease locus. Hum Mol Genet 1993; 2: 1295-1297.

21 Warburg M. Norrie's disease: a congenital progressive oculo-acoustico-cerebral degeneration. Acta Ophthalmol 1966; 89: 1-147.

22 Dudgeon J. Familial exudative vitreoretinopathy. Trans Ophthalmol Soc UK 1979, 99: 45-49.

23 Plager DA, Orgel IK, Ellis FD, et al. X-linked recessive familial exudative vitreoretinopathy. Am J Ophthalmol 1992; 114: 145-148.

24 Fullwood P, Jones J, Bundey S, et al. X-linked exudative vitreoretinopathy: clinical features and genetic linkage analysis. Br J Ophthalmol 1993; 77: 168-170.

25 Clement F, Beckford CA, Corral A, Jimenez R. X-linked familial exudative vitreoretinopathy. Report of one family. Retina 1995; 15: 141-145.

26 Criswick VG, Schepens CL. Familial exudative vitreoretinopathy. Am J Ophthalmol 1969; 68: 578-594.

27 Godel V, Goodman RM. X-linked recessive primary retinal dysplasia: clinical findings in affected males and carrier females. Clin Genet 1981; 20: 260-266.

28 Woodruff G, Newbury-Ecob R, Plaha DS, Young ID. Manifesting heterozygosity in Norrie's disease? Br J Ophthalmol 1993; 77: 813-814.

29 Ohba N, Yamashita T. Primary vitreoretinal dysplasia resembling Norrie's disease in a female: association with X autosome translocation. Br J Ophthalmol 1986; 70: 64-71.

30 Capella JA, Kaufmann HE, Lill FJ, Cooper G. Hereditary cataracts and microphthalmia. Am J Ophthalmol 1963; 56; 454-458.