MUTATION IN BRIEF

Three Novel and Two Recurrent Mutations of the Norrie Disease Gene in Patients With Norrie Syndrome

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We describe three novel mutations together with two recurrent point mutations in the Norrie disease gene of five unrelated patients with Norrie syndrome. The two recurrent sequence alterations were identified as de novo mutations in the cases studied.

Norrie disease is a rare X-linked inherited neurodevelopmental disorder characterized by congenital blindness, occasionally accompanied by progressive sensorineural deafness and different degrees of mental retardation (for review, see Warburg et al., 1966). Since the isolation and characterization of the disease gene (Berger et al., 1992a; Chen et al., 1992), several different sequence variations, mainly missense and nonsense point mutations, have been identified in patients with Norrie disease (Berger et al., 1992b; Meindl et al., 1992; Fuentes et al., 1993; Wong et al., 1993; Zhu and Maumenee, 1993; Fuchs et al., 1994).

We analysed the genomic DNA of five patients by polymerase chain reaction (PCR) amplification of the three exons and adjacent intronic sequences of the Norrie disease gene followed by single-strand conformation polymorphism (SSCP) analysis and direct sequencing of PCR products (for experimental procedures and oligonucleotide PCR primers see Berger et al., 1992b).

Patient 1 is a nine-year-old member of a three-generation family with Norrie disease. In addition to the typical ophthalmological features, affected males in the family exhibit both mental disturbances and hearing impairment. Molecular analysis revealed a G-to-A substitution of the first nu-

cleotide in intron 1 (pos. 209 + 1G→A according to the sequence given in Berger et al., 1992a) of the Norrie disease gene. This sequence alteration destroys the highly conserved splice donor site (AGgt) of exon I/intron 1. The G-to-A transition eliminates an Eco81I site present at this position in the wild-type sequence (Fig. 1A). Using Eco81I-digestion, family analysis was carried out (results not shown). A cosegregation of the mutation and the Norrie phenotype was found.

As the first exon of the Norrie disease gene is not translated (Meindl et al., 1992) the open reading frame, which starts in exon 2, should not be altered by the mutation found. However, incorrect splicing can lead to changes in important sequence information for transcript stability or translational efficiency, or both. Indeed, it has been shown for several different genes, e.g., the human ferritin gene (Aziz and Munro, 1987) that 5'-untranslated RNA regions may have translational control function.

Patient 2 is a two-year-old boy with bilateral leukocoria and hypoplastic iris, as well as posterior synechiae and microcornea in the left eye. Ultrasound and computer tomography scan showed that both eye chambers were filled up with material. The patient had no signs of deafness or psychomotor retardation. The family history was negative. DNA analysis revealed a G-to-C transversion of the first

TABLE 1. Novel Mutations Detected in the Norrie Disease Gene of Patients With Norrie Disease

Exon	Mutation	Nucleotide change	Amino acid change	Restriction site
I	Splice site	209 + 1G→A	?	−Eco81I
II	Splice site	590 + 1G→C	?	+DdeI
III	C126X	TGT→TGA	Cys→ter	−MaeIII

nucleotide in intron 2 (pos. 590 + 1G→C). This substitution creates a new DdeI restriction site at this position and can therefore be detected by DdeI digestion analysis. By this means, we showed that the patient's mother is heterozygous for this sequence alteration (Fig. 1B). The G-to-C substitution destroys the consensus splice donor site (AGgt) of exon II/intron 2. Because 9 bp downstream in the intron a sequence motif exists that represents a potential splice donor site (pos 590 + 9: aggt), it is possible that this cryptic site is used for splicing. If this was the case, the resulting transcript would contain an 11-bp insertion causing a shift in the open reading frame from codon 38 onward and a premature termination of translation after incorporation of 49 amino acids unrelated to the Norrie protein. Hence, as a consequence of this splice site mutation, the Norrie protein would lack a major part of its genuine amino acid sequence. It is interesting to note that a mutation (590 + 5G→C) affecting the fifth nucleotide of the same splice site has already been reported (Berger et al., 1992b; unpublished observation). In spite of the difference in the nucleotide exchange, the biological consequence of this latter mutation should be similar to that discussed for patient 2.

Patient 3 is a one-year-old boy. His parents noted that there was no fixation of objects with both eyes when he was 2.5 months of age. The only reaction to direct illumination was closure of the lids in bright light. In the right eye he presented with leukocoria, corneal edema, flat anterior chamber, iris atrophy with posterior synechiae, anterior traction on ciliary processes, medium diffuse lens opacities, and dense opacities in the vitreous with no visibility of the retina. The results of the examination of the left eye exhibited a milder stage of the same malformation. Clear cornea and lens, normal anterior chamber, and only mild traction on ciliary processes were seen. There was a dense central vitreous opacity posterior to the lens, and the retina showed a severe traction to the temporal side with a retinal fold from the optic disk to the upper temporal periphery. Ultrasonography revealed a temporal mass on the right eye comparable to the retinal fold in the left eye.

In the patient's DNA, a TGT-TGA substitution in codon 126 produced a stop codon (C126X). The resulting protein would lack the last 8 amino acids of the wild-type Norrie protein, including three conserved cysteine residues involved in the formation of the "cystine knot motif," a structural feature thought to represent a functionally important domain by adopting a three-dimensional structure similar to that of transforming growth factor-β (TGF-β) [Meindl et al., 1992; Meitinger et al., 1993). The expected gross changes should lead to instability of the mutant protein. The T-to-A substitution destroys a MaeIII restriction site present at this position in the wild-type sequence. MaeIII restriction analysis revealed that the patient's mother and maternal grandmother carried the same mutation (Fig. 1C).

The mother of the patient has had low vision on both eyes since birth. She underwent retinal surgery on the right eye before age one. Visual acuity in the right eye was 20/60 at age 31. On the same eye, severe temporal dragging of the retina, including the macula, has been detected. Because of lens opacity, the fundus was not visible in the phthitic left eye. The maternal grandmother had lost one eye attributable to unknown cause. The other eye was said to be normal, as was the vision in all other family members. However, detailed clinical data are not available. The eye condition of the patient's mother is puzzling. The overall clinical picture is highly suggestive of a heterozygote manifestation. In general, carriers of Norrie disease are asymptomatic. Nonetheless, a manifesting carrier with severely impaired vision at the age of two years and heterozygous for the C69S-mutation has recently been reported (Chen et al., 1993). The most plausible explanation for the clinical manifestation in heterozygotes is skewed X-inactivation (in the retina). However, in the mother's blood peripheral leukocytes, there was only a slight shift in the pattern of X-inactivation, as was the case for the other manifesting carrier described by Chen et al. (1993).

Patient 4 is a 12-year-old boy with pseudoglioma of both retinas compatible with Norrie disease and a missense mutation (C-to-G) in codon 74 (R74C) that has previously been reported in two other pa-
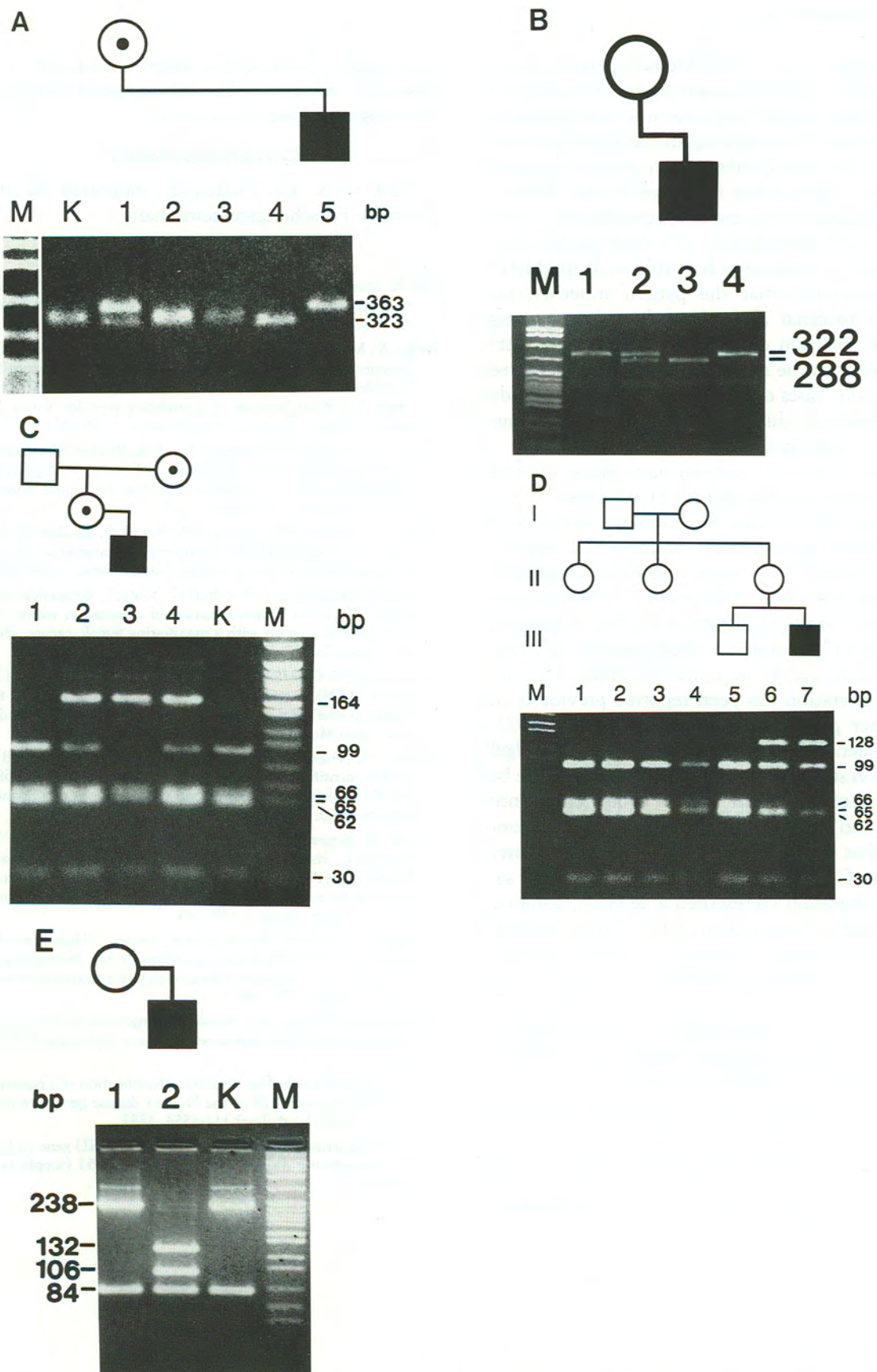
FIGURE 1. Detection of mutations in the Norrie disease gene by restriction analysis. Fragment lengths are given in base pairs (bp). M: DNA molecular weight marker pBR322, MspI digested. K: unrelated control DNA. ⊙: obligate carrier (A) Detection of the splice site mutation in exonI/intron 1 by Eco8II restriction. Lanes 2-4: female family members without the mutation. (B) Detection of the splice site mutation in exonII/intron2 by DdeI restriction. Lanes 1 and 4: unralated control DNA, lane 2 and 3: patient's mother and patient, respectively. (C) Detection of the T→A transversion in codon 126 by MaeIII restriction analysis. (D) Detection of the C→G transversion in codon 74 by MaeIII restriction. (E) Detection of the T→C transition in codon 110 by HgaI restriction.

tients (Berger et al., 1992; Meindl, personal communication). This mutation is located near the "cystine knot motif" and may well interfere with its formation by introducing an additional cysteine residue. The C-to-G substitution destroys a MaeIII restriction site and was identified in the patient's mother but not in his maternal grandmother or in other family members (Fig. 1D). Segregation analysis using a CA-repeat polymorphism at the MAO A locus showed that the patient inherited his mother's paternal allele (not shown) suggesting that the mutation occurred de novo during spermatogenesis in the maternal grandfather. As three independent cases of R74C mutation have already been identified, this change may represent a mutation hot spot in the Norrie gene.

Patient 5 is a 19-year-old man whose clinical examination revealed phthisis bulbi, cataract, and amaurosis in both eyes. Additionally, the patient has bilateral sensorineural deafness. His intelligence is normal. No family history of comparable symptoms is known. Analysis of the Norrie gene revealed a T-to-C transition of the first nucleotide in codon 110 of exon III, predicting the substitution of cysteine by arginine (C110R). This sequence alteration has been reported previously in one other patient (Zhu and Maumenee, 1993). The sequence change found creates a new HgaI restriction site at this position and can therefore be easily detected. Both direct sequencing (not shown) and HgaI restriction analysis (Fig. 1E) confirmed that the mother of the patient did not carry the T-to-C transition, suggesting that this sequence alteration represented a de novo mutation in the Norrie disease gene of the patient studied. The replacement of cysteine-110 should considerably alter the structure (and function) of the Norrie protein. In the same codon, a nonsense point mutation (C110X) has already been described in a patient with Norrie disease (Berger et al., 1992b).

The three novel gene mutations that we have identified in patients with Norrie disease represent further examples of sequence aberrations that alter the structural and/or functional property of the Norrie protein and are therefore considered the primary genetic cause of the disease in the respective families. None of the mutations identified in this study was detected in 60 unrelated control X chromosomes (data not shown).

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