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Identification of a Recurrent Missense Mutation in the Norrie Disease Gene Associated with a Simplex Case of Exudative Vitreoretinopathy

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Disorders such as Norrie disease, X-linked familial exudative vitreoretinopathy, retinopathy of prematurity and X-linked primary vitreoretinal dysplasia have very similar clinical manifestations. They exhibit retinal fold, retinal detachment, retinal traction and the formation of retrolental fibrovascular membrane. In order to identify carriers for these disorders and provide precise genetic counseling of the relatives, a molecular genetic analysis will be helpful. This report describes the results of Norrie disease gene analysis in simplex cases of exudative vitreoretinopathy. The identification of a recurrent mutation in the Norrie disease gene in a simplex case of exudative vitreoretinopathy further strengthens the notion that Norrie disease and exudative vitreoretinopathy are allelic disorders. © 1998 Academic Press

Familial exudative vitreoretinopathy (FEVR) is an inherited eye disease first reported by Criswick and Schepens (1). It is characterized by premature arrest of vascularization of the peripheral retina. It is inherited as an autosomal dominant (2), autosomal recessive (3) and X-linked recessive (4) trait with high penetrance and variable expressivity. Mild forms of the disease predominate and are often asymptomatic, showing only peripheral vascular abnormalities such as a peripheral avascular zone, vitreoretinal adhesions, and a v-shaped area of retinochoroidal degeneration. More severe manifestations include neovascularization, sub- and intraretinal hemorrhage and exudates and vascularized preretinal membranes which can lead to retinal folds, macular ectopia and retinal detachment due to vitreoretinal traction. Many of these findings are identical to those observed in retinopathy of prematurity (ROP), but affected individuals with FEVR have a normal gestational period and lack a history of low birthweight and exposure to supplemental oxygen therapy (5).

While ROP tends to either spontaneously regress or reach a cicatricial stage and stabilize at a postconceptual age of 46-48 weeks, FEVR tends to be a slowly progressive disorder with detachments, often not occurring until the first or second decade of life. The candidate genes responsible for autosomal dominant and recessive FEVR have not been identified to date. However, molecular genetic analyses have recently demonstrated that X-linked FEVR and Norrie disease (ND) are allelic in some families (6-11) and some families show genetic heterogeneity (12). In addition, certain sporadic ND, FEVR as well as advanced ROP patients were shown to harbor mutations in the ND gene suggesting its role in vasculogenesis and in developing a spectrum of clinical ocular disorders (11, 13, 14).

Norrie disease is an X-linked recessive disorder with an asymptomatic female carrier and is characterized by bilateral retinal dysgenesis followed by retinal detachment which leads to congenital blindness (15). Norrie patients also often develop sensorineural deafness, a varied degree of mental retardation, cataract and corneal opacities in early childhood. The locus for Norrie disease has been mapped to chromosome Xp11.4-p11.3 and a putative gene has been recently isolated by positional cloning (16, 17). The gene consists of 3 exons (the first exon is untranslated) and two introns and encodes a protein of 133 amino acids. It is expressed in brain and eye tissues and intragenic deletion and several point mutations have been described recently in Norrie disease patients (18, 19). This report describes the results of Norrie disease gene analysis in simplex cases of exudative vitreoretinopathy.

MATERIALS AND METHODS

For the purpose of mutation analysis, we collected venous blood from affected and unaffected members of the family and the leukocyte DNAs were amplified by the polymerase chain reaction (PCR). The study was approved by the Oakland University Institutional Review Board. Three pairs of oligonucleotide primers were used: exon 1, 5′-GCGCCTCACATTTCCGTGGC-3′ and 5′-CTCGGGTTTGGAAAGAAGCGA-3′; exon 2, 5′-GGAGGTGAAGCCATTTCCAATT-3′ and 5′-CTTGCCTGTTTCTGAGGG-3′; and exon 3, 5′-CCTGGCTAAGGTTGTGGC-3′ and 5′-CACAGCAGCGGGCCTCAG-3′. These primers were previously re-

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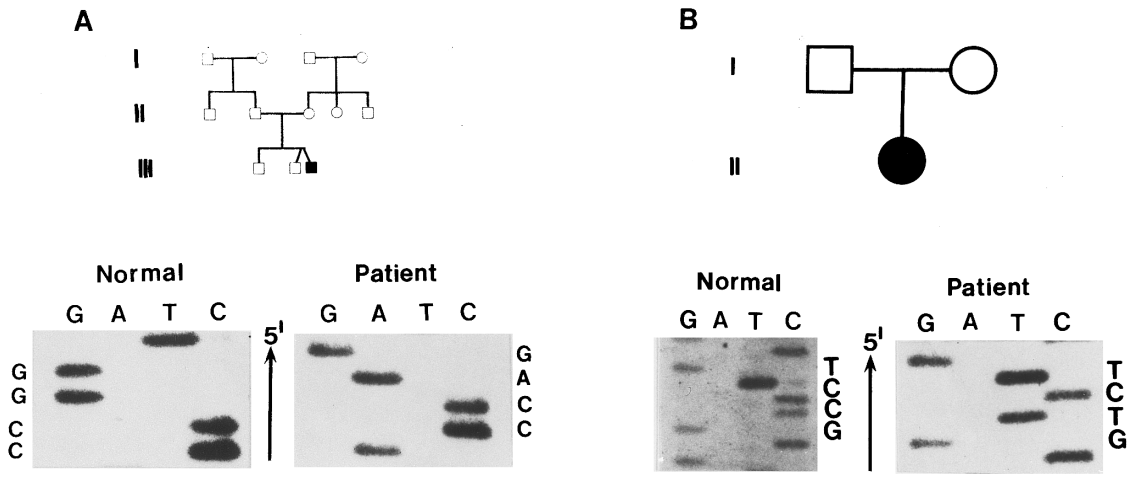

FIG. 1. Panel A: A sporadic case of an exudative vitreoretinopathy family used for the analysis. An affected male is indicated by a filled square and unaffected individuals by open symbols. The nucleotide sequence of the mutant part of the exon 3 is shown below the pedigree. The sequence change in the patient is C to T, which results in the substitution of the amino acid arginine to tryptophan. Panel B: Mutational analysis of the peripherin/RDS gene. The nucleotide sequence of the mutant part of the exon 3 is shown. The sequence change in the patient is G to A, which results in the substitution of the amino acid glycine to aspartic acid.

ported by others (18) and were commercially synthesized. The genomic DNA (0.1 μg) from each patient was amplified by using the procedure described before (8). The amplified products were gel isolated, purified and subcloned into a plasmid vector or directly used for sequencing with a cycle sequencing kit (Perkin-Elmer) according to the manufacturer's protocol. DNA sequencing was carried out using dideoxynucleotide termination reactions with [α-^{35}S]dATP or [α-^{32}P]dATP. Whenever the cloned DNAs were sequenced multiple randomly selected individual subclones from each sample were analyzed. The reactions were analyzed on 6% polyacrylamide wedge gels containing 8M urea and the bands were visualized by autoradiography. Whenever necessary, 7-deaza dITP was used to resolve GC-band compression.

The sequence changes were further verified by restriction analysis of the freshly amplified genomic DNA. For this purpose DNA, was amplified from genomic DNA from affected infants and normal controls, and subjected to restriction enzyme digestion. The reactions were analyzed on a 9% polyacrylamide gel and visualized by autoradiography.

RESULTS AND DISCUSSION

The dizygotic twin brothers (Fig 1, panel A) were full-term infants with normal birthweight. There was no history of supplemental oxygen or auditory impairments. At the age of 2 years, an ophthalmoscopy examination of the proband showed peripheral exudates, a retinal fold and vitreous detachment. The unaffected brothers and their parents showed no visual problems when examined. Their visual acuity is 20/20 OD and 20/20 OS. A diagnosis of exudative vitreoretinopathy (EVR) was established on the basis of characteristic fundus findings for the individual lacking a history of auditory impairments, premature birth, low birthweight and exposure to supplemental oxygen.

Since some of the clinical features of ND are common with EVR, ROP and persistent hyperplastic primary vitreous (PHPV), it is possible that mutations in the ND gene might contribute to the sporadic case of FEVR. In order to test this hypothesis, leukocyte DNAs from affected and unaffected family members were amplified by the PCR using three pairs of primers (18) designed to amplify three exons and splice junctions of the ND gene. When the amplified products were sequenced, a single base pair substitution (C to T) was detected in one family at codon 121 of the ND gene (Fig 1). The sequence derived from both parents' DNA revealed the wild-type sequence, indicating that the observed mutation was sporadic. In addition, DNA sequencing of the ND gene of 70 unrelated healthy controls, selected randomly from the general population, did not show this alteration. This mutation destroys one of the Msp1 restriction sites normally present in exon 3 of the ND gene and changes the encoded amino acid arginine to tryptophan. Since there are no tryptophans in the normal ND protein, this change is likely to cause a highly specific disruption of protein function.

In order to verify the sequence changes, freshly amplified genomic DNA from an affected individual was subjected to Msp1 digestion. As expected, all clinically unaffected family members as well as normal individuals yielded the expected 169, 72 and 56 base pair fragments while the affected individual exhibited a unique 128 base pair product (instead of 72 and 56bp), indicating the destruction of one of the two Msp1 sites normally present in the wild-type ND gene (data not shown). Since the mutation described above was also shown to be involved in X-linked FEVR, ND and ROP (8, 9, 14, 20) which are clinically similar to one another, and this mutation has not been found in the general population (240 normal X-chromosomes from our own study as well as from other laboratories from around

the world), it is tempting to speculate that the phenotype in this family is due to the mutation in the exon 3 of the ND gene.

Since mutations in the ND gene have also been described in female carriers manifesting Norrie disease (21, 22, 23), we next examined one sporadic case of ND involving a female patient with the assumption that a similar mutation is responsible for the pathogenesis of this disease. The propositus (Fig 1, panel B), a female infant, aged 5 months, was admitted to the hospital for ocular examination. She was born with a gestation of 39 weeks and a birthweight of 5 pounds and 12 oz. A hearing abnormality was noticed at the age of 3 months. Detailed ophthalmological examination revealed retinal detachment and microphthalmia of the right eyeball. The retinal detachment along with the hearing loss and microphthalmia permitted the diagnosis of a Norrie disease-like feature. Additionally, the family history indicated that none of the other family members presented any clinical abnormalities.

To provide further insight into the usefulness of the screening technique, this particular case was investigated in more detail at the gene level. All three exons of the ND gene were amplified and sequenced as detailed above. The results indicate that there are no deletions, inversions, insertions, rearrangements and point mutations in the coding regions, splice junctions and 90bp upstream of exon 1 which contains putative promoter regions of the ND gene. However, this still does not exclude the possibility that this gene is involved in the pathology because mutation in the far upstream promoter and other regulatory regions cannot be ruled out at present. The above analysis suggests that, although classic Norrie disease involves translocation, inversion, deletion and point mutations of the ND gene, some sporadic cases seem to be associated with different genes.

Since mutations in the peripherin/RDS gene is associated with multiple phenotypes, the author questioned whether mutations in this gene could contribute to the disease phenotype in the present case. To address this question, the entire peripherin/RDS gene, including splice sites has been analyzed using the primers reported by others (24, 25). DNA sequencing of exon-3 revealed a missense mutation (G338D) at codon 338 (Fig 1, panel B). This mutation changes the encoded amino acid glycine to aspartic acid and is not found in unrelated normal individuals. Similar analysis of exon-1 and exon-2 did not reveal any additional sequence alterations. It is not possible at present, however, to conclude that the mutation is disease related, because it has been described in certain normal populations (26), although our normal controls did not show this variation. It may represent a polymorphic variation, which does not produce pathological problems, similar to the mutation described in the factor IX gene. The complex phenotype observed in the present case is therefore attributable to different genes.

Although the relationship between genotype and phenotype is not straightforward, similarities in the clinical presentation of several distinct disorders may suggest allelic heterogeneity at a single locus. Mutations in the ND gene are associated with simplex cases of ND (13), FEVR (11) and two X-linked families in which females are exhibiting Norrie disease (21, 22, 23). The present results further strengthen the above suggestion and provide a firm basis for the evaluation of the sporadic cases of FEVR, particularly when the clinical pictures are ambiguous for this related retinal phenotypes. It also provides an opportunity to identify the carriers and counsel the relatives of the proband.

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