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Norrie disease and exudative vitreoretinopathy in families with affected female carriers

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ABSTRACT: Purpose. *Norrie disease (ND) is a rare X-linked recessive disorder characterized by congenital blindness, which is often associated with sensorineural hearing loss and mental retardation. X-linked familial exudative vitreoretinopathy (FEVR) is a hereditary disorder characterized by an abnormality of the peripheral retina and is not associated with systemic diseases. X-linked recessive disorders generally do not affect females. Here we show that female carriers can be associated with manifestation of an X-linked disorder.*
Methods. *A four-generation family with an affected female, and a history of congenital blindness and hearing loss, was identified through the pro-band. A second family, with a full-term female infant, was evaluated through ophthalmic examinations and found to exhibit ocular features, such as retinal folds, retinal detachment and peripheral exudates. Peripheral blood specimens were collected from several affected and unaffected family members. DNA was extracted and analyzed by single-strand conformation polymorphism (SSCP) following polymerase chain reaction (PCR) amplification of the exons of the Norrie disease gene. The amplified products were sequenced by the dideoxy chain termination method.*
Results. *In an X-linked four-generation family, a novel missense (A118D) mutation in the third exon of the Norrie disease gene, was identified. The mutation was transmitted through three generations and cosegregated with the disease. The affected maternal grandmother and the unaffected mother carried the same mutation in one of their alleles. In an unrelated sporadic family, a heterozygous missense mutation (C96Y) was identified in the third exon of the Norrie disease gene in an affected individual. Analysis of exon-1 and 2 of the Norrie disease gene did not reveal any additional sequence alterations in these families. The mutations were not detected in the unaffected family members and the 116 normal unrelated controls, suggesting that they are likely to be the pathogenic mutations.*
Conclusions. *The results further strengthen the proposal that X-linked disorders can occur in female carriers, due likely to an unfavorable X-inactivation. (Eur J Ophthalmol 1999; 9: 238-42)*

KEY WORDS: Heterozygous, Norrie disease, Mutation, Recessive, Vitreoretinopathy, Sporadic

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INTRODUCTION

Norrie disease (ND) is a bilateral X-linked recessive syndrome characterized by ocular dysgenesis, progressive mental deterioration and auditory impairment (1). Bilateral blindness is frequently observed at birth. Characteristic findings include retinal detachment, persistent hyperplastic primary vitreous, vitreous hemorrhage, cataract, glaucoma, optic nerve atrophy and ultimately phthisis bulbi. The patients often present with vascularized retrolental masses, simulating retinoblastoma. Mild to severe mental retardation has been described in up to two - thirds of cases while progressive sensorineuronal hearing loss occurs in

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about 30% of cases (1-3). Histopathological studies have demonstrated the presence of inner and outer neuroblastic layers containing rod and cone precursors and complete absence of retinal blood vessels, suggesting an arrest in embryonic retinal development during the third to fourth months of gestation (4). In the majority of cases only males are affected, with female carriers revealing no retinal or electrophysiologic abnormalities (5). In rare cases in which females manifest the disorder, non-random X-inactivation is likely the cause (6-8).

Familial exudative vitreoretinopathy (FEVR) is a vitreoretinal dystrophy characterized by premature arrest of vascularization of the peripheral retina (9,10). The disorder can appear in a highly penetrant form and can progress to an exudative process leading to macular traction, retinal detachment and retinal folds which can lead to blindness. The disorder is always bilateral, usually symmetric and exhibits highly variable expressivity. It is inherited with autosomal dominant (10, 11), autosomal recessive (12) and X-linked traits (13). X-linked FEVR has been shown to involve mutations of the ND gene in some families (14-17) and is a genetically heterogeneous disorder (18). Affected individuals of FEVR do not have systemic abnormalities. The female carriers of X-linked FEVR are clinically undetectable. In an effort to understand the retationship between the genotype and phenotype we have evaluated a large ND family and a sporadic case of FEVR in which females are affected.

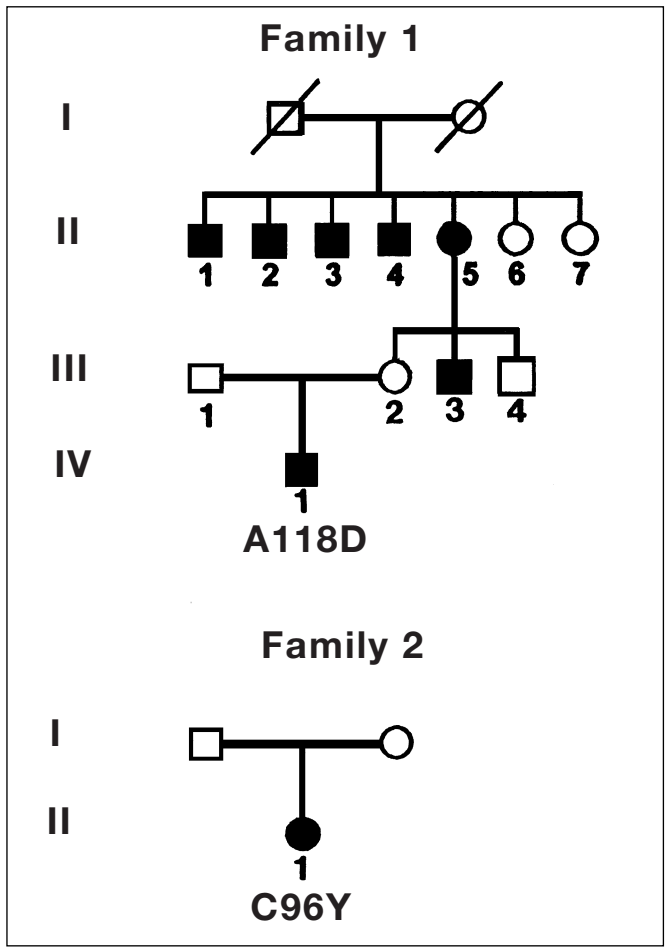

Fig. 1 - *Pedigree of the ND (family 1) and FEVR (family 2) families under study. Squares and circles denote males and females respectively. Unblackened and blackened symbols represent unaffected and affected individuals respectively. Slashed symbols indicate deceased individuals.*

PATIENTS AND METHODS

The families used in the present study are shown in Figure 1. A full term male infant, in family 1 (IV-1), was born in 1998 to healthy parents with an uncomplicated delivery. His maternal uncle, maternal grandmother and maternal grand uncles had a history of congenital blindness and hearing loss. The proband when examined at the age of two weeks after birth showed bilateral tractional retinal detachments and vitreous hemorrhage with normal anterior segments and clear lenses. His mother is phenotypically completely normal. An examination of the records of the attending ophthalmologists regarding the patient's relatives showed that the maternal grandmother (II-5) and all of her affected brothers (II-1to4) had suffered severely with congenital blindness and deafness and had been diagnosed as having ND. Their physical examination records were normal. In family 1, the disease is transmitted through three generations without male-to-male transmission but through the unaffected mother who has affected maternal uncles, all consistent with an X-linked pattern of inheritance. An unaffected male child (III-4) born to the affected mother (II-5) defines the carrier status of the mother (II-5).

In family 2, a full term female infant (II-I) was born to healthy non-consanguinous parents with an uncomplicated delivery. Ophthalmic examinalion showed a retinal fold extending towards the retinal periphery, an intravitreous fibrous mass and membrane, vitreous detachment and peripheral exudates at the

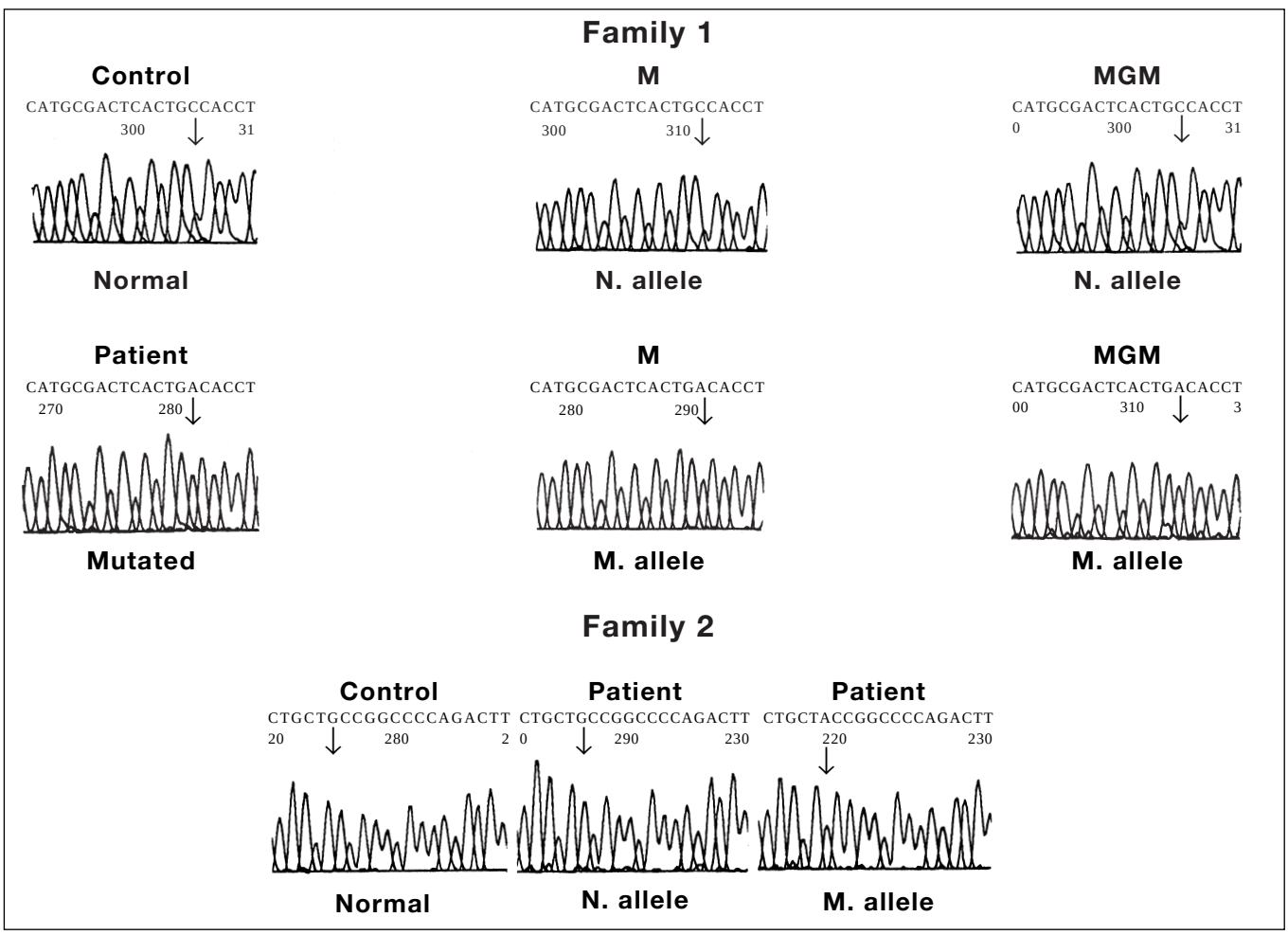

Fig. 2 - *Nucleotide sequence of the mutant part of the exon 3 of the ND gene. The nucleotide sequence change (family 1) in the patient is C to A which results in the amino acid aspartic acid (GAC) instead of the normal alanine (GCC). In family 2, the nucleotide sequence change in the patient is G to A which results in the amino acid tyrosine (TAC) instead of the conserved amino acid cysteine (TGC) which is involved in the cystine knot tertiary structure of the protein norrin. N. allele and M. allele represent normal and mutated alleles respectively. M = mother, MGM = maternal grandmother.*

junction between the avascular and vascular retina. When the other family members were examined, they showed no clinical symptoms. The diagnosis of FEVR was established on the basis of characteristic fundus findings. The patient did not have a premature birth or exposure to supplemental oxygen.

DNA analysis

Peripheral blood samples from several affected and unaffected family members were collected and DNA was isolated as described (15). All three exons of the ND gene were amplified by the polymerase chain reaction (PCR) as detailed previously (15, 16). The amplified products were cloned into a pT7 blue vector and sequenced with fluorescent primers using Applied Biosystems Data analysis software, following the manufacturer's protocol. Multiple, randomly selected individual subclones from each sample were sequenced to obtain a consensus sequence.

X-inactivation assay

To perform X-chromosome inactivation analysis, we have used a PCR based method at monoamine oxidase A (MAOA) and human androgen receptor (HUMARA) loci. This method is based on two assumptions: the presence of a polymorphism to discriminate

between the maternal and paternal X-chromosomes and a difference in the methylation pattern on the active versus inactive X-chromosome. For this assay, an aliquote of lymphocyte DNA was digested with the methylation sensitive enzyme Hpa II. The digested DNA was then used to amplify the MAOA and HUMARA loci using the flanking primers . The amplified products were then analyzed on a 9% nondenaturing polyacrylamide gel and were visualized by autoradiography.

RESULTS AND DISCUSSION

In this report, we have analyzed one large ND kindred and a sporadic case of FEVR with affected females for the mutations in the ND gene. Our extensive analyses identified two missense mutations in the third exon of the ND gene. These mutalions were not found in 116 normal and randomly selected individuals. In family 1, the sequencing of exon 3 revealed a missense mutation at codon 118 (Fig. 2) in all affected individuals but not in unaffected family members. To our knowledge, this mutation was not reported previously and hence represents a new mutation in the ND gene. The affected maternal grandmother (II-5) and the unaffected mother (III-2) showed the same mutation in one of their alleles (Fig.2). This mutation has changed the encoded amino acid alanine to aspartic acid, thereby altering the structure and possibly the function of the protein. Since the mutation is transmitted through three generations, is segregated with the disease and is not found in either unaffected family members or 116 normal controls, it is very likely that the altered gene product is pathogenic in this family. The maternal grandmother, although carrying the mutation in only one of her alleles, has been diagnosed as having ND. In family 2, sequencing of exon 3 similarly revealed a missense mutation at codon 96 in one of the alleles of an affected female child, but not in her parents. This mutation has changed the encoded highly conserved amino acid cysteine to tyrosine and is likely to result in loss of function of the protein (20). Since this amino acid is involved in the cystine knot formation and the same mutation is reported to cause ND in a family (21), disruption of this cystine knot motif is likely to be responsible for the phenotype in this patient. DNA sequence analysis of the probands for the remaining coding regions did not reveal any additional sequence changes.

X-linked recessive disorders generally do not affect females due to random X-inactivation. However, occasionally females do manifest clinical symptoms of X-linked diseases such as the Wiskott-Aldrich syndrome (22), Duchenne muscular dystrophy, hemophilia B (23) and X-linked primary immunodeficiencies (19). ND has also been previously reported in two carrier females (7,8) carrying C69S and I123N mutations in the ND gene. In the present case (family 1), it is interesting that although both the grandmother (II-5) and her daughter (III-2) carry the same mutation heterozygously, only the grandmother is affected This result can not be explained by the dominant effect of the mutation, since the grandmother's mutant chromosome was inherited by her daughter (III-2) who is asymptomatic. It is also unlikely that a second *de novo* mutation in the patient's normal allele is responsible for her phenotype because the sequencing of the entire ND gene, (for both families) including about 90 bp upstream region (containing the promoter), did not reveal additional alterations. In addition, the live birth of an unaffected son, in family 1 (III-4), clearly establishes the presence of one normal allele in his mother. The most likely explanation for the expression of the disease in our families is an unfavourable X-inactivation, as proposed by others (7, 8, 22).

In order to test this possibility, we have carried out an X-inactivation assay using a methylation sensitive enzyme (Hpa II) and lymphocyte DNA at MAOA and HUMARA loci. The results showed that one of the HUMARA alleles in the maternal grandmother (II-5) was amplified 2-fold greater than the other allele after digestion (before digestion both alleles were equally amplified), indicating the possibility of skewed X-inactivation. Interestingly, when such an analysis was carried out using the unaffected carrier mother's DNA (III-2), both alleles were equally amplified after digestion (both alleles were again equally amplified before digestion) suggesting random X-inactivation in the unaffected carrier mother (data not shown). However, methylation of these sites does not correlate with X-inactivation in the affected and unaffected male children, possibly due to variability in the pattern of methylation between the eyes and lymphocytes as well as between males and females. Similarly, methylation pattern at MAOA locus does not correlate with X-inactivation in both families. It will be interesting to ana-

lyze in the future (if the opportunity arises) the X-inactivation pattern in the eye tissues of this family. Alternatively, if the mutant allele is activated at a very critical stage of peripheral retinal development, it is possible that female carriers may exhibit disease symptoms. This suggestion is supported by the fact that peripheral retinopathy can occur in offspring of carriers of ND gene mutation (24). Whatever the mechanisms of the expression of the disease, knowledge that an X-linked recessive disease can occur in women is important for both genetic counseling and diagnosis, especially when there is no evidence of disease or a history of disease in other family members, as is shown in the present study (family 2).

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