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# Lack of Association of the Norrie Disease Gene with Retinoschisis Phenotype

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**Purpose:** It has been reported recently that mice carrying a disrupted Norrie disease gene produced alterations in the murine eye that are similar to congenital retinoschisis. Therefore, it was of interest to determine whether mutations in the Norrie disease gene can account for the disease in families with retinoschisis that do not carry mutations in the retinoschisis gene.

**Methods:** The patient set comprised 5 cases of retinoschisis (1 familial and 4 sporadic), all unrelated to each other. Fundus examination of affected individuals showed foveal and peripheral schisis, and the visual acuity range was 20/40–20/60. Peripheral blood specimens were collected from affected and unaffected family members. DNA was extracted and amplified by polymerase chain reaction amplification of exons of the Norrie disease gene. The amplified products were sequenced by the dideoxy chain termination method.

**Results:** The data revealed no disease-specific sequence alterations in the Norrie disease gene.

**Conclusion:** Although we cannot completely exclude the possibility of the Norrie disease gene as a candidate gene, the above results suggest that the structural and functional changes in the Norrie disease gene are not associated with clinically typical retinoschisis families that do not contain mutations in the coding regions and splice sites of the retinoschisis gene. **Jpn J Ophthalmol 2000;44:627–629** 

**Key Words:** Mutation, Norrie disease, retinoschisis.

## Introduction

X-linked juvenile retinoschisis or congenital retinoschisis (RS) is a recessively inherited vitreoretinal disorder that develops early in life, and can result in poor visual acuity. It is bilateral and can be a progressive disease with variable clinical manifestations. The disorder is characterized by intraretinal schisis cavities, central (cartwheel) and peripheral vitreous hemorrhage, intra-schisis hemorrhage, and retinal detachment.[1] Although the mechanism of the pathogenesis of the disorder is unknown, histopathological and electroretinographic (reduced b wave) studies suggested that Mueller cells are involved in the disease. The majority of carriers have normal vision with no abnormalities in the retina. Retinoschisis also occurs sporadically with negative family history[2,3] and can be differentiated from the familial form by an adult onset of symptoms and absence of specific macular changes. The gene responsible for RS has been recently isolated[4] and a spectrum of mutations has been described.[5] There is no locus heterogeneity for RS among different sets of families. It has been reported recently that a disrupted Norrie disease (ND) gene carried by mice produced alterations in the eye that are similar to senile and juvenile human RS.[6] Although the genetic loci for RS (Xp22.13–p22.3) and ND (Xp11.3–p11.4) are clearly distinct from each other, the above transgenic mouse experiment still raised the possibility that mutations in a gene other than the RS gene may be involved in RS. Therefore, it was of interest to determine whether mutations in the ND gene can account for the disease in families with RS but without an RS gene mutation, and to establish the relationship between the genotype and phenotype.

Received: December 15, 1999
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Published by Elsevier Science Inc.

0021-5155/00/$–see front matter
PII S0021-5155(00)00288-4

Jpn J Ophthalmol
Vol 44: 627–629, 2000

## Materials and Methods

For the purpose of evaluating the ND gene, we have studied five well-characterized RS families who did not show mutations in the RS gene. The pedigree of the one familial case of RS is shown in Figure 1. The other 4 cases, which are sporadic RS cases, are similar to the familial case with respect to their fundus appearance. All patients underwent a complete ophthalmological examination by one of the authors. The study was approved by the Institutional Review Board of Oakland University and the Human Subject Investigation Committee of the William Beaumont Hospital. All patients were informed of the purpose of the study and their written consent was obtained. In the pedigree shown in Figure 1, there is no male-to-male transmission and all affected individuals are males. Although it is not possible to confirm the pattern of inheritance in the family shown, it is possible that it is an X-linked recessive disorder. The familial and 4 sporadic cases are unrelated to each other. The diagnosis of RS was established on the basis of characteristic fundus findings (foveal and peripheral schisis, dotted-swiss appearance of the peripheral retina). The visual acuity range was (20/40–20/60) at the time of the last clinical examination.

Since the family (Figure 1) showed co-segregation of markers across the RS region,[7] we first examined the leukocyte DNA for mutations in the RS gene, as described previously.[8] However, no disease-causing mutations have been identified in any of the 5 families, although mutations in the upstream regions and introns cannot be excluded. We then carried out haplotype analysis of the markers MAOA and DXS 228 (familial case), which are tightly linked to the ND locus. However, the gene locus did not segregate with the disease. Because this experiment cannot rule out completely the involvement of the ND gene or determine the status of the ND gene, we screened the ND gene directly for mutations by means of direct sequencing. All three exons of the ND gene were amplified by polymerase chain reaction (PCR), using three pairs of commercially synthesized primers spanning all three exons, splice site, and the 5′-upstream region containing the promoter elements as described previously.[9] The conditions were: 30 cycles of 1.5 minutes at 60°C, 2 minutes at 72°C in a buffer containing 2–4 units polymerase (Applied Biosystems, Foster City, CA, USA), 10 pmol each of the primers, 50 μM each of four deoxynucleotides, 1.5 mM $MgCl_2$, and 10 mM Tris-HCl (pH 8.3). The products of PCR reaction were gel isolated, purified by phenol:chloroform extraction and ethanol precipitation, and subcloned into a plasmid vector. DNA sequencing was performed using standard dideoxynucleotide termination reaction with $\alpha^{35}$S-deoxyadenosine triphosphate. Multiple randomly selected individual subclones from each sample were sequenced to obtain the consensus sequence. Whenever necessary, 7-deaza dITP was used to resolve GC band compression. The reactions were analyzed on 6% polyacrylamide gels containing 8 M urea, and the bands were visualized by autoradiography.

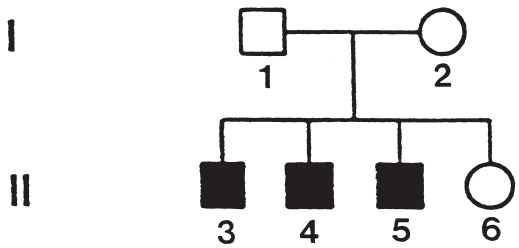


**Figure 1.** Pedigree showing segregation of retinoschisis in a family. Open and closed symbols represent unaffected and affected individuals, respectively. Squares and circles denote males and females, respectively. Four other families studied are sporadic type.

## Results

The ND gene contains three exons and exon-1 is an untranslated region. The availability of the sequence information between the intron-exon boundaries and the small size of the ND gene made it possible for us to screen for the mutations effectively by means of direct sequencing. All three exons including splice sites and the approximately 90-bp untranslated 5′-flanking region (constituting the promoter) of the ND gene were sequenced for the affected individuals. Our extensive mutational analyses failed to identify any disease-causing mutations in any of the families analyzed, suggesting that the ND gene is not responsible for the pathogenesis of RS in these families.

## Discussion

Norrie disease is characterized by ocular dysgenesis, progressive mental deterioration, and auditory impairment.[10] Bilateral blindness is typically observed at birth. Characteristic findings include retinal detachment, persistent hyperplastic primary vitreous, and vitreous hemorrhage. Histological studies have demonstrated a marked generalized avascularity of the retina and severe retinal dysplasia Little is known about the mechanisms of the ocular pathogenesis of RS and ND. There are probably not too

many reasons to search for a mutation in the ND gene in retinoschisis patients. However, three considerations have led us to investigate whether the ND gene had a role in the ocular pathology of X-linked and senile RS families. First, some of the ocular features, such as formation of retrolental fibrovascular membrane, retinal traction, and retinal detachment, are common features in ND and RS. Second, it has been shown recently[6] that mice carrying a disrupted ND gene produced retinoschisis-like alterations in the eye, similar to senile and juvenile human retinoschisis, suggesting that mutations in a gene other than the RS gene may cause a retinoschisis-like phenotype. Third, the families used in this study, although they have a clinically typical retinoschisis-type pathology, did not show mutations in the RS gene by DNA sequence analysis. Therefore, it appeared logical to extend our study to include the ND gene. Our extensive sequencing data failed to identify mutations in either the ND or the RS gene (in exons and splice sites). However, we cannot completely exclude the possibility of ND gene involvement in these families because our analyses would not detect mutations in the introns and the upstream regions. Because sequencing data failed to identify mutations in the ND and RS genes in the families analyzed, it is possible that RS could be caused by different genes in these families. This is supported by the finding that the RS gene is found to be mutated in 90% of RS patients,[5] and senile RS did not show mutations in the RS gene in several cases.[11] Although the relationship between genotype and phenotype is not straightforward, it is simply not possible to deduce the underlying causative gene based on the clinical phenotype or an animal model. It has also been reported recently[12] that even the electroretinogram, which has been extensively used in the diagnosis of RS, should be employed cautiously in the differential diagnosis of RS. These findings clearly demonstrate an exceptional genetic and clinical complexity of inherited retinal disorders. Further identification of the causative gene may facilitate the elucidation of the molecular basis of the disorder in the families analyzed.

This work was supported in part by a grant from the Retinopathy of Prematurity Foundation (ROPARD) of Royal Oak, MI, USA (BSS), and a Core grant for Vision Research from the National Eye Institute (EY 05230) Bethesda, MD, USA.

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