·Basic Research·

A novel Norrie disease pseudoglioma gene mutation, c.-1_2delAAT, responsible for Norrie disease in a Chinese family

Xin-Yu Zhang¹, Wei-Ying Jiang², Lu-Ming Chen², Su-Qin Chen²

¹Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, Guangdong Province, China
²Department of Medical Genetics, Zhongshan Medical College, Sun Yat-sen University, Guangzhou 510080, Guangdong Province, China
Co-first authors: Xin-Yu Zhang and Wei-Ying Jiang
Correspondence to: Su-Qin Chen. Department of Medical Genetics, Zhongshan Medical College, 74 Zhongshan Road II, Guangzhou 510080, Guangdong Province, China. chensq@mail.sysu.edu.cn

Abstract

• **AIM:** To investigate the genetic findings and phenotypic characteristics of a Chinese family with Norrie disease (ND).

• **METHODS:** Molecular genetic analysis and clinical examinations were performed on a Chinese family with ND. Mutations in the Norrie disease pseudoglioma *(NDP)* gene were detected by direct sequencing. Haplotypes were constructed and compared with the phenotypes in the family. Evolutionary comparisons and mutant open reading frame (ORF) prediction were also undertaken.

• **RESULTS:** Two family members with ocular manifestations were diagnosed with ND. No signs of sensorineural hearing loss were observed in either patient, while one of them showed signs of mild mental retardation. A novel heterozygous mutation in the *NDP* gene, c.-1_2delAAT, was detected in both patients. The mutation and the mutation bearing haplotype co-segregated with the ND phenotype in males and was transmitted from their mothers and/or grandmothers (II: 2). The male without ND did not harbor the mutation. The mutation occurred at the highly conserved nucleotides. DRF finder predicted that the mutation would lead to the production of a truncated protein that lacks the first 11 N-terminal amino acids.

• **CONCLUSION:** A novel mutation, c.-1_2delAAT in the *NDP* gene, was identified in a Chinese family with ND. This mutation caused ND without obvious sensorineural hearing loss. Mental disorder was found in one but not the other patients. The clinical heterogeneity in the family indicated that other genetic variants and epigenetic factors may also play a role in the disease presentation.

• **KEYWORDS:** Norrie disease; pseudoglioma; mutation; Chinese

INTRODUCTION

Norrie disease (ND, MIM#310600) is a rare X-linked recessive disorder characterized by bilateral congenital blindness in males due to degenerative and proliferative changes in the neuroretina. Approximately 30% -50% of patients show some forms of progressive mental disorder and/or sensorineural hearing loss[1,2].

The Norrie disease pseudoglioma gene (*NDP*, NM_00266) is the causal gene that underlies ND and is located on chromosome Xp11.4[3,4]. It contains three exons, but only the second and the third exons are translated. This gene encodes a 133-amino-acid secretory protein, norrin, with a cystein-knot motif that plays a critical role in retinal vascular development[5-8].

More than 143 *NDP* mutations (HGMD Professional 2013.2) (http://www.hgmd.cf.ac.uk) have been identified to date in patients with ND or other *NDP*-related retinopathies, including persistent hyperplastic primary vitreous (PHPV)[9-11], X-linked familial exudative vitreoretinopathy (XL-FEVR)[9,10,12,13], retinopathy of prematurity(ROP)[9,10,14,15] and Coats disease[16]. In this study, we identified a novel *NDP* mutation responsible for ND in a Chinese family with three affected males, two of which were evaluated in this study.

SUBJECTS AND METHODS

subjects The family from Guangdong province, China comprised 4 generations with 3 affected patients (Figure 1). Pedigree analysis indicated that the mode of inheritance was most likely to be X-linked recessive. Informed consent was obtained from all recruited subjects or their parents or guardians. The study was approved by the Ethics Committee of the University for Human Study. The principles outlined in the Declaration of Helsinki were followed. Eight subjects from the family were recruited for genetic analysis, among which 2 patients underwent comprehensive clinical examinations. One hundred unrelated healthy individuals from the same province and ethnic background were recruited as controls.

Mutation detection and haplotype construction Genomic DNA was extracted from peripheral blood samples using a standard phenol-chloroform technique. Mutations were detected by sequencing the PCR products flanking all 3 exons and intron-exon splice sites of the *NDP* gene. The guidelines for describing sequence variations and numbering (http://www.hgvs.org/mutnomen/) were followed [17]. Haplotypes were constructed using the informative intragenic *single nucleotide polymorphisms* (SNPs).

Alignment of *NDP* cDNA orthologues CLUSTAL X (1.83) was used to compare human *NDP* cDNA sequence (NM_000266.3) with the nucleotide sequences of chimpanzee (XM_001139783.2), gorilla (XM_004064036.1), cow (NM_001046090.2), pig (XM_003469362.2), mouse (NM_010883.2), rat (NM_001108814.1) and chicken (NM_001278086.1)[18].

Mutant open reading frame prediction Open reading frame (ORF) finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to predict the ORF in the mutant DNA.

RESULTS

Clinical findings Of the 3 affected subjects, 2 were alive (III:5 and IV-1) and one died at young age from an accident (III:3) (Figure 1). The proband, IV-1, was born in September 2007 after an uncomplicated pregnancy. He was found after birth by his parents for not following moving light stimuli. He was taken to Zhongshan Ophthalmic Center for examination when he was 3-month old. The cornea of both eyes were transparent, while the diameter of left side cornea (9mm) was smaller than that of the right (10.5mm); The lens of both eyes were clear, while the anterior surface of lens attached to the posterior surface of the cornea in the right eye; Gray-whitish opacity was observed behind the lens in both eyes. Ultrasound B-scan examination revealed massive opacity in the vitreous cavity with complete retinal detachment in both eyes. He was diagnosed with ND and
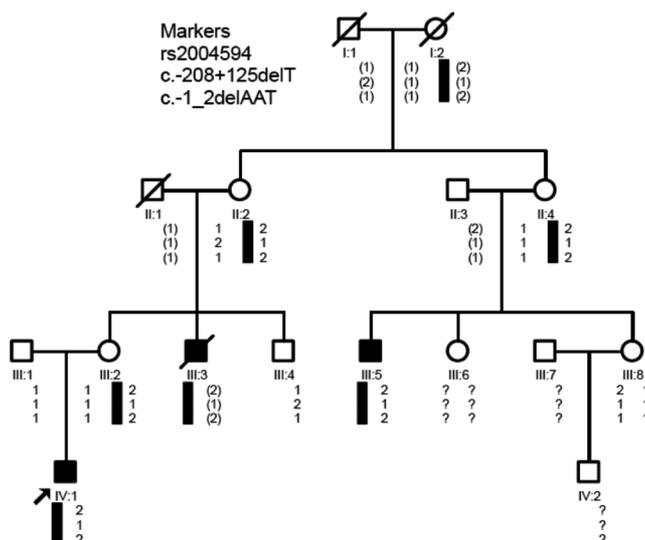

Figure 1 Pedigree and haplotype construction for a family with Norrie disease Markers are listed on the upper left of the figure. The arrow indicates the proband. Black bars indicate the affected haplotype. Inferred genotypes are indicated in parentheses, and unknown genotypes in question mark.
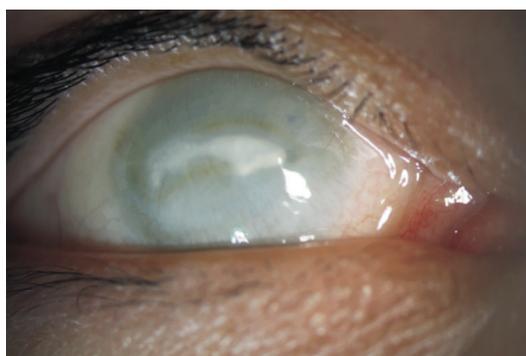

Figure 2 Slit –lamp photograph of patient III:5 It showed sunken eye and corneal opacification.

pars plana vitrectomy and lensectomy were performed in the left eye. Extensive retinal proliferation was observed during surgery, and retinal reattachment was not achieved. Since the surgical outcome of the left eye was not satisfactory, no surgical intervention was attempted in the right eye. No obvious sensorineural hearing loss was observed. During the five-year follow-up, signs of mild mental retardation were documented for this patient.

Patient III:5 was also found to be blind in both eyes after birth and didn't receive any eye examination until 31 years of age. Both eyes were enophthalmos and both cornea appeared milky white (Figure 2). Ultrasound B-scan revealed calcification of ocular wall, massive vitreous opacity and complete retinal detachment (Figure 3). No obvious signs of mental retardation or sensorineural hearing loss were observed based on clinical observation. No medical intervention was attempted for this patient.

Fundus examination found no obvious abnormality in the proband's 30-year-old mother (III:2).
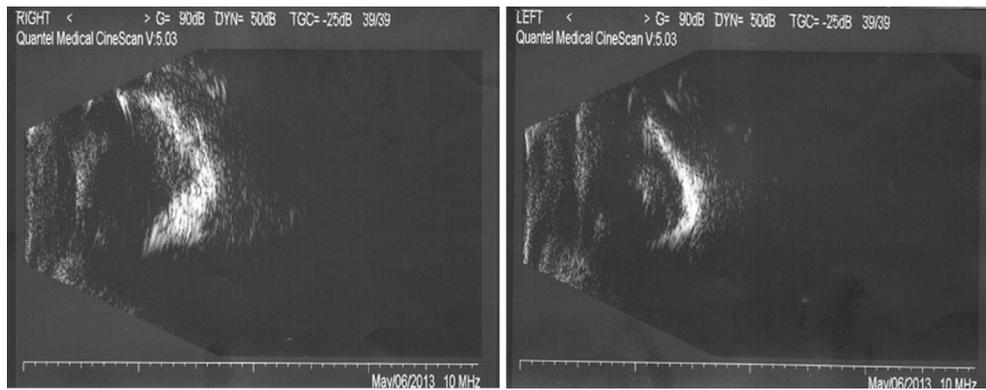
Figure 3 Ultrasound B−scan of patient III:5 It showed calcification of the ocular wall, massive vitreous opacity and complete retinal detechment in both eyes.
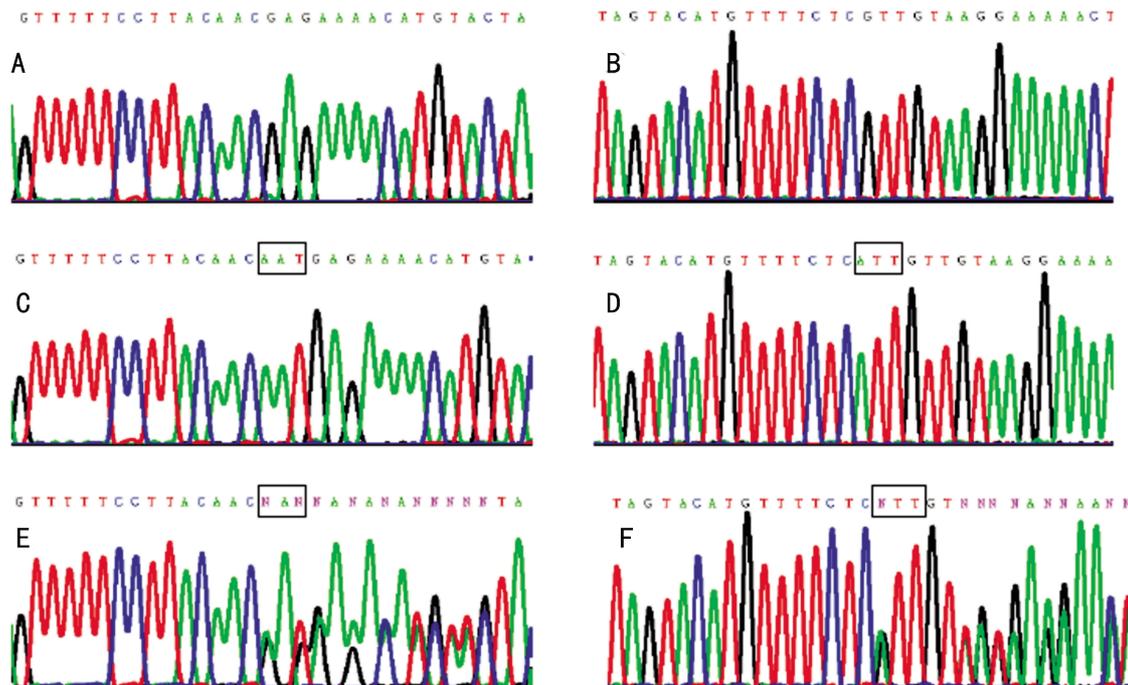

Figure 4 Partial nucleotide sequences of exon 2 of the *NDP* **gene in affected, unaffected and heterozygous family members** The forward (A) and the reverse (B) sequences of the affected family members show the AAT deletion at position c. -1_2. The forward (C) and the reverse (D) sequences of the other unaffected family members and the general population show no nucleotide changes. The forward (E) and the reverse (F) sequences of the patients' mothers and grandmother show heterozygous AAT deletion.

Genetic findings Direct sequencing on both DNA strands in individuals with ND (III:5 and IV:1) revealed a novel mutation, c.-1_2delAAT, in exon 2 of the *NDP* gene (NM_00266.3) (Figure 4). This mutation was found to be heterozygous in their mothers (II:4 and III:2) and/or grandmothers (II:2), but was absent in the other unaffected family members and normal controls. Three intragenic SNPs were identified in this family: one was rs2004594, the insertion of additional GGCCTCTT repeat at 79 bases upstream of the untranslated exon 1; the other two SNPs were in the intron 1, rs1159223 and a novel SNP c. -208+125delT. Haplotypes were constructed using the mutation and the two informative intragenic SNPs, rs2004594 and c.-208+125delT.

Haplotypes constructed showed that the mutation-bearing haplotype co-segregated with the ND phenotype in males (III:5 and IV:1) which was transmitted from their mothers (II:4 and III:2) and/or grandmothers (II:2). The male without ND inherited normal haplotype.

Alignment of the gene in different species showed conserved c.-1_2 AAT in the cDNA sequence in chimpanzee, gorilla, cow, pig, mouse, rat and chicken (Figure 5).

The c.-1_2delAAT mutation deletes the last nucleotide A of the 5'UTR and the first two nucleotides AT of the initiation codon, changing the start of translation initiation site. ORF finder predicts that the mutant ORF begins 33 bp further downstream which causes the production of a truncated protein lacking the first 11 N-terminal amino acids.

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Human       CAAC AAT GAGAAAACATGTACTAGCTGCATCCTTT
Chimpanzee  CAAC AAT GAGAAAACATGTACTAGCTGCATCCTTT
Gorilla     CAAC AAT GAGAAAACATGTACTAGCTGCATCCTTT
Cow         CAAC AAT GAGAAATCATGTACTAGCTGCATCCTTT
Pig         CAAC AAT GAGAAATCATGTACTAGCTGCATCCTTT
Mouse       CAAC AAT GAGAAATCATGTACTAGCTGCATCCATT
Rat         CAAC AAT GAGAAATCATGTACTAGCTGCATCCATT
Chicken     CAAC AAT GGGAAGTCATGTACTTGCAGCTTCCATT
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Figure 5 Alignment of *NDP* cDNA orthologues It shows that the nucleotides AAT where the mutation occurs (c.-1_2) are conserved in these species (highlighted by square frame).

DISCUSSION

Norrie disease (ND) is an X-linked recessive disorder in which retinal degeneration occurs before or very shortly after birth [1,2]. The ocular abnormalities include retinal dysplasia with early vascular proliferation (pseudoglioma), retinal detachment, corneal opacities, cataract and atrophic irides. In addition to the ocular findings, some affected males have varying degree of mental retardation or show cognitive decline with age. About a third of cases develop progressive sensorineural hearing loss that is usually apparent in the second decade of life. In this study two patients were diagnosed with ND based on their ocular manifestations. No signs of sensorineural hearing loss were observed in either patient, while one of them showed signs of mild mental retardation.

ND is caused by mutations in the *NDP* gene [3,4]. The gene encodes norrin, a 133-amino-acid secretory protein with two primary domains: a signal peptide (the first 24 amino acids of the N-terminus) responsible for anchoring the molecule and a cysteine knot that plays important roles in receptor binding and signal transduction activation[5-8]. Over 143 *NDP* mutations have been identified, which have been associated with various phenotypes including ND [9,10,12,13], PHPV [9-11], XL-FEVR [9,10,12,13], ROP [9,10,14,15] and Coats disease [16]. It has been observed that mutations disrupting the cysteine-knot motif are associated with severe retinal dysgenesis, whereas noncysteine mutations are present in various abnormality in vascular and retinal development [10,13]. The 5'UTR contains elements that regulate gene expression, mutations in the region may alter gene regulation [19]. The 5'UTR mutations in *NDP* have been reported in ROP [9,10]. In this study, a novel mutation responsible for ND, c.-1_2delAAT, was identified. The mutation deletes the last nucleotide A of the 5'UTR and the first two nucleotides AT of the initiation codon. Since the translation start site and its context sequence play an important role in the control of translation efficiency and the correct translation of eukaryotic mRNA[20], the c.-1_2delAAT mutation is expected to cause the failure of ND gene translation or the production of an aberrant protein. ORF

finder predicted that it would eliminate the first 11 N-terminal amino acids of norrin, disrupting its putative signal peptide. Loss of the signal peptide would lead to altered secretory pathway and absence of functional protein. The other initiation codon point mutations, c.1A>G (ATG>GTG, p.Met1Val?) and c.2T>G (ATG>AGG, p. Met1Arg?), have been reported to be responsible for ND[21,22]. Congenital blindness is common among patients with either of the two mutations or the c.-1_2delAAT mutation in this study. However, three Japanese patients with c.1A>G mutation remained unremarkable in neurological and otological studies, while the patient with c.2T>G mutation had hearing loss and autistic features[22]. The difference in the mutations and the subsequent differences in the translation efficiency and aberrant protein products may contribute to the differences in phenotypes[21]. Patients with c.-1_2delAAT mutation in this study had no obvious sensorineural hearing loss. One patient showed mild mental retardation, but not the other one. The clinical heterogeneity in the same family with the same *NDP* mutation has also been found in another report and indicates that other genetic and epigenetic factors may also play a role in the disease presentation [9,22,23]. Characterization of the mutant protein and the regulation of *NDP* gene expression would provide further understanding on the mechanism of this disease.

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