

A novel *NHS* mutation in a Chinese family with Nance-Horan Syndrome

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Abstract. Nance-Horan syndrome (NHS) is a rare X-linked disorder with various clinical manifestations. The present study aimed to identify the pathogenic mutation causing NHS in a three-generation Chinese family with 4 individuals presenting primarily with congenital cataracts. The genomic DNA of 5 individuals was collected, and family history and clinical information were recorded. Whole exome sequencing was performed on the proband, and candidate mutations were filtered by a series of screening processes and validated by Sanger sequencing. The identified pathogenic mutation was confirmed by co-segregation analysis. Finally, a novel frameshift mutation (NM_001291867.1: c.302dupA; p.Ala102fs) was identified in the NHS actin remodeling regulator (*NHS*) gene, which co-segregated with congenital cataracts in this family. Carrier females exhibited similar but milder clinical symptoms compared with the affected male. These clinical symptoms were consistent with the phenotypic features of the *NHS*-associated disease, NHS. In summary, the present study identified a novel *NHS* mutation in a Chinese family with atypical NHS; the results broaden the known pathogenic mutation spectrum of *NHS* and will aid in the genetic counseling of patients with NHS. The data from the present study also suggest that genetic analysis may be required for the diagnosis of this disease.

Introduction

Nance-Horan syndrome (NHS; Online Mendelian Inheritance in Man no. 302350), also known as cataract-dental syndrome, is a rare X-linked inherited disease first described in 1974 (1-3). It is characterized by congenital cataracts, dental and craniofacial abnormalities, and mental retardation in ~30% of affected males (4-6). All affected males have bilateral severe congenital cataracts, usually requiring cataract surgery at an early age, and the majority also have congenital microcornea, nystagmus, strabismus or microphthalmia (7,8). Dental anomalies are a typical feature that may distinguish NHS from other types of congenital cataracts. Dental anomalies observed in patients with NHS include screwdriver-shaped incisors, supernumerary maxillary incisors (mesiodens) and a space between two teeth (diastema) (2). Dysmorphic facial features are usually mild and are often overlooked by ophthalmologists, but include a prominent nose and nasal bridge, a long narrow face, and large anteverted pinnae (2,9). Heterozygous carrier females often exhibit similar but milder, symptoms compared with affected males, including posterior Y-sutural lens opacities, little or no loss of vision, and occasional dental and facial abnormalities (10,11).

In the present study, a clinical and genetic analysis of a Chinese family with congenital cataracts and no obvious dental or craniofacial abnormalities was performed. A novel frameshift mutation in the NHS actin remodeling regulator (*NHS*) gene (c.302dupA; p.Ala102fs) was identified using whole exome sequencing (WES), and validated by direct sequencing. This result broadens the known phenotypic and genotypic spectrum of *NHS*-associated diseases.

Materials and methods

Subjects. A three-generation Chinese family was enrolled in the present study (Fig. 1). Ophthalmic examinations and disease diagnosis were performed in Liuzhou Maternal and Child Health Care Hospital (Liuzhou, China). The study adhered to the guidelines of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology statement on human subjects. All procedures were approved by the Ethics Committee of the National Research Institute for Family Planning (Beijing, China) and informed consent was obtained from the patients.

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Abbreviations: NHS, Nance-Horan Syndrome; WES, whole exome sequencing

Key words: Nance-Horan syndrome, NHS actin remodeling regulator, congenital cataracts, whole exome sequencing, co-segregation analysis

Whole exome sequencing and variant screening. The genomic DNA of 5 individuals of the family was extracted using the HiPure Blood DNA Mini kit (Qiagen GmbH, Hilden, Germany). WES of the proband (III:2) was performed using the SureSelect Human All Exon V5 kit (Agilent Technologies, Inc., Santa Clara, CA, USA) to capture all exons and enrich the DNA fragments. Sequencing was then performed using the HiSeq4000 sequencer (Illumina, Inc., San Diego, CA, USA), with Burrows-Wheeler Alignment software (bio-bwa.sourceforge.net/bwa.shtml; version bwa-0.7.12) to align the sequences against the human reference genome. Variants were labelled using the Genome Analysis Toolkit (www.broadinstitute.org/gatk/; version GATKv3.4.0), including single nucleotide variants and indels, and annotated using SnpEff (snpeff.sourceforge.net/SnpEff.html; version SnpEff_v4.1g).

Annotated variants were filtered by a series of processes: Firstly, exonic non-synonymous variants of cataracts-causing genes (12) were extracted, including missense, nonsense, frameshift and splice site mutations. Secondly, high frequency mutations with minor allele frequencies >1% in 1000Genomes (www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), ESP6500 (evs.gs.washington.edu/EVS/) or ExAC (exac.broadinstitute.org/) databases were excluded. Then, genes with inheritance patterns that did not match the family were removed. Finally, genes responsible for syndromic phenotypes where cataracts are one of the features, but not the primary symptom, were excluded.

Candidate mutation validation and co-segregation analysis. The candidate mutation of the proband was validated by Sanger sequencing (performed by Beijing Liuhe Huada Gene Technology Co., Ltd., Beijing, China) with primers designed using Primer Premier 5 software (version 5.00; PREMIER Biosoft International, Palo Alto, CA, USA). Forward and reverse primers used to identify the *NHS* mutation were: 5'-GGACTGGCTGGACTGATTTGCT-3' and 5'-CGCTCCCTTCTGCTCCTTCT-3', respectively. Sanger sequencing was then used to perform co-segregation analysis of mutation and disease phenotypes.

Results

Clinical manifestations. The proband (III:2), a 2-year-old boy, had diagnosed cataracts in both eyes following birth. Ophthalmic examinations revealed congenital cataracts with microcornea (horizontal corneal diameter = 8 mm) and nystagmus. No other facial or dental abnormalities were observed, with the exception of slightly large anteverted pinnae. He underwent lentectomy without artificial lens implantation due to his young age. His mother (II:3) exhibited a congenital cataract with microcornea and strabismus of the right eye, and received cataract surgery. Fundus examination demonstrated unclear optic atrophy and tigroid fundus of the right eye, and the left eye was normal (Fig. 2A). The maternal aunt of the proband (II:1) also exhibited a congenital cataract of the right eye, and compound myopic astigmatism of the left eye with no obvious cataract. Fundus examination revealed similar results to those of II:3 (Fig. 2B). Detailed clinical features are summarized in Table I.

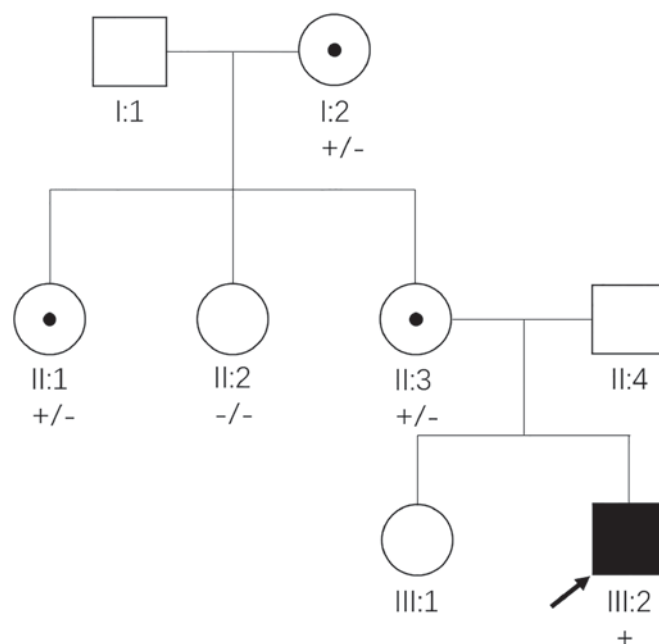


Figure 1. Pedigree of a three-generation Chinese family with Nance-Horan Syndrome. The black arrow indicates the proband. Square, male; circle, female; black square, patient; blank symbol, unaffected member; dotted circle, carrier; +, mutant allele; -, normal allele.

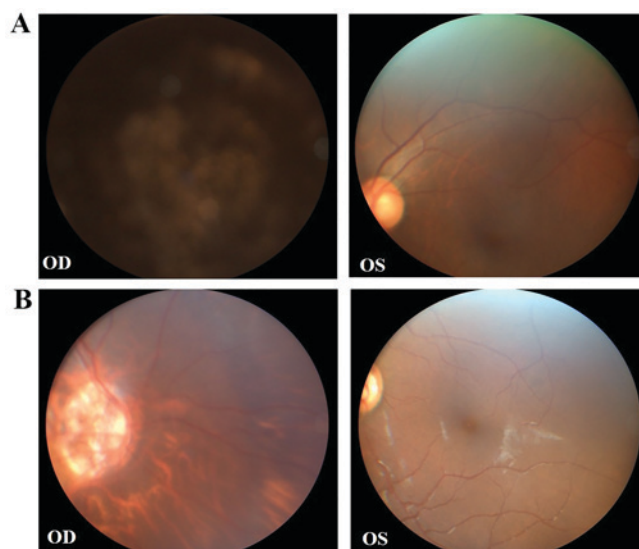


Figure 2. Fundus images of the mother and maternal aunt of the proband. (A) Left image: The right eye of the mother, with unclear optic atrophy and tigroid fundus. Right image: A normal left eye. (B) Left image: The right eye of the maternal aunt with unclear optic atrophy and tigroid fundus. Right image: Tiny retinal vessels in the left eye. OD, right eye; OS, left eye.

Identification of the pathogenic mutation. WES identified 79,533 single nucleotide polymorphisms and 9,307 indels in the proband. A total of 7 mutations in different genes were identified following the first two filtering processes, including 5 missense mutations, one splice acceptor mutation and one frameshift mutation (Table II). Variants of genes inherited in an autosomal recessive pattern were excluded, as the family tree suggested an autosomal dominant or X-linked inheritance pattern. A total of 3 genes, *NHS*, aldehyde dehydrogenase 18 family member A1 (*ALDH18A1*) and myosin heavy chain 9

Table I. Clinical symptoms of carriers and affected members in the Chinese Nance-Horan Syndrome family.

| Characteristics | Disease phenotype | II:1 (carrier) | II:3 (carrier) | III:2 (affected) |
|---------------------|--------------------------------|----------------|----------------|------------------|
| Age, years/sex | - | 41/F | 36/F | 2/M |
| Ocular features | Bilateral congenital cataracts | + | + | + |
| | Microcornea | - | + | + |
| | Nystagmus | - | - | + |
| | Strabismus | - | + | - |
| | Microphthalmia | - | - | - |
| Non-ocular features | Dental anomalies | + | + | - |
| | Long narrow face | - | - | - |
| | Prominent nose | - | - | - |
| | Large anteverted pinnae | - | - | + |
| | Mental retardation | - | - | - |

F, female; M, male; +, positive; -, negative; right, right eye.

Table II. Candidate mutations identified following the first two filtering processes.

| Gene | Locus | Inheritance | Mutation | Group of genes in a cataract review (12) |
|-----------------|---------|-------------|---------------------------------------|--|
| <i>PEX5</i> | 12p13.3 | AR | NM_001300789.1; c.362G>A; p.Arg121His | Syndromic cataract genes |
| <i>RECQL4</i> | 8q24.3 | AR | NM_004260.3; c.2296+1delC | Syndromic cataract genes |
| <i>NHS</i> | Xp22.13 | XL | NM_001291867.1; c.302dupA; p.Ala102fs | Syndromic cataract genes |
| | | | | Syndromic genes only with cataract |
| <i>LRP2</i> | 2q24q31 | AR | NM_004525.2; c.1199A>G; p.Asn400Ser | Syndromic cataract genes |
| <i>ALDH18A1</i> | 10q24.3 | AD/AR | NM_002860.3; c.868G>A; p.Gly290Arg | Syndromic cataract genes |
| <i>MYH9</i> | 22q13.1 | AD | NM_002473.4; c.3676C>T; p.Arg1226Trp | Syndromic cataract genes |
| <i>GALK1</i> | 17q24 | AR | NM_000154.1; c.735A>T; p.Glu245Asp | Only cataract genes |

AR, autosomal recessive; XL, X-linked; AD, autosomal dominant; *PEX5*, peroxisomal biogenesis factor 5; *RECQL4*, RecQ like helicase 4; *NHS*, NHS actin remodeling regulator; *LRP2*, LDL receptor related protein 2; *ALDH18A1*, aldehyde dehydrogenase 18 family member A1; *MYH9*, myosin heavy chain 9; *GALK1*, galactokinase 1.

(*MYH9*), were identified as candidates. However, *ALDH18A1* and *MYH9* are syndromic cataract genes. *ALDH18A1* is one of the pathogenic genes of autosomal dominant or autosomal recessive inherited cutis laxa (13), which is primarily characterized by redundant, sagging, inelastic and wrinkled skin with variable systemic involvement (14). *MYH9* is the pathogenic gene of Fechtner syndrome, which is characterized by hereditary nephritis, hearing loss, cataracts, macrothrombocytopenia and leucocyte inclusions, present in varying combinations (15). In cutis laxa and Fechtner syndrome, cataracts are one of the clinical manifestations, but are not the main phenotype. In addition, members of this family with cataracts did not present other clinical symptoms of cutis laxa and Fechtner syndrome. Therefore, *ALDH18A1* and *MYH9* were excluded. *NHS* is the pathogenic gene of NHS, which is characterized by congenital cataracts, and dental and craniofacial abnormalities, and mental retardation in ~30% of affected males (5,11). However, there are also certain NHS families with atypical clinical manifestations, that only exhibit congenital cataracts, and occasionally other concurrent ocular abnormalities (16).

Tug *et al* (8) suggested that 100% of patients with NHS have bilateral congenital cataracts, 90% have large anteverted pinnae and only 65% have dental abnormalities. Therefore, *NHS* was identified as the only candidate gene.

The *NHS* frameshift mutation (c.302dupA; p.Ala102fs) identified in the Chinese family examined in the present study was absent in 1000Genomes, ESP6500 and ExAC databases, suggesting that it is a novel mutation. Sanger sequencing verified the presence of the *NHS* mutation in the proband (III:2). Subsequent co-segregation analysis among the 5 available individuals in this family detected c.302dupA in the 4 members with congenital cataracts (I:2, II:1, II:3 and III:2), but not in the unaffected member (II:2) (Fig. 3). The three female members (I:2, II:1 and II:3) were all heterozygous for this mutation, and exhibited similar but milder clinical symptoms to the affected male (III:2). These phenotypic discrepancies and segregation pattern conform to the disease features of NHS. Therefore, the *NHS* frameshift mutation, which altered the open reading frame and resulted in a premature truncated protein, was the pathogenic mutation of this family.

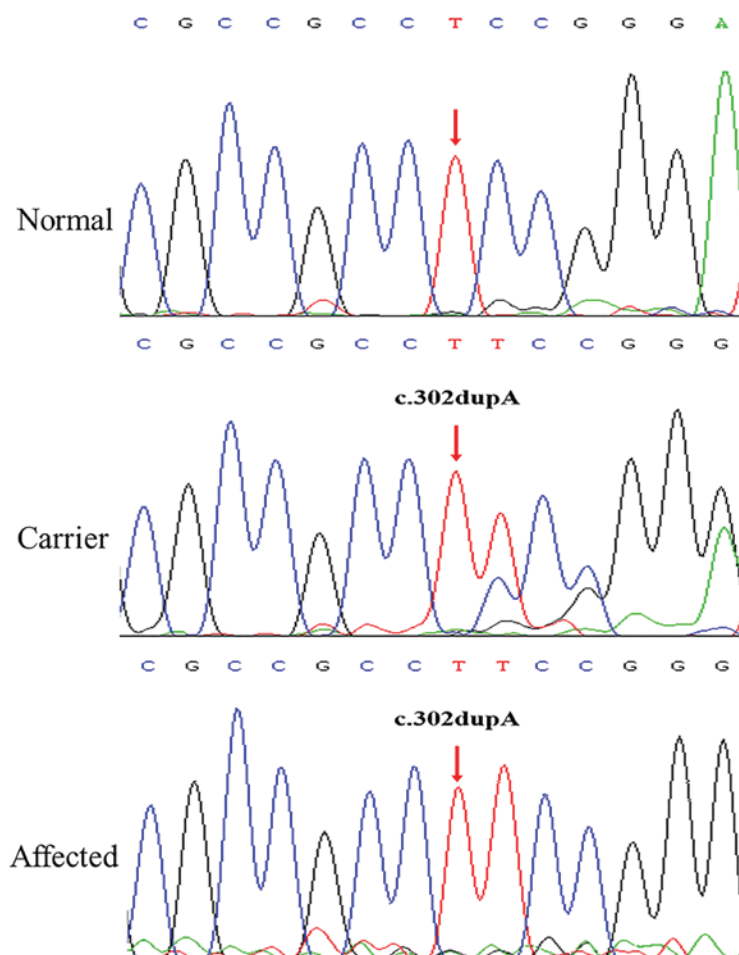


Figure 3. Reverse sequencing of a normal individual, female carrier and the male patient (III:2). Red arrow indicates the mutation site.

Discussion

In the Chinese family examined in the present study, the proband (III:2), his mother (II:3), maternal aunt (II:1) and maternal grandmother (I:2) exhibited congenital cataracts with other ocular abnormalities, but no marked craniofacial or dental anomalies. Therefore, all congenital cataracts-associated genes were initially screened. Subsequent to exclusion of high-frequency variants, genes with recessive inheritance patterns and those causing syndromic cataracts, a novel *NHS* frameshift mutation (c.302dupA; p.Ala102fs) was identified, which altered the open reading frame and resulted in a premature truncated protein. Sanger sequencing demonstrated that the mutation co-segregated with the primary phenotype; congenital cataracts.

NHS, located on Xp22.13, contains 10 exons and encodes at least 5 isoforms resulting from alternative splicing (16,17). *NHS*-A and *NHS*-1A are the two primary isoforms, encoding proteins of 1,630 and 1,651 amino acids, respectively, and are transcribed from exon 1. These 2 isoforms differ in that *NHS*-1A contains exon 3a and *NHS*-A does not (17). *NHS*-B is transcribed from exon 1b, is translated from exon 4, and encodes a protein 1,335 amino acids in length. *NHS*-C is transcribed and translated from exon 1a and encodes a protein containing 1,453 amino acids (6-8). Null mutations in *NHS* have been identified as causative of NHS (5,11). At present, ≥ 40

different *NHS* mutations have been described, originating in India, China, Australia, the United Kingdom, Turkey, Canada and the United States of America (<http://cat-map.wustl.edu/>). A number of these are nonsense mutations or indels, while a few are splice site mutations or copy number variations.

NHS is conserved in humans and other vertebrates, including mice (18). It is expressed in multiple tissues that regulate morphology and development, including the lens, craniofacial mesenchyme, dental primordia and brain (19). *NHS* isoforms exhibit spatial differences in expression. Isoforms containing exon 1 are expressed in the epithelia and are localized to the cell periphery, while isoforms lacking exon 1 are expressed in non-epithelial tissue and localized to the cytoplasm (7). Therefore, the pleiotropic features of *NHS* may be attributed to differences in subcellular localization and *NHS* protein isoforms. The N-termini of *NHS*-A and *NHS*-1A have a functional Wiskott-Aldrich syndrome protein-family verprolin-homologous protein homology domain, which serves an important role in the regulation of actin remodeling and the maintenance of cell morphology by interacting with Abl interactor proteins (17). *NHS* mutations in exon 1 were demonstrated to only disrupt the *NHS*-A and *NHS*-1A isoforms, while mutations in exons 3, 5 and 6 appear to affect ≥ 1 isoform type (20,21). The frameshift mutation identified in exon 1 (c.302dupA; p.Ala102fs) in the present study may cause the NHS disease phenotype by disrupting *NHS*-A and *NHS*-1A function.

In summary, the present study described the clinical manifestations of an affected male and carrier females in a Chinese family with NHS, and identified a novel frameshift mutation (c.302dupA; p.A1a102fs) in the *NHS* exon 1. The results of the present study broadens the known pathogenic mutation spectrum of *NHS* and will aid genetic counseling for patients with NHS. The identification of atypical NHS in the present study, with congenital cataracts and other ocular abnormalities but no marked craniofacial or dental anomalies, caused by an *NHS* mutation suggests that diagnosis may require a combination of clinical assessment and genetic analysis. There are certain limitations in the present study. Firstly, a frameshift insertion variant of *NHS* that was completely co-segregated with the disorder was identified. This provided only genetic evidence to the pathogenicity of the variant. Therefore, future experiments may be required to confirm these data. For example, whether the insertion affects the mRNA production, for example nonsense-mediated mRNA decay, may be verified by reverse transcription polymerase chain reaction. Concomitantly, the variant produced a truncated protein, which requires additional confirmation by performing western blot analysis. Finally, whether this frameshift variant is specific to this family or common in the Chinese NHS population was not determined. Future studies will require the analysis of more patients, to include a range of *NHS* variants.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MW, AQ, XM and BW were involved in the conception and design of the study. HM, and KW were responsible for the acquisition of data. MW, AQ, HM and KW were responsible for analysis and interpretation of the data. MW and AQ drafted the manuscript, and XM and BW critically revised the manuscript for important intellectual content. All authors read and approved the final version to be published.

Ethics approval and consent to participate

Approval of the present study was obtained from the Ethics Committee of the National Research Institute for Family

Planning. All procedures adhered to the guidelines of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology statement on human subjects. Written informed consent was obtained from the patients.

Patient consent for publication

Written informed consent for publication of clinical details and clinical images was obtained from the patients.

Competing interests

The authors declare that they have no competing interests.

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