A Novel Frameshift Mutation in The NHS Gene Causes Nance-Horan Syndrome in a Chinese Family

Huaming Wen

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

Huang Xiaosheng

Shenzhen University

Qianwen Li

Shenzhen Stomatology Hospital Affiliated to Shenzhen University

Yan Wang

Shenzhen University

Mei Shaoyi

Shenzhen University

Cai Jiamin

Baoan Central Hospital of Shenzhen

Jun Zhao (✉ doctorzhaojun@163.com)

Shenzhen People's Hospital, Jinan University, Southern University of Science and Technology)

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Abstract

Background: Nance-Horan syndrome (NHS), also known as cataract-tooth syndrome, is a rare X-linked genetic disorder characterized by congenital cataract as well as dental and craniofacial abnormalities caused by mutations in the *NHS* gene. In this study, we describe a Chinese family with a frameshift mutation in *NHS* associated Nance-Horan syndrome, thus expanding the mutational spectrum of this gene.

Methods: Four members (including three patients) had their ocular bodies examined in the presence of congenital cataracts featuring dental and craniofacial abnormalities. DNA samples of family members were extracted from peripheral venous blood, and known pathogenic genes of congenital cataracts were panel sequenced.

Results: In the proband, a novel frameshift mutation (c.1694_1697delGAATinsCATTCG) was identified in the *NHS* gene. Sanger sequencing of family members verified that the mutation completely co-segregated with the disease in the pedigree.

Conclusion: The congenital cataract family was diagnosed as having Nance-Horan syndrome (NHS), and the *NHS* frameshift mutation was determined to cause the disease in this family. This is a novel *NHS* gene mutation that has not been reported previously.

Background

Congenital cataract is a leading cause of visual disability in children, and currently ranks second as the cause of child blindness (1). Cataract can be isolated or can occur in association with a large number of different metabolic diseases or genetic syndromes. Nance-Horan syndrome (NHS), also known as cataract-tooth syndrome, is a rare X-linked genetic disorder characterized by congenital cataract as well as dental and craniofacial abnormalities caused by mutations in the *NHS* gene (2). NHS was reported and described simultaneously in 1974 by Walter E. Nance and Margaret B. Horan (3,4). Approximately 30% of affected males may have varying levels of mental retardation (5-7). Most male patients with this syndrome present with severe congenital cataract in both eyes, and also have congenital microcornea, nystagmus, strabismus, or congenital microphthalmia (8,9). Dental anomalies are typical features that distinguish NHS from other types of congenital cataract, and the clinical features mainly include conical or serrated incisors, extra maxillary incisors, and excessive interdental spaces (10). Craniofacial anomalies primarily include bulging alae and bridge of the nose, long and narrow face, and large and anteverted pinnae (10,11). Approximately 43 *NHS* mutations have been identified, most of which are nonsense mutations, insertions, or deletions, and a few are splice-site variants or copy number variations. The *NHS* mutations are mostly located in exon 6. It is of great importance to discover causative genes of rare diseases as this will not only facilitate the precise diagnosis of rare diseases and reduce the birth of deficient infants, but also facilitate the development of drugs and therapies. In the present study, a novel frameshift mutation in the *NHS* gene was identified from a Chinese family affected with NHS.

Methods

Pedigree and patients

A congenital cataract family consisted of five patients affected with NHS, including the proband (III:4) and 4 family members (I2: II:3, II:7 and III:7), was recruited from Shenzhen, Guangdong Province, China (**Fig. 1**). All patients underwent detailed medical history collection and physical examination. Disease history, family history, and pregnancy history, ocular history, Snellen visual acuity, best-corrected visual acuity, non-contact tonometry, along with slit-lamp examination, anterior segment examination and photography, and posterior segment examination of family members were recorded. The study protocol was approved by the Ethics Committee of Shenzhen Eye Hospital and conducted according to the standards of the Declaration of Helsinki. All participants provided written informed consent.

DNA extraction and target region sequencing

DNA extraction: Peripheral blood samples (4–5 ml) were collected in EDTA anticoagulant vacuum tubes and stored at -20°C. Genomic DNA was extracted using [provide the information for the kit] and determined using a Nanodrop™ 2000 spectrophotometer (Thermo Fisher Scientific Co. Ltd. Boston, MA).

Genomic library construction: An ultrasonic DNA oscillator broke DNA into 180–280 base pair fragments, the adaptors on each end were then ligated, end repair and phosphorylation was conducted, and then the ligation products were purified using magnetic beads. After purification via agarose gel electrophoresis, suitable fragments were enriched by PCR amplification.

Target region gene capture: The gene fragments were hybridized to the probe (whole-exon P039-Exome probes), and adsorbed to the beads through biotin and streptavidin-biotin. The nonspecific binding DNA fragments were then washed out and the target gene was enriched.

NextSeq500 high-throughput sequencing: All sequencing was performed on a NextSeq500 (Illumina, San Diego, CA) using bridge amplification and the Flowcell sequencing chip (Illumina). The NextSeq500 performs intelligent cycle imaging, in which individual cycle reactions can be extended with only one correct complementary base, and the base species are confirmed on the basis of distinct fluorescent signals followed by multiple cycles to yield the complete nucleic acid sequence.

Bioinformatics analysis

Primary sequencing data were aligned to the human reference genome (hg19) after filtering out low-quality reads and potential adaptor contamination sequences. Sequences were aligned using Burrows-Wheeler Aligner software (Bwa:bwa-0.7.10; <http://bio-bwa.sourceforge.net/>). The collected data were processed through a standard information analysis pipeline (<https://samtools.sourceforge.net/>), including detection, annotation, and analysis of single nucleotide polymorphisms (SNPs) as well as insertion and deletion mutations. At the same time, sequencing data were analyzed to assess whether the sequencing depth was sufficient for transcriptome coverage. The GATK (<https://www.broadinstitute.org/gatk/>) genome analysis web tool library was used to retrieve the number of SNPs and missing marker loci. The reference databases were the human HAPMAP, dbSNP138, Exome Sequencing Project, and Exome Aggregation Consortium databases. Candidate causative genes were screened by stepwise filtering.

Validation of the candidate gene mutation by Sanger sequencing

The candidate *NHS* mutation was verified using Sanger sequencing. The coding regions of the gene were amplified and sequenced. PCR primers were designed using Primer 3.0 online software (Applied Biosystems ABI, Foster City, USA). The sequences of the forward primer and the reverse primer were

5'-TTCGCCAAGCGGATCGTGGA-3' and 5'-TTAGGGTCAAGCGTGCTGAGGA-3' respectively. Sanger sequencing was also performed for all family members to determine whether this gene mutation was co-separated with the disease.

Functional prediction of the mutation protein

SOPMA was used to calculate the secondary structure features of the mutant protein (NPS@ : SOPMA secondary structure prediction (ibcp.fr)). PSORTII was used to predict the subcellular location of the mutant protein (<https://psort.hgc.jp/form2.html>). The protein structure were predicted with Phyre2 protein fold recognition server (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>).

Results

Clinical characteristics of the patients in the family

The 3-generation family consisted of 5 patients affected with NHS and 13 unaffected family members (**Fig. 1**). No members of the family had any influence with respect to radiation, drugs, or toxic factors. The genetic pattern in this family was determined to be X-linked dominant inheritance. The proband (III:4) was a 30-years-old female patient, who had diagnosed cataract at 9 years old. Ophthalmic examinations revealed congenital cataract with narrow palpebral fissures, intraocular lens, and nystagmus. No other facial or dental abnormalities were observed. B ultrasound showed binocular vitreous opacity with posterior detachment, long ocular axis, and posterior scleral staphyloma. She underwent lensectomy and artificial lens implantation (**Fig. 2**). Her mother had no cataract or dental abnormalities (II:4). Her father (II:3) and uncle (II:7) exhibited congenital cataract with nystagmus and strabismus, and underwent cataract surgery. They had typical craniofacial genetic characteristics, including a long narrow face, prominent nose, large auricle, and serrated incisors with wide interdental spaces. B ultrasound examination further revealed similar results to those of III:4 (**Fig. 3**). Detailed clinical characteristics of the patients in the family were summarized in Table 1.

Mutation analysis of candidate genes

The sequencing coverage of the gene panel consisted of 188 lens pathogenic related genes was >99% (10x), and the sequencing depth was 200(±30)x. A heterozygous mutation in the *NHS* gene was detected, c.1694_1697delGAATinsCATTCG, which is a frameshift mutation (p.R565Pfs). As verified by Sanger sequencing, this heterozygous mutation co-segregated with the disease in this family. Three patients (III:4, III:3 and III:7) carried the mutation while the unaffected member (II:4) possessed it (**Fig. 4**). Since the mutation was not found in the public databases including the Human Gene Mutation Database (HGMD), the dbSNP database, the 1000 Genomes Project (1KG), the Exome Sequencing Project (ESP), and genome aggregation database (gnomAD), it was considered to be a novel mutation. The mutation was evaluated as a suspected pathogenic variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Functional prediction of the mutation protein

The amino acids number of mutation NHS protein is 648 and its molecular weight is 70336.37. Theoretical pI is 9.76. The instability index (II) has been computed to be 77.32. The stability classifies of the protein is unstable. PSORTII was used to predict the subcellular location of the mutant protein and the k-NN prediction results of query showed that 69.7% protein located in nuclear. SOPMA was used to predict the secondary structure of the mutant protein, the results showed that were α -helix (31.33%) and random coil (58.18%), extended strand (7.72%) and β -sheet (2.78%) (**Fig.5**). Phyre2(string method, ab initio calculation mode) was used to predict the tertiary structure of the mutant protein, the results had been shown in figure 6 (**Fig.6**). According to the sequence homology, the best template structure c3p8cD was selected, the hydrophobic structure had the highest similarity, and the reliability rate was 99.9%.

Discussion

It has been reported that the majority of NHS cases are X-linked recessive, and female carriers usually feature similar but milder clinical features than male patients, mainly including punctate or coralliform opacities in the posterior Y-suture of the crystals and lesser visual impact, occasionally accompanied by some dental and facial abnormalities (12). In the present study, the inheritance pattern in the family was consistent with a dominant X-linked trait, and the proband (III:4) was a female patient who underwent cataract extraction and intraocular lens implantation at the age of 9 years, with subsequent robust postoperative visual acuity. However, her visual acuity was poor owing to high myopia in both eyes, posterior scleral staphyloma, and retinal detachment. The patient had no obvious dental or facial anomalies, but had some eye manifestations, such as small palpebral fissures, nystagmus, and long eye axis. The father (II:3) and uncle (II:7) of the proband had typical ocular and craniofacial phenotypes, including congenital cataract, nystagmus, long narrow face, prominent nose, large auricle, and serrated incisors with wide interdental spaces. Based on these findings, we propose that the NHS pedigree with X-linked dominant inheritance pattern has similar phenotypes as X-linked recessive inheritance. Male patients show more severe disease phenotypes than female patients. Accordingly, this family showed that male patients had more obvious facial and dental abnormalities than female patients, while female patients had better visual acuity than male patients.

According to the classic definition, gene mutations are sudden heritable changes in DNA. At the molecular level, mutations refer to changes in gene structure that modify the composition or sequence of base pairs, which can be generally grouped into point mutations and frameshift mutations. Point mutations are classified into synonymous mutations, missense mutations, and nonsense mutations. Nonsense mutations are the most frequent type of NHS pathogenic variants, leading to a lack of functional NHS protein production, which are the main reason for the incidence of NHS (7). Missense mutations, frameshift mutations, and aberrant splicing can all lead to premature stop codons that may produce truncated proteins. In addition, duplication and large fragment deletion of the *NHS* gene can also be pathogenic through abnormal transcription of the *NHS* gene or non-production of the NHS protein. NHS mutations are mainly located in exon 6, 43 NHS gene mutation sites have been listed in detail in this article, of which 19 mutations are located in exon 6. Approximately 20 mutations are point mutations and 18 mutations are frameshift mutations (6-9,12-28).[Table 2](#).

In this study, we identified a heterozygous frameshift mutation (c.1694_1697delGAATinsCATTCG) in exon 6 of the *NHS* gene in the family (**Fig. 4**). Since this mutation was not reported in the public human gene mutation databases, we considered it as a novel mutation. Moreover, the mutation was co-segregated with the disease in the pedigree (**Fig. 1**). According to the guidelines of the American Society of Medical Genetics and Genomics, this frameshift mutation was judged as a likely pathogenic variant. Protein secondary structure refers to the folding and coiling of the amino acid residues of the protein polypeptide chain which include the α -helix, β -sheet, extended strand and random coil formed. The mutant NHS protein contains α -helix (31.33%) and random coil (58.18%). The function of a protein is closely related to its tertiary structure. The prediction and analysis of protein high-level structure will help to understand the correlation between protein structure and function. However, the specific pathogenic mechanisms of this mutation and its effect on protein function require further study.

In summary, the congenital cataract family has typical ocular phenotypes, as well as teeth and facial abnormalities. Target region gene capture detected a novel frameshift mutation in the NHS gene. Sanger sequencing of the proband's family members revealed that the mutation completely co-segregated with the disease in the pedigree. The present identification of a novel mutation not only further supports the diagnosis of this NHS pedigree, but also expands the spectrum of NHS mutations, which will contribute to the genetic diagnosis and counseling of families with NHS.

Conclusion

The congenital cataract family was diagnosed as having Nance-Horan syndrome (NHS), and the *NHS* frameshift mutation was determined to cause the disease in this family. This is a novel *NHS* gene mutation that has not been reported previously.

Declarations

Aknowledgements

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Authors'contributions

J.Z. and H.M.W. conceived and supervised the study. J.Z., H.M.W. and X.S.H. recruited patients and evaluated the patients' clinical manifestations. J.Z., H.M.W., Q.W.L, Y.W., S.Y.M and J.M.C. carried out mutation screening for candidate genes. H.M.W. and X.S.H. performed data collection and analysis. J.Z., H.M.W. and X.S.H. drafted and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analysed during the current study are available in the [SRA] repository, [<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA745396/>].

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shenzhen Eye Hospital and was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment.

Consent for publication

All participants provided written informed consent to publish this information was obtained. The information included images or clinical details of participants.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Clinical characteristics of the patients in the family

Patient	Ⓐ:4	Ⓑ:3	Ⓒ:7
Gender	Female	Male	Male
Age (years)	30	58	48
Eyelid	Narrow palpebral fissures, double blepharoplasty	Narrow palpebral fissures	Narrow palpebral fissures
Corneal	Transparency	Transparency	Transparency
Lens	Intraocular lens	Aphakia	Aphakia, residual cortex
Eye position	Normal position, nystagmus	Strabismus, nystagmus	Strabismus, nystagmus
B ultrasound	Binocular vitreous opacity with posterior detachment, long ocular axis, posterior scleral staphyloma	Binocular vitreous opacity with posterior detachment, long ocular axis, posterior scleral staphyloma;	Binocular vitreous opacity with posterior detachment, long ocular axis, posterior scleral staphyloma
Facial features	Normal	Long narrow face, prominent nose, large auricle	Long narrow face, prominent nose, large auricle
Teeth	Normal	Serrated incisors, teeth gap-wide	Serrated incisors, teeth gap-wide

Table 2. 43 NHS gene mutation sites and amino acid changes

Base site	Nucleotide	Amino acid	Reference	Base site	Nucleotide	Amino acid	Reference
Exon 1	c.400delC	p.R134fs*61	Brooks et al. (2004)(6)	Exon 6	c.2232delG	p.K744Afs*15	Khan et al. (2012)(23)
Exon 1	c.302dupA	p.Ala102fs	Wei et al. (2019)¶13¶	Exon 6	c.1989C>T	p.P551S	Chograni et al.(2011)(24)
Exon 1	t(X;1)	/	Laura et al. (2019)¶14¶	Exon 6	c.1693C>T	p.R565*	Li D et al. (2016)(25)
Exon 1	c.556G>T	p.E186X	Sun et al. (2014)¶15¶	Exon 6	c.1694_1697delGAATinsCATTCG	p.R565Pfs	This study(2021)
Exon 1	Deletion NHS downstream	No protein product	Coccia et al. (2009)(7)	Exon 6	c.4449C>G	p.Tyr1483Ter)	Ling et al. (2019)(26)
Exon 1	c.322G>T	p.E108*	Hong et al. (2014)(16)	Exon 6	c.2387insC	p.S797fsX35	Burdon et al. (2003)(12)
Exon 1	c.558insA	p.E186Efs*11	Tug et al. (2013)(9)	Exon 6	c.3908del11bp	p.I1302fs*4	Reches et al. (2007)(27)
Exon 1	c.115C>T	p.Q39*	Ramprasad et al.(2005)(17)	Exon 6	c.4129C>T	p.Q1358*	Huang et al. (2007)(21)
Exon 1	c.472C>T	p.Q158*	Coccia et al. (2009)(7)	Exon 6	c.3624C>A	p.C1208*	Huang et al. (2007)(21)
Exon 2	c.614delC	p.P206fs*7	Coccia et al. (2009)(7)	Exon 6	c.3596insA	p.K1198fs*44	Sharma et al.(2008)(19)
Exon 3	c.718insG	p.E240fsX277	Burdon et al. (2003)¶12¶	Exon 6	c.2601-2602ins G	p.K868Efs*5	Florijn et al. (2006)(20)
Exon 3	c.742C>T	p.R248*	Shoshany et al.(2017)(18)	Exon 6	c.2550_2553del4b	p.K850fs*3	Huang et al. (2007)(21)
Exon 3	c.792delA	p.P264fs*18	Sharma et al.(2008)(19)	Exon 6	c.2915 C>A	p.Ser972*	Shoshany et al.(2017)(18)
Exon 4	c.852delG	p.S285Pfs*13	Li et al. (2015)(8)	Exon 6	c.2635C>T	p.R879*	Florijn et al. (2006)(20)
Exon 5	c.1117C>T	p.R373*	Florijn et al. (2006)(20)	Exon 8	Intragenic genomic rearrangement	Protein truncation	Coccia et al. (2009)(7)
Exon 5	c.1108C>T	p.Q370*	Huang et al. (2007)(21)	Intron 1	Intragenic segmental deletion	Altered transcriptional regulation	Coccia et al. (2009)(7)
Exon 5	c.1117C>T	p.R373*	Sharma et al.(2008)(19)	Intron 2	c.IVS2-3 C>G	p.E240fsX277	Burdon et al. (2003)(12)
Exon 6	c.3738-3739delTG	p.C1246Afs*15	Brooks et al. (2004)(6)	Intron 3	c.853-2A>G	/	Sun et al. (2014)(15)
Exon 6	c.2687delA	p.Q896fs*10	Brooks et al. (2004)(6)	Intron 3	c.IVS3-2A>G	p.S285fsX27	Florijn et al. (2006)(20)
Exon 6	An extensive	/	Liao et al. (2011)¶22¶	Intron 4	c.1045 + 2T > A	/	Tian et al. (2017)(28)
Exon 6	c.2716_2719delTTAG	p.L906MfsX24	Sun et al (2014)¶15¶		Segmental duplication -triplication	Altered transcriptional regulation	Coccia et al. (2009)(7)
Exon 6	c.3459delC	p.L1lj54fsX28	Burdon et al. (2003)¶12¶				

Figures

Figure 1

The pedigree map of the NHS family. The black arrow indicates the proband. Square, male; circle, female; black square, patient; blank symbol, unaffected member.

Figure 2

Phenotypic and clinical examination images of the proband (III:4). A1/A2: Transparent cornea, cataract-postoperative, intraocular lens; B1/B2: Vitreous opacity with posterior detachment, long ocular axis, posterior scleral staphyloma; C1/C2: Right retinal detachment, high myopia retinopathy.

Figure 3

Phenotypic and clinical examination images of the other patients in the NHS family (II:3 and II:7). A1/A2: Corneal transparency, posterior capsule calcification, cataract postoperative, aphakia; A3/A4: Cortical residue after cataract surgery, aphakia; B1-B2: Vitreous opacity with posterior detachment, long ocular axis, posterior scleral staphyloma; D1/D2: patient II:3, Dental abnormality, serrated incisors with wide interdental spaces.

Figure 4

NHS gene mutation site with Sanger validation sequencing peak map: A, B, D: Proband III:4 and patients II:3 and II:7 sequencing peak map; red arrow indicates mutation site; C: normal phenotype II:4 sequencing peak map; red arrow indicates no mutation).

Alpha helix	(Hh)	:	203	is	31.33%
3 ₁₀ helix	(Gg)	:	0	is	0.00%
Pi helix	(Ii)	:	0	is	0.00%
Beta bridge	(Bb)	:	0	is	0.00%
Extended strand	(Ee)	:	50	is	7.72%
Beta turn	(Tt)	:	18	is	2.78%
Bend region	(Ss)	:	0	is	0.00%
Random coil	(Cc)	:	377	is	58.18%
Ambiguous states (?)		:	0	is	0.00%
Other states		:	0	is	0.00%

SOPMA was used to predict the secondary structure of the mutant NHS protein, the results showed that were α -helix (31.33%) and random coil (58.18%), extended strand (7.72%) and β -sheet (2.78%).