• Brief Report •

A novel Nance-Horan syndrome mutation identified by next-generation sequencing in a Chinese family

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Abstract

● **AIM:** To identify the disease-causing mutation in a four-generation Chinese family diagnosed with Nance-Horan syndrome (NHS).

● **METHODS:** A Chinese family, including four affected patients and four healthy siblings, was recruited. All family members received ophthalmic examinations with medical histories provided. Targeted next-generation sequencing approach was conducted on the two affected males to screen for their disease-causing mutations.

● **RESULTS:** Two male family members diagnosed with NHS manifested bilateral congenital cataracts microcornea, strabismus and subtle facial and dental abnormalities, while female carriers presented posterior Y-sutural cataracts. A novel frameshift mutation (c.3916_3919del) in the NHS gene was identified. This deletion was predicted to alter the reading frame and generate a premature termination codon after a new reading frame.

● **CONCLUSION:** The study discovers a new frameshift mutation in a Chinese family with NHS. The findings broaden the spectrum of NHS mutations that can cause NHS in Chinese patients.

● **KEYWORDS:** Nance-Horan Syndrome; cataract; next-generation sequencing; NHS gene
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INTRODUCTION

Nance-Horan syndrome (NHS; MIM 302350), a rare X-linked syndrome characterized by congenital bilateral cataracts and dental abnormity, was first reported in 1974 by two studies independently[1-2]. So far, this syndrome has been reported in different ethnic groups and reveal variable clinical features[3-6]. Male patients manifest severe bilateral congenital nuclear cataracts, including opacity in the fetal nucleus and posterior Y-suture; thus, surgical intervention should be performed at an early age[7]. Other ophthalmological abnormalities include microcornea, microphthalmia and nystagmus. Non-ophthalmic abnormalities include dental anomalies (supernumerary maxillary incisors, screwdriver-shaped incisors, and diastema), mental retardation and lateral brachymetacarpalia. As an X-linked hereditary disorder, heterozygous females exhibit similar but milder features than affected males, including posterior Y-sutural cataracts, little or no loss of vision and occasional dental abnormalities[8-9].

NHS gene locates to Xp22.31 to p22.13b between short tandem repeat markers DXS1195 and DXS999[9-10]. It is highly conserved among human and other vertebrates including rat, mouse, and zebrafish. NHS protein plays an important role in the development of ocular lens, tooth, midbrain, thus its mutations can lead to congenital cataract, dental anomalies and, in some cases, mental retardation[10]. Previous studies have discovered more than 40 mutations in the *NHS* gene including frameshift mutations, nonsense mutations, missense mutations, deletion mutations and genomic rearrangements[11-13]. Here, we report a novel a frameshift deletion in the *NHS* gene (c.3916_3919del) and characterize the clinical features of a Chinese pedigree with this syndrome.

SUBJECTS AND METHODS

Ethical Approval Our study, conformed to the tenets of the Declaration of Helsinki, was approved and prospectively reviewed by the Ethics Committee on Human Research of

Table 1 Clinical features of included family members

Members ID	Mutation	Age (y)/ sex	BCVA		Lens		Nystagmus	Microcornea	Strabismus
			OD	OS	OD	OS			
LZ-II:2	*NHS* c.3916_3921del	72/F	20/60	20/60	Posterior Y-sutural cataracts and cortical opacities	Posterior Y-sutural cataracts and cortical opacities	No	Yes	No
LZ-III:1	None	56/F	20/40	20/60	Normal	Normal	No	No	No
LZ-III:2	None	53/F	20/20	20/40	Normal	Normal	No	No	No
LZ-III:3	None	50/M	20/40	20/60	Normal	Normal	No	No	No
LZ-III:5	*NHS* c.3916_3923del	46/F	20/60	20/20	Posterior Y-sutural cataracts	Posterior Y-sutural cataracts	No	Yes	No
LZ-IV:1	*NHS* c.3916_3924del	23/M	20/400	20/160	Underwent bilateral lensectomy due to congenital nuclear cataract	Underwent bilateral lensectomy due to congenital nuclear cataract	No	Yes	Yes
LZ-IV:2	*NHS* c.3916_3925del	18/M	20/120	20/400	Underwent bilateral lensectomy due to congenital nuclear cataract	Underwent bilateral lensectomy due to congenital nuclear cataract	No	Yes	Yes

BCVA: Best corrected visual acuity; OD: Right eye; OS: Left eye.

Nanjing Medical University. Written informed consents were signed by the participants or their legal guardians before enrollment.

Subjects and Clinical Evaluations A Chinese family (family LZ) with the initial symptom of poor central vision was recruited from the First Affiliated Hospital of Nanjing Medical University (Figure 1). Eight family members, including four affected patients and four healthy siblings, participated in our study. All included members from family LZ received ophthalmic examinations with their medical histories collected. Systemic examinations were conducted on the four patients. Another 150 unrelated healthy controls free of major ocular diseases were also recruited. Peripheral venous blood samples were collected from all participants from family LZ and 150 additionally unrelated healthy controls free of major ocular diseases using 5 mL tubes containing ethylenediamantetetraacetic acid (EDTA). Genomic DNA isolation was performed using a QIAmp DNA blood kit (Qiagen, Valencia, CA, USA) per the manufacturer's protocols. DNA samples were stored at -20°C before used.

Targeted Next-Generation Sequencing and Mutation Validation Targeted next-generation sequencing (NGS) approach was conducted on patients LZ-IV:1 and LZ-IV:2 using a previously described microarray targeting 316 ophthalmic disease relevant genes[14-16]. Library preparation, qualification, NGS with the Illumina HiSeq2000 platform (Illumina, Inc., San Diego, CA, USA), and bioinformatics analyses were performed as detailed previously[15,17-18]. Coverage and mean depth for NGS were calculated. All initially detected variants were subsequently filtered against five SNP databases, including dbSNP138, HapMap Project, 1000 Genome Project, YH database, and Exome Variant Server. Sanger sequencing was subsequently conducted for intrafamilial cosegregation analysis and prevalence test in 150 unrelated healthy controls. Standard protocol for Sanger sequencing has been discussed previously[19].

RESULTS

Phenotypic Descriptions This family included two affected

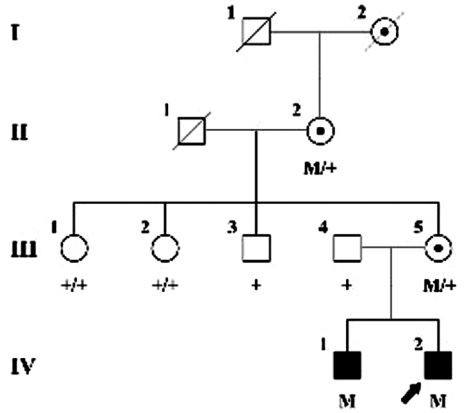

Figure 1 Pedigree of family LZ with the NHS genotypes annotated below the pedigree symbols Black filled and blank symbols represent affected and unaffected status, respectively. Square signify male and circles females. Arrows mark the index patients. M refers to the mutant allele and normal allele.

males. And all family members were further investigated for clinical features to provide precise information. Considering the unbalanced incidence between male and female members, we suggest the mode of inheritance was X-linked.

Detailed description for clinical features is provided in Table 1. The two male patients (LZ-IV:1 and LZ-IV:2) presented bilateral congenital nuclear cataracts and both of them received cataract surgeries at an early age (Figure 2). Additional ocular features include microcornea and strabismus (Figure 2). LZ-IV:1 manifest subtle facial and dental abnormalities including a long face and narrow mandible, screwdriver-shaped incisors and diastema, while LZ-IV:2 did not show these non-ocular abnormalities. Other systemic abnormalities (cardiovascular abnormalities, mental retardation and brachymetacarpalia) were not shown in these two male patients.

Two heterozygous female carriers in this family (LZ-II:2 and LZ-III:5) presented fine, punctate opacities outlining the posterior Y-suture without visual acuity affected. LZ-III:2 manifested bilateral cortical opacities. No facial or dental abnormalities related to NHS were observed in these two carriers.

Genetic Assessments Targeted NGS approach was selectively conducted on patients LZ-IV:1 and LZ-IV:2. Coverage of the targeted region was 98.53% for patient LZ-IV:1 and 98.61% for patient LZ-IV:2. The mean depth of targeted region for the two patients reached 133.31- and 160.88-fold, respectively. A total of 3672 (3274 SNPs and 398 Indels) variants were initially revealed by targeted NGS approach for patient LZ-IV:1, and 3844 (3444 SNPs and 400 Indels) for patient LZ-IV:2. Only two variants retained after the filtration against the 5 SNP databases and were shared by the two tested patients, including a missense substitution in the *PLEC* gene (c.7181C>T) and a frameshift deletion in the *NHS* gene (c.3916_3919del). Since the pedigree of family LZ suggested the possibilities of both autosomal dominant and X-linked inheritance patterns, we therefore tried to determine the disease causative mutation for this family in both manners. In the autosomal dominant model, *PLEC* c.7181C>T failed the co-segregation analysis and was thus discarded, while in the X-linked way, *NHS* c.3916_3919del was shared among all affected patients and was further proved absent in 150 additional normal controls (Figure 3). This deletion was predicted to alter the reading frame and generate a premature termination codon (PTC) after a new reading frame of 8 amino acids (p.Ser1306Thrfs*9).

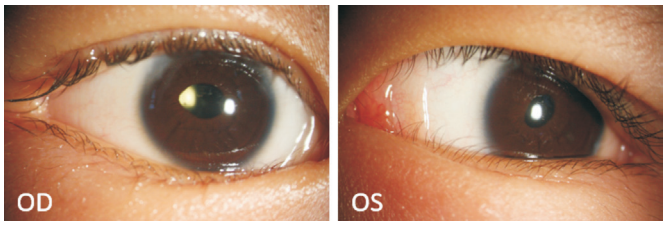

Figure 2 LZ-IV:2 manifest exotropia of the left eye.

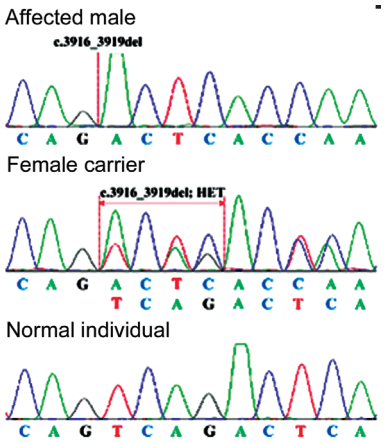

Figure 3 DNA sequence chromatograms of an affected male patient, a female carrier, and a normal individual.

DISCUSSION

Our study discovered a Chinese family with NHS syndrome and identified a novel NHS frameshift mutation (c.3916_3919del) in the two infected males through NGS approach. This mutation has been further confirmed absent in 150 normal controls. We speculated this deletion mutation alters the reading framework and resulted in a PTC after new reading frame of 8 amino acids (p.Ser1306Thrfs*9).

NHS is an X-linked inheritance pattern involving bilateral congenital cataracts, dental anomalies and craniofacial dysmorphisms[20-22]. Mild mental retardation has been reported in about 20% of affected patients[23]. The proband (LZ-IV:2) and his brother (LZ-IV:1) exhibited congenital cataract with microcornea and strabismus which were similar to NHS syndrome. In order to find out the underlying causes, we performed targeted NGS, and found a novel mutation in the NHS gene. The pedigree family was recalled for further examination after this identification. The two affected males manifested characteristic ocular clinical features of NHS. Other two female carriers manifested milder signs (posterior Y-sutural cataracts). So, the results of targeted NGS as well as clinical features indicated the existence of NHS mutation in this pedigree.

NHS is a highly phenotypic heterogeneous syndrome. In this syndrome, researchers failed to find out the correlation between genotype mutation and phenotype severity[24]. In this study, identical non-ocular features were subtle and none of them presented mental retardation. This may be the result of allelic heterogeneity or the additional function of modifier genes[3].

The NHS gene, located on Xp22.13, is expressed during the development of embryonic tissues, especially in midbrain, lens, retina, craniofacial mesenchyme and tooth[25-26]. And it is conserved among human and other vertebrate species[27-28]. It comprises 10 exons which encompass about 650 kb of genomic DNA, and at least 4 different isoforms resulting from alternative splicing[29]. NHS-A and NHS-1A are the two major isoforms transcribed from exon1, encoding 1630 amino acids and 1651 amino acids respectively. NHS-B, encoding 1335 amino acids, is transcribed from exon 1b and translated from exon 4. NHS-C, encoding 1453 amino acids, is transcribed and translated from exon 1a. The exact biological function of NHS protein is unclear. To date, more than 40 mutations associated with NHS have been reported, originating from China, Australia, India, the United Kingdom, the United States of America and Turkey[21,27]. Most of the identified mutations are nonsense or indel, while others are frameshift mutations, genomic rearrangements and missense mutations[12]. The underlying consequence of these mutations can be classified into two categories. One is function damaging, the other is aberrant cellular distribution. In this study, the mutation in the NHS gene produced a truncated NHS protein. In most cases, the premature protein can initiate the nonsense-mediated mRNA decay pathway (NMD) which is able to degrade the shortened mRNA and protect cells from potential toxic

effects resulting from dominant negative or gain-of-function effects[30]. Some mRNAs with PTCs, however, can avoid this translation coupled quality control system, resulting in truncated proteins. Weather this mutation is able to provide truncated protein requires further investigation. It has been reported that nonsense mutation can also lead to disrupted NH_2-terminus and COOH-terminus of NHS protein resulting in aberrant intercellular location in the epithelium of lens and retinas. As NHS proteins are co-located with tight junction protein ZO-1, this loss of subcellular localization can cause disease[13]. Considering this mutation happened from 3916 to 3919 and NH_2-terminus can act as a label for protein localization, the abnormal subcellular localization may not happen in this pedigree.

In summary, our study presented the clinical manifestations of affected males and female carriers in a Chinese family diagnosed with NHS, and identified a previously unreported frameshift mutation. The result of the present study suggested that the diagnosis of NHS or other genetic diseases require a combination of genetic analysis and clinical assessment. Besides, our result broadened the spectrum of NHS mutation and can provide genetic counselling for NHS patients. However, our study only identified a frameshift mutation and provided genetic evidence. Therefore, future experiments exploring the underlying mechanisms of this mutation are needed.

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REFERENCES

1 Horan MB, Billson F. X-linked cataract and hutchinsonian teeth. *Journal of Paediatrics and Child Health* 1974;10(2):98-102.

2 Nance WE, Warburg M, Bixler D, Helveston EM. Congenital X-linked cataract, dental anomalies and brachymetacarpalia. *Birth Defects Orig Artic Ser* 1974;10(4):285-291.

3 Li AJ, Li BZ, Wu LM, Yang LP, Chen NN, Ma ZZ. Identification of a novel NHS mutation in a Chinese family with Nance-Horan syndrome. *Curr Eye Res* 2015;40(4):434-438.

4 Florijn RJ, Loves W, Maillette de Buy Wenniger-Prick LJ, Mannens MMAM, Tijmes N, Brooks SP, Hardcastle AJ, Bergen AAB. New mutations in the *NHS* gene in Nance-Horan Syndrome families from the Netherlands. *Eur J Hum Genet* 2006;14(9):986-990.

5 Tug E, Dilek NF, Javadiyan S, Burdon KP, Percin FE. A Turkish family with Nance-Horan Syndrome due to a novel mutation. *Gene* 2013;525(1):141-145.

6 Damián A, Ionescu RO, Rodríguez de Alba M, *et al*. Fine breakpoint mapping by genome sequencing reveals the first large X inversion disrupting the *NHS* gene in a patient with syndromic cataracts. *Int J Mol Sci* 2021;22(23):12713.

7 Tian Q, Li YP, Kousar R, *et al*. A novel NHS mutation causes Nance-Horan syndrome in a Chinese family. *BMC Med Genet* 2017;18(1):2.

8 Miller C, Gertsen BG, Schroeder AL, Fong CT, Iqbal MA, Zhang B. Allelic and dosage effects of NHS in X-linked cataract and Nance-Horan syndrome: a family study and literature review. *Mol Cytogenet* 2021;14(1):48.

9 Gómez-Laguna L, Martínez-Herrera A, Reyes-de la Rosa ADP, *et al*. Nance–Horan syndrome in females due to a balanced X;1 translocation that disrupts the *NHS* gene: familial case report and review of the literature. *Ophthalmic Genet* 2018;39(1):56-62.

10 Toutain A, Dessay B, Ronce N, *et al*. Refinement of the NHS locus on chromosome Xp22.13 and analysis of five candidate genes. *Eur J Hum Genet* 2002;10(9):516-520.

11 Milunsky A, Milunsky JM, Dong WL, Hovhannisyan H, Oates RD. A contiguous microdeletion syndrome at Xp23.13 with non-obstructive azoospermia and congenital cataracts. *J Assist Reprod Genet* 2020;37(2):471-475.

12 Li HJ, Yang LZ, Sun ZX, Yuan ZS, Wu SJ, Sui RF. A novel small deletion in the *NHS* gene associated with Nance-Horan syndrome. *Sci Rep* 2018;8(1):2398.

13 Wei MR, Qi AH, Mo HM, Wu KL, Ma X, Wang BB. A novel NHS mutation in a Chinese family with Nance-Horan syndrome. *Mol Med Rep* 2019;19(5):4419-4424.

14 Rong WN, Chen X, Zhao KX, *et al*. Novel and recurrent MYO7A mutations in Usher syndrome type 1 and type 2. *PLoS One* 2014;9(5):e97808.

15 Wang YW, Xu M, Liu XX, Huang YH, Zhou Y, Liu QH, Chen X, Zhao C, Wang M. Targeted next-generation sequencing extends the mutational spectrums for OPA1 mutations in Chinese families with optic atrophy. *Mol Vis* 2019;25:912-920.

16 Liu XX, Xiao JJ, Huang H, *et al*. Molecular genetic testing in clinical diagnostic assessments that demonstrate correlations in patients with autosomal recessive inherited retinal dystrophy. *JAMA Ophthalmol* 2015;133(4):427-436.

17 Chen X, Liu Y, Sheng XL, *et al*. PRPF4 mutations cause autosomal dominant retinitis pigmentosa. *Hum Mol Genet* 2014;23(11):2926-2939.

18 Pan XY, Chen X, Liu XX, *et al*. Mutation analysis of pre-mRNA splicing genes in Chinese families with retinitis pigmentosa. *Mol Vis* 2014;20:770-779.

19 Zhao C, Lu SS, Zhou XL, Zhang XM, Zhao KX, Larsson C. A novel locus (RP33) for autosomal dominant retinitis pigmentosa mapping to chromosomal region 2cen-Q12.1. *Hum Genet* 2006;119(6):617-623.

20 Hernández V, Pascual-Camps I, Aparisi MJ, Martínez-Matilla M, Martínez F, Cerón JA, Pedrola L. Great clinical variability of Nance Horan syndrome due to deleterious NHS mutations in two unrelated Spanish families. *Ophthalmic Genet* 2019;40(6):553-557.

21 Shoshany N, Avni I, Morad Y, Weiner C, Einan-Lifshitz A, Pras E. *NHS* gene mutations in Ashkenazi Jewish families with nance-horan syndrome. *Curr Eye Res* 2017;42(9):1240-1244.

22 de Souza N, Chalakkal P, Martires S, Soares R. Oral manifestations of nance-horan syndrome: a report of a rare case. *Contemp Clin Dent* 2019;10(1):174-177.

23 Brooks SP, Ebenezer ND, Poopalasundaram S, Lehmann OJ, Moore AT, Hardcastle AJ. Identification of the gene for Nance-Horan syndrome (NHS). *J Med Genet* 2004;41(10):768-771.

24 Ramprasad VL, Thool A, Murugan S, *et al*. Truncating mutation in the*NHS* gene: phenotypic heterogeneity of nance-horan syndrome in an Asian Indian family. *Invest Ophthalmol Vis Sci* 2005;46(1):17.

25 Cammarata-Scalisi F, Avendaño A, Callea M. Main genetic entities associated with supernumerary teeth. *Arch Argent Pediatr* 2018;116(6):437-444.

26 Burdon KP, McKay JD, Sale MM, *et al*. Mutations in a novel gene, NHS, cause the pleiotropic effects of Nance-Horan syndrome, including severe congenital cataract, dental anomalies, and mental retardation. *Am J Hum Genet* 2003;73(5):1120-1130.

27 Accogli A, Traverso M, Madia F, Bellini T, Vari MS, Pinto F, Capra V. A novel Xp22.13 microdeletion in Nance-Horan syndrome. *Birth Defects Res* 2017;109(11):866-868.

28 Ling C, Sui RF, Yao FX, Wu ZH, Zhang X, Zhang SY. Whole exome sequencing identified a novel truncation mutation in the *NHS* gene associated with Nance-Horan syndrome. *BMC Med Genet* 2019;20(1):14.

29 Sun WM, Xiao XS, Li SQ, Guo XM, Zhang QJ. Exome sequencing of 18 Chinese families with congenital cataracts: a new sight of the NHS gene. *PLoS One* 2014;9(6):e100455.

30 Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur J Hum Genet* 2006;14(10):1074-1081.