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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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 ethylenediaminetetraacetic 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 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. 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 did not show these non-ocular abnormalities. Other systemic abnormalities (cardiovascular abnormalities, mental retardation and brachymetacarpalia) were not shown in these two male patients.

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.

Table 1 Clinical features of included family members

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

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

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 dysmorphism[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

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.
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 proteins. Weather this mutation is able to provide genetic evidence. Therefore, future experiments exploring the underlying mechanisms of this mutation are needed.

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REFERENCES


