

Case Report

A Microdeletion at Xp22.13 Associated with Nance-Horan Syndrome Identified by Copy Number Variation Sequencing

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Received: September 5, 2022 **Accepted:** November 18, 2022 **Published:** February 15, 2023

Abstract

Nance-Horan syndrome (NHS) represents the uncommon X-linked genetic disease with the features of congenital cataracts, tooth deformities, mental retardation along with facial deformities. It is mostly caused by small insertions, deletions, and nonsense mutations of NHS gene on Xp22.13, which may cause the NHS protein truncation. The present work discovered the 1.62-Mb microdeletion on Xp22.13 through copy number variation sequencing in a China girl with NHS syndrome, which included these genes: *NHS*, *Cyclin-dependent kinase-like 5*, *Scm polycomb group protein like 2*, *Retinoschisin1*, *Scm polycomb group protein like 1*, *RALBP1 associated Eps domain containing 2*, and *Retinoic acid induced 2*. Her parents did not find the microdeletion of Xp22.13, which was a novel microdeletions of Xp22.13 will cause haploinsufficiency of flanking genes, non-random X chromosome inactivation may increase the X-linked disease risk.

Keywords: Nance-Horan syndrome, copy number variation sequencing, Xp22.13 microdeletion, mental retardation, congenital cataract

Citation: Sun D, Guo Q, Chen W, Zhang J, Peng Y, Wang K, Zhang J. A Microdeletion at Xp22.13 Associated with Nance-Horan Syndrome Identified by Copy Number Variation Sequencing. *J Mod Pharmacol Pathol*, 2023; 1: 2. DOI: 10.53964/jmpp.2023002.

1 INTRODUCTION

Nance-Horan syndrome (NHS) [MIM 202350], with the features of congenital cataracts in both eyes, dysmorphic facial features, dental abnormalities like bud molars or screwdriver-shaped teeth, with occasional brachymetacarpia and mental retardation, represents the uncommon X-linked recessive hereditary disorder initially put forward in 1974 by Nance et al^[1]. NHS gene is mapped onto Xp22.13 at

the interval of 1.3-Mb^[2]. Its gene includes the genomic DNA (gNDA) of 650kb, which encodes one candidate nuclear protein of 1,630 amino acids. Research discovers a complex expression pattern of temporal and spatial regulation, accompanied by pleiotropic characteristics in NHS, indicating the role of the gene in regulating eye, teeth, craniofacial and cerebral development^[3]. NHS can be inherited on the X chromosome, and female heterozygous

carriers typically show a close yet moderate phenotype to infected men^[4,5], usually with posterior Y-suture lens opacities and innate cortical cataracts, with no congenital cataract^[6].

The present work clinically and genetically analyzed the 1.62Mb aninterstitial microdeletion on Xp22.13 detected by next generation sequencing in a 7 years old girl, who showed severe mental retardation, cataract, abnormal tooth development, low muscle tone, and severe epilepsy. The parents were not found to have chromosome copy number variation (CNV), as a result, X-linked deletion was derived from her daughter de novo. Furthermore, the girl's cytogenetic analysis revealed an apparently karyotype of 46, XX, t(17; 18)(q23; p11.3) that was transmitted from the carrier mother

2 CASE PRESENTATION

2.1 Clinical Report

A 7 years old girl. It is the third birth of her mother, full term delivery, birth weight 3.1kg, no asphyxia. The fundus screening after birth diagnosed congenital cataract in her right eye (Figure 1). When she was 5 months the epilepsy began. After the antiepileptic treatment, the frequency of seizures and clinical symptoms have not been significantly improved (3-4times/day, 2-3min/time). The child's psychomotor development is seriously backward. She can't lift her head, turn over, sit, stand, walk or speak. Her hands can't grasp. She neither knows her family nor have eye contact. Physical examination: body length 120cm, weight 10.5kg (<3SD), underdeveloped, poor nutrition, flat occipital fovea, congenital cataract in the right eye, large teeth gap, cardiopulmonary (-), low muscle tension of limbs, weak tendon reflex. Auxiliary examination: no obvious abnormality was found in chest X-ray, abdominal ultrasound, cardiac ultrasound and head magnetic resonance imaging. The parents were healthy, married without close relatives, denied the history of infectious diseases during pregnancy, denied the history of radiation exposure and family genetic history. With the approval of hospital medical ethics committee (No. 20220165) and the informed consent of the parents of the children, the peripheral blood samples of the children and their parents were collected for G-banding karyotype analysis, CNV sequencing (CNVseq) and fluorescence quantitative polymerase chain reaction (PCR) analysis.

3 MATERIALS AND METHODS

3.1 Chromosome Analysis

By adopting standard cytogenetic approaches, this work cultivated peripheral blood in serum-free lymphocyte medium for a 72-h period. Later, G-banding was adopted in chromosome analysis with trypsinization as well as Giemsa's staining. This work utilized altogether 20 metaphase spreads for analysis^[7], and depicted karyotypes in line with the international system for human cytogenomic

nomenclature (ISCN 2016).

3.2 Detection of Chromosomal CNVseq by Low Coverage Whole Genome High Throughput Sequencing

This work conducted CNVseq for detecting chromosome anomaly by low-coverage whole genome sequencing. In brief, gDNA was confirmed through 2 approaches together: (1) 1% agarose gels were utilized in DNA decomposition as well as contamination; (2) Qubit DNA assay kit was utilized to determine DNA contents with Qubit 2.0 Fluorometer (Life Technologies, CA, USA). Thereafter, CLEANNGS DNA kit was adopted to produce sequencing library in line with specific protocols, followed by addition of index codes into every specimen. Later, by adopting Novaseq 5000/6000 S4 Reagent Kit (Illumina), those index-coded specimens were clustered onto the cBot cluster generation system in line with specific protocols. Following cluster production, Illumina NovaSeq 6000 platform was employed to sequence DNA libraries to generate the 150bp paired-end reads. Through primary quality control, this work first processed fastq-format raw reads. Then, BWA software was employed to compare clean reads against reference human genome (UCSC hg19), following by conversion of results to the bam format as well as sorting with samtools software. At last, basic information was statistically analyzed and compared. Thereafter, SNP/InDels were identified with Verita TreKer. CNVs were detected by BIC-Seq and CNVnator, whereas SVs were discovered by Manta. Enliven was performed to do annotation for SNP/InDels/CNV/SV.

gDNA extraction Kit produced by Qiagen company in Germany was utilized to extract gDNA in line with kit protocols. DNA concentration was controlled at 50-250ng/ μ L. Then, sequencing libraries constructed were sequenced on the next-Seq CN500 platform. The sequencing results were analyzed, and each sequencing read was matched to its chromosome. Then the corresponding standardized Z-value analysis was done, and the chromosome abnormality was determined by Z-value. The clinical significance of CNV was determined by searching decipher, OMIM and DGV databases.

3.3 Fluorescence Quantitative PCR

Fluorescence quantitative PCR detection according to the results of high-throughput sequencing, the deletion region of the fragment was selected for fluorescent quantitative PCR verification. ABI7500 fluorescent quantitative PCR was used for relative quantitative analysis.

4 RESULTS

The cytogenetic analysis revealed an apparently karyotype of 46, XX, t(17;18)(q23;p11.3), which was inherited from her mother (Figure 2).

We found that there were 2 chromosomal copy variant

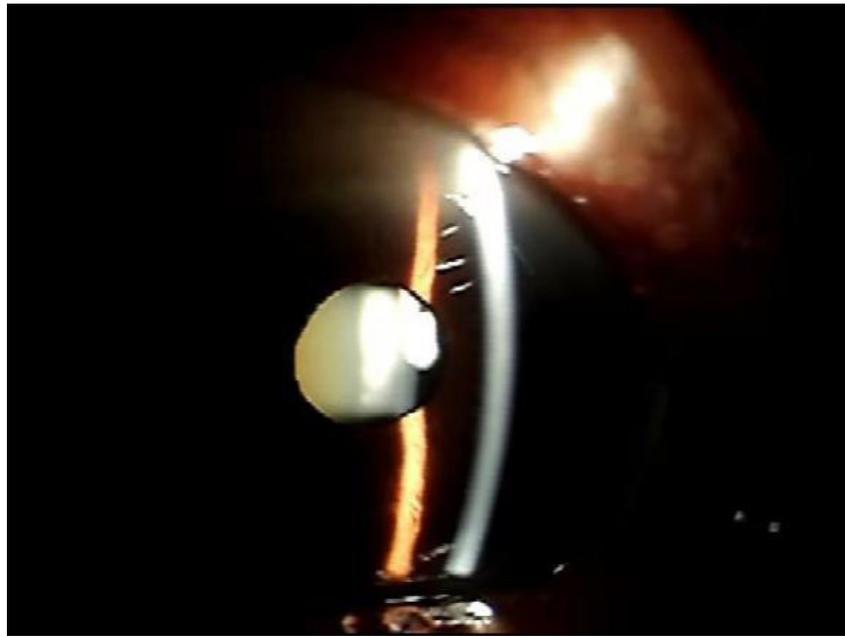


Figure 1. Image of fundus screening of the patient.



Figure 2. One balanced translocation that involved chromosomes 17 and 18 was identified by chromosome G-banding analysis within the child and her mother.

loci were detected by CNV-seq (Figure 3) with the first deletion being at nucleotide positions 17160001-18780000 (GRCh37/hg19 assembly), which were matched with cytogenetic band Xp22.13. Its deletion length was predicted to be 1.62Mb, which involved these genes: *NHS* [OMIM:300457], Cyclin-dependent kinase-like 5 (*CDKL5*) [OMIM:300203], *SCML2* [OMIM:300208], Retinoschisin1 (*RS1*) [OMIM:300839], *SCML1* [OMIM:30027], *RALBP1 associated Eps domain containing 2* (*REPS2*) [OMIM:300317], *Retinoic acid induced 2* (*RAI2*) [OMIM:300217]. The second one was on nucleotide positions 22540001-229600001 (GRCh37/hg19 assembly), which were matched with cytogenetic band

14q11.2. Its deletion length was predicted to be 0.42Mb, referring to relevant literature and databases, there was no gene in this region and it was polymorphic. Her parents did not find the microdeletion of Xp22.13. The chromosome 14 polymorphism was inherited from his father.

The results of fluorescence quantitative PCR were based on the *ALB* gene as the internal reference, and the *NHS* and *CDKL5* genes located in the Xp22.13 region were selected for primer design. Normal control samples and child and parents samples were tested by fluorescence quantitative PCR in the same group. Consequently, the *NHS* and *CDKL5* genes to normal control copy number ratio was

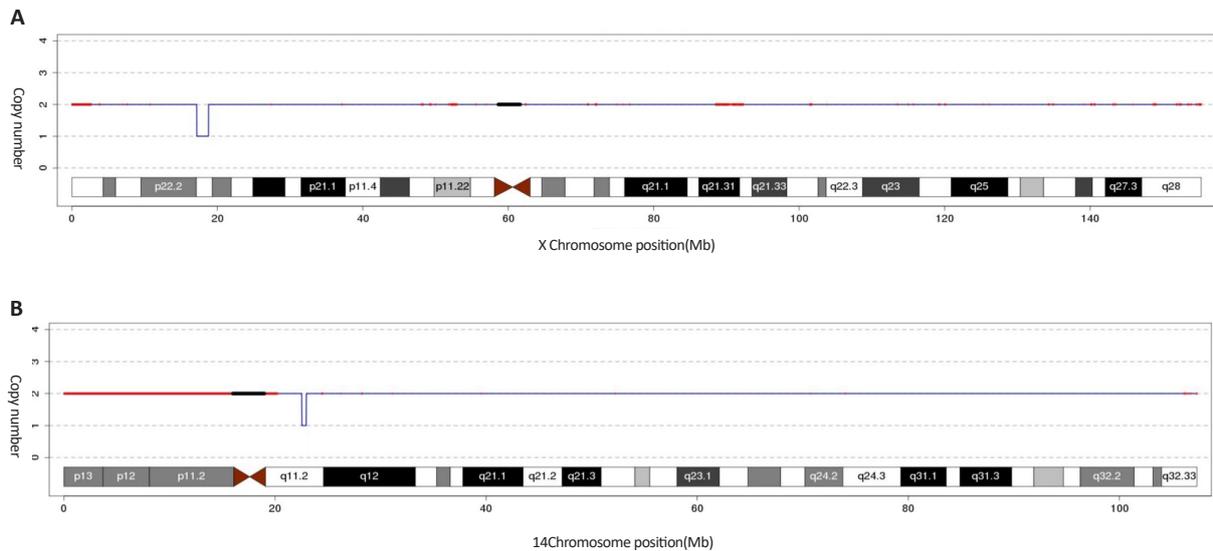


Figure 3. The identification of 1.62Mb at Xp22.13 (17160001-18780000) (GRCh37/hg19) by CNV-seq showing the microdeletion (A) and an interstitial microdeletion at 14q11.2 was identified by CNV-seq (B).

about 0.5, with loss of heterozygosity. The copies of the genes in this region were normal in both parents, suggesting that the chromosomal deletion was new (Figure 4).

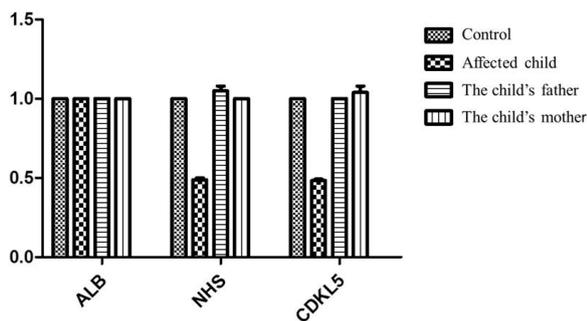


Figure 4. QF-PCR result showing heterozygous deletion of *NHS* and *CDKL5* genes in the affected subject.

5 DISCUSSION

Genetic factors account for 40% of developmental delay/mental disability, of them, chromosomal anomalies occupy 25%^[8]. G-banding karyotype analysis is a routine clinical cytogenetic diagnosis method. But <5Mb duplications and deletions cannot be detected even with high-resolution G-banding. Detection rate of conventional G-banding techniques for mental retardation, organ malformation, and growth retardation caused by a relatively small range of chromosomal imbalance aberrations is only 15% to 40%, and the missed diagnosis rate is as high as 60% to 85%^[9,10]. High throughput sequencing technology can detect chromosomal aberrations and gene CNVs at the submicroscopic level with high resolution, high throughput and high accuracy, which improves the diagnostic efficiency of genetic diseases.

The balanced translocation of this case was inherited from the mother, which did not lead to the deletion and

duplication of clinically significant genetic material. Therefore, balanced translocation is not the cause of a series of clinical manifestations. Molecular genetic testing found that there was a 1.62mb deletion at p22.13 on the X chromosome of the child, including *NHS*, *CDKL5*, Scm polycomb group protein like 2 (*SCML2*), *RSJ*, Scm polycomb group protein like 1 (*SCML1*), *REPS2*, *RAI2* genes. According to Van Esch et al.^[11] and Mathys et al.^[12], 2 distinct male children showing close phenotypes were identified, and they carried the 2,8-MB microdeletion on Xp22.13. The two microdeletions contain *CDKL5* and *NHS* genes. It was worth noting that such cases exhibited innate cataract in both eyes, epileptic encephalopathy, dental abnormalities and Fallot tetralogy. Liao et al.^[13] and Accogli et al.^[14] described three brothers developing NHS who carried one microdeletion on Xp22.13. Apart from characteristic NHS phenotype, these patients exhibited global developmental retardation and hypotonia. The main manifestations of our patient were congenital cataract, special facial features (narrow and long face, prominent nose, etc.), abnormal teeth, intellectual disability, nystagmus, finger deformities, etc., which were consistent with NHS. At the same time, the patient also had severe early-onset intractable epilepsy, no speech function, severe psychomotor retardation, hypotonia, etc. Notably, our patient was a girl, which was rare in previous reports.

After querying ClinGen database resources, pathogenicity of insufficient haploid doses of *NHS* [ISCA-35637] and *CDKL5* genes [ISCA-14810] was supported (haploinsufficiency score: 3). Khan et al.^[6] found the new mutation on *NHS* (p.Lys744AsnfsX15 [c.2232delG]) from 7 cases developing infantile or innate cataract, such as long face, bulbar nose and dentition abnormalities, and the mutation could also be seen among 4 asymptomatic female children who had Y-centered lens opacities, rather

than in additional 2 asymptomatic females or 3 males with clear lenses. Using whole exome sequencing, Tian et al.^[15] found that NM_198270: c.1045 + 2T >A, a donor splicing site mutation in *NHS* gene was the pathogenic mutation for the NHS family, carrier females had no obvious abnormality in intelligence, facial features, nystagmus, microcornea, strabismus and high myopia, while cataract was not seen because of cataract surgery before this work. One female carrier, who carried c.263_266delCGTC (p.Ala89TrpfsTer106), the new *NHS* gene small deletion, had bilateral posterior Y-suture lens opacities and slight dental anomalies^[16]. Miller et al.^[17] described *NHS* gene disruption because of balanced translocation t(X;19) (Xp22.13;q13.1), which was the specific pathogenesis of NHS referred *NHS* gene's dose effects on phenotype expression and manifestation, besides, female carriers showed congenital cataracts and Glaucoma. Maortua et al.^[18], Bahi-Buisson et al.^[19] and other authors^[20,21], who associate *CDKL5* gene mutations with various overlapping phenotypes, from autism and mental retardation to RTT with epilepsy phenotypes. Mei et al.^[22] also showed that *CDKL5* gene deletion/duplication may be the main cause of girls' early refractory seizures (unknown etiology).

Therefore, the clinical manifestations of the patient in this study may be related to the haploinsufficiency of *NHS* and *CDKL5* gene, there is no additional gene, which can possibly affect the affected girl health and clinical manifestations. Retinoic acid (RA) participates in regulating early embryogenesis and cell differentiation, while *RAI2* gene deletion has been elucidated that, the decreased RA signaling induces early cardiac defects in diverse animal models as well as mammalian embryos, and also lead to intellectual impairment^[23]. *REPS2* gene shows up-regulation within brain tissues and is associated with signaling pathway that involves small GTPases in Rho family, haploinsufficiency of *REPS2* gene may also lead to mental retardation^[13]. The *SCML1* gene was preferentially located within testicular germ stem cells, which regulates the ubiquitination of histone H2A to establish male germline epigenome^[24], so the impact on girls may be small. According to map location and homology analysis, *SCML2* was the possible gene related to Xp22-linked developmental disorders, like oral-facial-digital type I syndrome^[25]. According to Huopaniemi et al.^[26], *RSI* gene expression levels within different uterine and placental cells were analyzed, as a result, apart from retina, *RSI* expression could be detected within the uterus. Consequently, *RSI* protein may be related to embryonic survival and implantation.

6 CONCLUSION

Clinical manifestations caused by Xp22.13 microdeletion are complex and diverse. In the future, more investigations should be conducted for defining the Xp22.13 microdeletion phenotypic range, and the appropriate gene functions at the

specific locus should be specified.

Acknowledgements

We thank the patients and their families for their participation in this study. This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2019.316.

Conflicts of Interest

The authors declared that there is no conflict of interest regarding the publication of this paper.

Author Contribution

Guo Q and Sun DL designed the overall research strategy and supervised the whole process. Zhang J and Wang K recruited the case and performed the prenatal counseling. Chen WQ carried out sampling and part of the genetic experiments. Zhang J and Peng YY performed the other part of the genetic experiments, chromosome analysis and data analysis. Zang J and Sun DL together prepared this manuscript.

Abbreviation List

CDKL5, Cyclin-dependent kinase-like 5
CNV, Copy number variation
CNVseq, CNV sequencing
gNDA, Genomic DNA
NHS, Nance-Horan syndrome
PCR, Polymerase chain reaction
RAI2, Retinoic acid induced 2
REPS2, RALBP1 associated Eps domain containing 2
RSI, Retinoschisin1
SCML1, Scm polycomb group protein like 1
SCML2, Scm polycomb group protein like 2

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