De novo truncating variant in NSD2 gene leading to atypical Wolf-Hirschhorn syndrome phenotype

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Abstract

Background: Wolf-Hirschhorn syndrome (WHS) is a contiguous gene syndrome caused by partial 4p deletion highly variable in size in individual patients. The core WHS phenotype is defined by the association of growth delay, typical facial characteristics, intellectual disability and seizures. The WHS critical region (WHSCR) has been narrowed down and NSD2 falls within this 200 kb region. Only four patients with NSD2 variants have been documented with phenotypic features in detail.

Case presentation: Herein, we report the case of a 12-year-old boy with developmental delay. He had dysmorphic facial features including wide-spaced eyes, prominent nasal bridge continuing to forehead, abnormal teething and micrognathia. He also had mild clinodactyly of both hands. Using whole-exome sequencing, we identified a pathogenic mutation in NSD2 [c.4029_4030insAA, p.Glu1344Lysfs*49] isolated from peripheral blood DNA. Sanger confirmation of this variant revealed it as a de novo truncating variant in the family.

Conclusion: Here, we reported a boy with de novo truncating variant in NSD2 with atypical clinical features comparing with 4p16.3 deletion related WHS. Our finding further supported the pathogenesis of truncating variants in NSD2 and delineated the possible symptom spectrum caused by these variants.

Keywords: Wolf-Hirschhorn syndrome, NSD2 gene, Truncating variants
autism, and congenital cardiac disorders. Recent reported cases with de novo truncating variants on NSD2 and detail phenotypic features have offered a new insight into genetic causes of WHS [7–9]. NSD2 is thought to be the major, but not the unique gene for facial dysmorphisms, growth delay and intellectual disability. Here we report a boy with de novo variant in NSD2 with atypical WHS clinical manifestations, further supported the pathogenesis of truncating variant in NSD2 and delineated the possible symptom spectrum caused by these variants on a single gene.

Case presentation
The patient, a 12-year-old boy, the only child of healthy unrelated parents, was referred to the department of developmental behavior of Shanghai Children's Medical Center due to delayed development and growth. He was born with a cesarean section at term with a birth weight of 2.2 kg but without been told had intrauterine growth retardation during pregnancy period. The boy was purple after birth and improved after stimulation, but had feeding difficulties, so the boy was subsequent admission to the neonatal intensive care unit for weight management. Feeding difficulties and developmental delay were observed in infancy. He only sat at 10 months, walked at 18 months and spoke his first sentences at 2 years old. Growth delay persisted (current weight is 31.5 kg [P3–10], height 146.8 cm [P10–25], body mass index 14.62 kg/m² [P3–5], and occipitofrontal circumference 52 cm [−0.14SD] according to Chinese domestic data. Mild clinodactyly of both hands and abnormal facial features including wide-spaced eyes, prominent nasal bridge continuing to forehead, abnormal teething and micrognathia were noticed (Fig. 1 & Table 1). There was no history of seizures. Brain magnetic resonance image (MRI), EEG, X-ray of chest and spine, echocardiography and abdominal ultrasound were normal. Molecular karyotyping was normal.

Ethical approval for this study was obtained from the ethics committee of Shanghai Children's Medical Center,
| WHG1C variant | Facial Features | Prenatal onset | Growth deficiency | Developmental delay/ intellectual disability | Seizure disorder | Feeding difficulties | Hypotonia and muscle underdevelopment | Birthweight |
|---------------|----------------|---------------|------------------|-----------------------------------------------|-----------------|-------------------|---------------------------------------|------------|
| Patient 1a    | c.2518 + 1G > A | Yes Yes No Yes (mild) Yes Yes No Yes (mild) Yes Yes Yes Yes Yes Yes Yes Yes Yes 1.5 kg P3 | |
| Patient 2a    | c.2803C > T p.Arg935* | Yes (mild) Yes No Yes Yes No No No Yes Yes Yes (mild) Yes Yes Yes No Yes Yes 2.3 kg P3 | |
| Patient 3b    | c.3412C > T p.Arg1138* | No Yes Yes No Yes Yes No No No No No No Yes No No Yes No 3.05 kg P20 | |
| Patient 4c    | c.1676_1679del p.Arg559Thrfs*38 | No No No No No Yes Yes Yes No No Yes (mild) Yes No Yes No Yes Yes (mild) 2.58 kg P6 | |
| Our case      | c.4029_4030insAA p.Glu1344Lysfs*49 | Yes No Yes No Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes 2.2 kg P2 | |

a Deira et al.; b Lozier et al.; c Boczek et al.; d Jiang et al.
Whole exome capture was performed with Agilent SureSelect V6 enrichment capture kit (Agilent Technologies, Inc., Woburn, MA, U.S.) according to the manufacturer’s instructions. The captured library was then sequenced on an Illumina HiSeq 2500 System (Illumina, Inc., San Diego, CA, U.S.). Raw sequencing data was process as previously described [10]. All detected variants were analyzed on the TGex (Translational Genomics Expert) platform featuring with the VarElect scoring system [11]. An insertion variant c.4029_4030insAA leading to frameshift mutation (p.Glu1344Lysfs*49) on NSD2 was detected and thought to have high probability as a candidate mutation.

The primers for amplification of the NSD2 gene (NM_ 133,330.2) were designed using UCSC Exon Primer online software (http://genome.ucsc.edu/index.html) and synthesized. The primer sequences for the truncating variant to be confirmed were forward5′-agtttgtctgcccgtcctgt-3′ and reverse 5′-TGAGGTCCTAGTGGTG-3′.

The target fragments were amplified from the patients as well as his parents using polymerase chain reaction (Takara Biotechnology, Co., Ltd., Dalian, China). The PCR products were sequenced using an ABI3730XL sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, U.S.) with the forward and reverse primers. The sequence data were analyzed using Mutation Surveyor® software Version 4.0.4 (SoftGenetics, LLC). The truncating variant has been confirmed as de novo variants in the proband.

Discussion and conclusion

Critical pathogenic regions responsible for microdeletion syndromes are mapped by overlapping deletions and could in some cases help to reveal a single causative gene for the syndrome. For patients with WHS, NSD2 is the possible pathogenic gene located in the overlapping critical region previously defined by clinical and genomic information [12]. NSD2, nuclear receptor-binding SET domain-protein 2 (also known as WHSC1, Wolf-Hirschhorn syndrome candidate 1), is a SET domain histone methyltransferase, responsible for the methylation of H3K36. The importance of histone modification in brain development was gradually recognized as many pathogenic allele in genes encoding histone modification components was identified [13]. The histone substrate specificity of NSD2 encoded protein may explain differences in clinical phenotypes in related patients [14]. NSD2 gene is thought to be intolerant of loss of function variants (pLI = 1.00). Sequencing data from large cohorts of developmental delay, autism, and congenital cardiac problems also revealed several de novo variants in NSD2 that may be related to disease [15]. Case report with de novo truncating mutations on NSD2 with very detailed clinical information further supported that loss-of-function variants in NSD2 likely contributes to atypical or part of the clinical spectrum of WHS [7–9]. Herein, we reported another patient with disease-related frameshift mutation on NSD2 and reviewed clinical manifestation of previously reported patients.

Minimal diagnostic criteria for WHS have been proposed by including typical facial appearance, mental retardation, congenital hypotonia, growth delay and seizures [6]. The “Greek warrior helmet” appearance of the nose (wide bridge of the nose continuing to the forehead) is the most recognizable facial features for patients with WHS, but might become less evident after puberty. Our patient was considered for genetic testing initially due to observation of obvious abnormal appearance of the nose. Reviewing four reported cases and our case, their facial features are less recognizable comparing to typical 4p16.3 deletion WHS patients. Developmental delay or intellectual disability was observed in all four detailed reported cases. Battaglia et al. found that the degree of intellectual disability varies in patients with typical 4p deletion caused WHS [2]. The severity of intellectual disability seems to be much milder in reviewed and our patient. Seizures occur in 90–100% of children with WHS with a peak onset incidence around six to 12 months and represent one of the greatest problems in the clinical management of WHS and they act as an independent prognostic factor for the final degree of intellectual disability. Interestingly, none of the five patients reviewed with de novo truncating NSD2 variants had epileptic symptoms, indicating that other genes located within 4p16.3 region might be responsible for seizure pathogenesis. LETM1 and additional genes residing on 4pter distally to it have been suggested as candidate genes for seizure disorders in WHS patients in a comorbidity model of pathogenesis [16]. Thus, patients with truncating variants in NSD2 and without seizure attack before the age of one might have better intellectual performance. Skeletal anomalies were observed in our patients. He has mild clinodactyly on both hands. The 16 month-old boy reported by Lozier et al. also manifest clinodactyly of fifth fingers of the left hand [9]. Hand malformation is a much milder symptom compared to other skeletal anomalies like kyphosis/scoliosis with malformed vertebral bodies, accessory or fused ribs, clubfeet, and split hand found in 60–70% of WHS patients. Thus, we believe that truncating variants on NSD2 gene will lead to atypical clinical manifestations of WHS. Those patients may not have typical facial features and
epileptic symptoms. The proposed minimal diagnostic criteria may not be suitable for these patients, and the optimal diagnosis criteria for them should include identification of null variants on NSD2 gene by genetic testing.

In conclusion, we identified a de novo truncating variant on NSD2 gene in a patient with mild phenotypic spectrum of WHS, further supported the pathogenesis of null variants in NSD2 in syndromic intellectual disability and developmental delay and these variants lead to a mild form of WHS.

Abbreviations
NSD2: Nuclear receptor-binding SET domain-protein 2; pLi: Probability of loss-of-function variants intolerance; WHS: Wolf-Hirschhorn syndrome; WHSCR: WHS critical region

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Authors’ contributions
RY and FJ designed and organized the study. YJ, QL, and HS sampled the family members and acquired the clinical data. RY and JW carried out the molecular genetic testing. RY and WJ analyzed and interpreted the genetic testing and clinical data. RY wrote the manuscript, which was then edited by FJ and YJ. All authors have read and approved the final version of the manuscript submitted by RY.

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Availability of data and materials
The datasets (whole-exome sequencing and Sanger sequencing files) used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the Committee on Ethics of the Shanghai Children’s Medical Center (SCMCIRB-K2016013) and was performed in accordance with the Declaration of Helsinki.

Consent for publication
Written informed consent was obtained from the patient’s father for publication of this research.

Competing interests
The authors declare that they have no competing interests.

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