

CASE REPORT

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NSDHL-containing duplication at Xq28 in a male patient with autism spectrum disorder: a case report

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Abstract

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder in which genetics plays a key aetiological role. The gene encoding NAD(P)H steroid dehydrogenase-like protein (*NSDHL*) is expressed in developing cortical neurons and glia, and its mutation may result in intellectual disability or congenital hemidysplasia.

Case presentation: An 8-year-old boy presented with a 260-kb *NSDHL*-containing duplication at Xq28 (151,868,909 – 152,129,300) inherited from his mother. His clinical features included defects in social communication and interaction, restricted interests, attention deficit, impulsive behaviour, minor facial anomalies and serum free fatty acid abnormality.

Conclusion: This is the first report of an ASD patient with a related *NSDHL*-containing duplication at Xq28. Further studies and case reports are required for genetic research to demonstrate that duplication as well as mutation can cause neurodevelopmental diseases.

Keywords: Xq28 duplication, *NSDHL*, Autism, CNV

Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is defined in DSM-5 [1] as persistent deficits in social communication and social interaction across multiple contexts in conjunction with restricted, repetitive patterns, interests, or activities as manifested by at least two prototypically inflexible behaviours [2]. A high degree of heritability and modest environmental influences contribute to the aetiology of ASD [3, 4]. The genetic architecture of autism has proven to be complex and heterogeneous through linkage or association based on whole-genome or exome sequencing [5, 6]. In recent decades, 10–20% of ASD cases have been attributed to ASD susceptibility genes [6]. In the X chromosome, double expression of these related genes leads to functional disomy [7–9]. Among the pathogenic X-linked duplications, the most prominent and well-studied is that of the Methyl-CpG-binding Protein 2 gene (*MECP2*; OMIM:

300005), located at Xq28, causing severe X-linked intellectual disability (XLID) [10], and its loss of function causes Rett syndrome (OMIM: 613454) [11, 12]. Duplication of chromosome 15q11–13, which includes a series of imprinting and non-imprinting genes, results in the recurrent cytogenetic abnormalities associated with ASD and represents one of the most frequently reported CNVs in ASD [13].

Duplication involving the int22h-1/int22h-2 LCR-flanked region in distal Xq28 was recently linked to intellectual and developmental disabilities [14, 15]. NAD(P)H steroid dehydrogenase-like protein (*NSDHL*), also located at Xq28, is expressed in developing cortical neurons and glia and encodes an enzyme in post-squalene cholesterol biosynthesis [16]. Mutations of *NSDHL* have been related to neurodevelopmental diseases including CHILD syndrome (OMIM: 308050) and CK syndrome (OMIM: 300831). In CHILD syndrome, *NSDHL* function is presumably eliminated or greatly reduced [17]; CK syndrome affects only males [16]. CK syndrome caused by *NSDHL* mutations is characterized by mild to severe cognitive impairment, seizures beginning in infancy, microcephaly, cerebral cortical malformations and a thin body habitus [18]. Most male patients with CK syndrome manifest behaviours of aggression, attention

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deficit hyperactivity disorder (ADHD), and irritability. Using Autism Diagnostic Interview-Revised (ADI-R) [19] or the Autism Diagnostic Observation Schedule (ADOS) [20], the affected males fulfil partial criteria for ASD [18]. However, little is known about the clinical consequences of *NSDHL* duplication, and no *NSDHL* whole-gene duplication with ASD has been reported in the literature.

Here, we report an 8-year-old boy diagnosed with ASD and possessing a 260-kb *NSDHL*-containing duplication at Xq28 inherited from his mother; this constitutes the first report of an ASD patient with a related *NSDHL*-containing duplication at Xq28. The patient underwent careful clinical observation, detailed examination, and comprehensive medical history recording and demonstrated symptoms of social deficiency, restricted interests, attention deficit and impulsive behaviour but normal cognition.

Case presentation

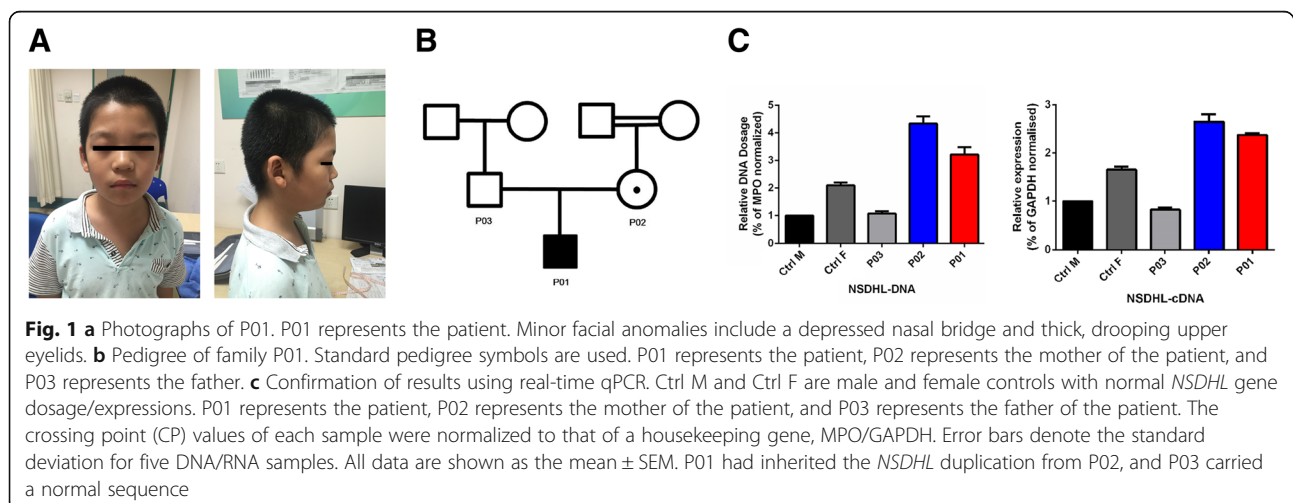
Subject

The patient was referred to our clinic because of indiscipline and impulsive behaviour. He was born at full-term after a normal pregnancy by caesarean section and had a birth weight of 2800 g. During the neonatal period, he had been hospitalized twice, once for pathological jaundice (at 2 months of age) and again for hernia (at 8 months of age). Otherwise, development was nearly normal. The patient walked unsupported at the age of 15 months but exhibited poor motor coordination and slight hypotonia. He had asthma at 3–4 years of age but gradually improved, although with continued allergic rhinitis. He seldom exhibited sharing behaviours or facial expressions and exhibited repetitive patterns of behaviour. He displayed poor linguistic competence, deficits in social reciprocity, and difficulty in maintaining normal back-and-forth conversation with normal eye contact. His ADOS-M3 [20] and ADI-R scores [19] were all

above the cutoff (See Additional file 1: Table S1). In primary school, it was difficult for the subject to obey class discipline due to his impulsive behaviours and attention deficit. SNAP-IV [21] was administered to screen for ADHD (See Additional file 1: Table S1). According to the clinical data and assistant examination, the patient fulfilled the diagnostic criteria for ASD and ADHD. Physical examination revealed minor facial anomalies, including a depressed nasal bridge and thick, drooping upper eyelids (Fig. 1a).

At reexamination at the age of 9 years and 8 months, the patient remained weak in language comprehension and had a short attention span. His height was 146.5 cm, and his weight was 42.15 kg (within the 90 to 95 centile for his age). Based on the Wechsler Intelligence Scale for Children (WISC) [22], his IQS of Verbal Scale was 96, and his IQS of Performance Scales was 90, producing a Full Scale IQ 92 (See Additional file 1: Table S1). The results showed that he had normal cognition with a high score for calculation but a low score for comprehension. After examination of the serum lipids, he showed abnormal serum free fatty acids (875 $\mu\text{mol/L}$ (age 9) and 1165 $\mu\text{mol/L}$ (age 10)); the normal levels were 172–588 $\mu\text{mol/L}$ in age-matched healthy children. No significant abnormalities were detected via electroencephalogram (EEG) or head magnetic resonance imaging (MRI).

Both of the patient's parents completed the combined Raven's Test (CRT) [23] and scored in the normal range. When consulting with both parents, we observed that the mother, who carried the *NSDHL*-containing duplication, exhibited a quick temper and irritability. Tracing the history of the pregnancy and delivery indicated that she may have suffered from postpartum depression. Although she did not have ASD symptoms, she did exhibit some of the phenotype. Her score for the Broader Autism Phenotype Questionnaire was high-normal. She reported no health issues or miscarriages. The father of



the patient had a moderate temperament and was otherwise normal.

The gathered family history showed that the maternal grandparents of the patient had intermarried (they are cousins). DNA samples could not be obtained from either grandparent. Both parents of the patient were the only child in their respective families. Both families declared no significant health or psychological issues. (Fig. 1b).

Genomic DNA and RNA were extracted from the peripheral blood of the patient and his parents. The Agilent array-based comparative genomic hybridization (aCGH) microarray was employed to perform a genome-wide CNV scan of the patient using SurePrint G3 Human CGH microarrays 4 × 180 K (Agilent Technologies) following the manufacturer’s instructions. For WES of the family trio, the SureSelect Human All Exon V5 Kit (Agilent), the Illumina HiSeq X™ HD PE Cluster and SBS kit were used for exon capture, and the Illumina HiSeq X10 system was for paired-end parallel sequencing. To verify the source of *NSDHL* duplication in this patient and his parents, a primer set was designed to target the fragment along the gene through an online Primer Quest Tool (<https://pga.mgh.harvard.edu/primerbank/>) (See Additional file 1: Table S2). The presence of the duplication was also examined in the patient’s parents. X-chromosome inactivation patterns (XCIP) of the mother’s genomic DNA were tested by determining differential CpG-methylation. Genomic DNA/RNA samples from normal male and female individuals were used simultaneously as controls. Subsequent reverse transcription of RNA to cDNA was carried out using the Takara cDNA Synthesis Kit. qPCR was carried out in triplicate using the SuperReal PreMix Plus (SYBR

Green) PCR reagent kit according to the manufacturer’s protocol.

The patient fulfilled the criteria for ASD diagnosis. His clinical features included defects in social communication and interaction, restricted interests, attention deficit, impulsive behaviour, minor facial anomalies and showed abnormal serum free fatty acids. Array-CGH analysis identified a 260-kb duplication on chromosome Xq28 (151,868,909 – 152,129,300; GRCh 37/hg19) in the patient (Fig. 2a). The duplicated region (Fig. 2b), starting from *MAGE2* to a partial copy of *ZNF18*, included the following genes: melanoma antigen (*MAGE*) family (*MAGE2*, *MAGEA3*, *MAGEA6*, *MAGEA12*), chondrosarcoma-associated gene (*CSAG1*, *CSAG2*, *CSAG3*, *CSAG4*), centrin (*CETN2*), NAD(P)H steroid dehydrogenase-like protein (*NSDHL*), and a part of zinc finger protein 185 (*ZNF18*). The qPCR result of DNA confirmed that the patient had inherited the *NSDHL* duplication from his mother and that his father carried a normal sequence; RNA analysis showed that the patient expressed almost threefold the RNA compared to normal males (Fig. 1c). We tested the X-chromosome inactivation patterns (XCIP) of the mother by determining the differential CpG-methylation. According to the pyrosequencing results, there was no significantly skewed X inactivation in the mother (See Additional file 2). The WES of the core family trio did not reveal any causative variants (See Additional file 3).

Discussion and conclusions

The patient in our study manifested core symptoms of ASD with accompanying ADHD and impulsiveness.

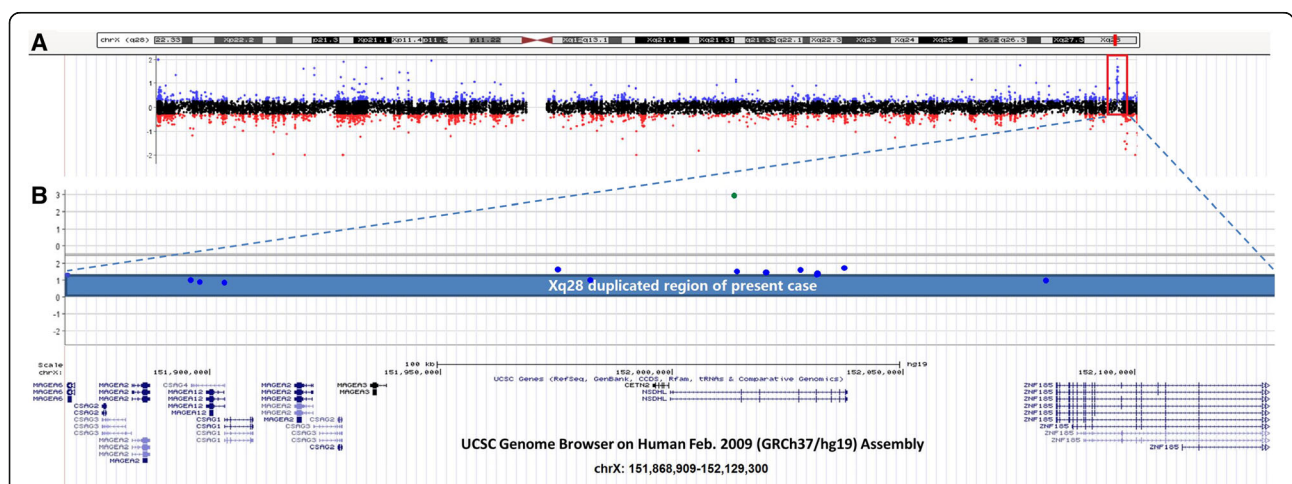


Fig. 2 a The chromosomal status of the patient. Dots represent relative intensities reported in log2 ratios and the genomic locations of the oligonucleotide probes employed in the aCGH assay. A value of zero indicates a balanced chromosomal status. For the patient (P01), red (loss), black (no change), and blue (gain) dots represent log2 ratio deviations from the horizontal line of 0. Regions with copy number gains are indicated with red frames. **b** *NSDHL*-containing 260 kb Xq28 duplication in the patient. The blue bar indicates the Xq28 duplication region of the patient (chrX:151,868,909–152,129,300, converted to GRCh37/hg19) displayed using the UCSC genome browser. Blue (gain) dots represent log2 ratio deviations from the horizontal line of 0, and genes in the duplication region are indicated using the UCSC Genes prediction track

CNV scanning revealed that the patient had a Xq28 duplication encompassing 8 genes, and that the most disease-associating gene *NSDHL* was among this duplication region; this is the first report of an ASD patient with a related *NSDHL*-containing duplication at Xq28.

We hypothesized that the *NSDHL*-containing duplication at Xq28 was responsible for the ASD. Four *MAGE* family genes [24] have been reported to be expressed at high levels in numerous tumours of various histological types, whereas in a large panel of healthy tissues, expression was observed only in the testis and placenta. *CSAG* family genes [25], which are expressed at quite low levels in brain, are mostly expressed in the testis, and *CSAG1* is expressed in some higher-grade chondrosarcomas, chondrosarcoma-derived cell lines, and melanoma cell lines, as well as in some types of cartilage. *CETN2* [26] is associated with the centrosome, whereas *ZNF185* [27] is mostly expressed in the human adult prostate, testis, ovary, placenta, peripheral blood, and embryonic kidney; a partial copy was contained in this region (encompassing a minimum of 16 and a maximum of 18 exons). Since the

levels of *CETN2* and *ZNF185* are also high in the brain, we searched more databases, including OMIM, Gene Cards and ClinVar, but still did not find any report indicating associations among *CETN2*, *ZNF185* and neural development disorders. Diseases associated with *CETN2* include Xeroderma pigmentosum [28], while diseases associated with *NSDHL* include CK Syndrome and CHILD Syndrome. Thus, most genes in the duplicated region are expressed beyond the brain or exhibit little relation to autism or neurodevelopmental disease (See Additional file 1: Table S3–1, S3–2). *NSDHL*, which is located at Xq28, is expressed in developing cortical neurons and glia and encodes an enzyme that functions in post-squalene cholesterol biosynthesis [16], thus affecting the steps of the cholesterol biosynthetic pathway. Research has shown that perturbations of cholesterol metabolism are associated with a variety of CNS disorders found in ASD [29] and other mental disorders [30]. Nevertheless, we still need more evidence to evaluate the effects of other genes in the duplicated region and more functional studies to show the importance of *NSDHL* to the development of ASD.

Table 1 Clinical features of patients with similar duplications reported in the DECIPHER database^a

Patient NO	Sex	Chromosome	Size	Inheritance	Gene number	Phenotype
258546	46XY	X 151958561-152031784	73.22 kb	From an unaffected mother	2	Autism Spectrum Disorder
268469	46XY	8 13745710-14028750	273.31 kb	From a normal parent	1	Obesity Low anterior hairline Intellectual disability
		X 151897058-152170367	283.04 kb	From a normal parent	11	
251730	46XX	X 151901372-153232466	1.33 Mb	De novo	45	Bulbous nose Short palm Muscular hypotonia
288269	46XX	X 122322494-1223858575	63.38 kb	Unknown	1	Global developmental delay
		X 151492204-152101413	609.21 kb	Unknown	18	
290161	46XY	1 225217624-225307001	89.38 kb	Unknown	1	Hypoplastic male external genitalia Obesity
		16 29586128-30195608	609.48 kb	Unknown	32	
		X 151958995-152119014	160.02 kb	Unknown	3	
249396	46XY	X 140664743-152216545	11.55 Mb	De novo	122	Low-set ears Hypertelorism Cryptorchidism Micrognathia Muscular hypotonia Intellectual disability Prenatal short stature

^aThis study makes use of data generated by the DECIPHER [34] community. A full list of centres who contributed to the generation of the data is available from <http://decipher.sanger.ac.uk> and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. Firth, H.V. et al. (2009). *Am.J.Hum.Genet* 84, 524–533 (Doi:dx.doi.org/10.1016/j.ajhg.2009.03.010)

NSDHL mutations, which have been implicated in CK syndrome [18] in males, result in rare X-linked intellectual disabilities and cognitive impairment. Affected males also have behavioural problems, including aggression, ADHD, irritability, and seizures [18] and partially fulfil the criteria for ASD [18]. Our patient exhibited the characteristics of attention deficit and irritability but not seizure and satisfied the criteria for ASD diagnosis. The patient had an *NSDHL* duplication at Xq28, which was inherited from his mother. *NSDHL* is likely the most important disease-related gene within the duplicated region of this patient because the other genes in this duplicated region have little relevance to ASD. In contrast to patients with CK syndrome [16], our patient exhibited normal cognition, possibly because the *NSDHL* gene was duplicated and not mutated. His mother, who carried the *NSDHL* duplication on only one X chromosome, exhibited a few symptoms of ASD and had normal physical features. Her intellect and irritable disposition were consistent with previous reports of female carriers of *NSDHL* mutations [16]. Studies have demonstrated that female carriers of an *NSDHL* mutation generally do not manifest signs or symptoms of CK syndrome [31]. These carriers have normal physical features, intellect, and brain imaging findings but exhibit behavioural problems, such as irritability and aggression.

We also checked the common variation in DGV (<http://dgv.tcag.ca/dgv/app/home>) for the same CNV duplication region. We found that Jakobsson's [32] study showed an observed gain including the entire *NSDHL* gene. Tracing the duplication region, we found out that a female had the duplication by checking the information of the raw data (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM264524>; sample ID HGDP00959). We assumed that female carriers with *NSDHL* duplication exhibit few symptoms and tend to be normal. To our knowledge, the frequency of the CNV in the normal population remains uncertain.

Patients with similar duplications reported in the DECIPHER database share some phenotypes and exhibit intellectual disabilities, muscular hypotonia and obesity. Table 1 shows an overview of similar duplications in DECIPHER. We found one boy (No. 258546) who had inherited from his unaffected mother a similar duplication region as that in our patient also had Autism Spectrum Disorder (We contacted the clinician for more information on these patients via DECIPHER. However, some patients did not have any further follow-up. A negative finding for ASD diagnosis in DECIPHER does not necessarily indicate that the cases do not have ASD; therefore, the importance of *NSDHL* cannot be ignored). Furthermore, because *NSDHL* functions in the removal of two C-4 methyl groups in one of the later steps of cholesterol biosynthesis, it is more likely that patients

with similar duplications will exhibit obesity, including our patient, who showed higher serum levels of free fatty acids. These data support the dysfunction of *NSDHL* in cholesterol biosynthesis and the development of CNS.

Here, we have identified an 8-year-old patient carrying a 260-kb *NSDHL*-containing duplication inherited from his mother. Since the data in DGV and DECIPHER are not considered part of a peer-reviewed publication, this is the first report of an ASD patient with a related *NSDHL*-containing duplication at Xq28. The patient fulfilled the criteria for ASD and shared some characteristics with CK syndrome patients [16, 18, 33] but exhibited normal cognition. Further studies and case reports are required to establish that both mutations and duplications of *NSDHL* cause neurodevelopmental diseases. The relationship between *NSDHL* and ASD also requires further study to obtain conclusive support.

Additional files

Additional file 1: Table S1. Results showing the Swanson Nolan and Pelham, Version IV (SNAP-IV), Autism Diagnostic Observation Schedule (ADOS) M3, Autism Diagnostic Interview-Revised (ADI-R), and Wechsler Intelligence Scale for Children (WISC) scores for the *NSDHL*-containing patient. **Table S2.** The DNA and cDNA primers used to analyse the *NSDHL* variant in the study. **Table S3–1.** The tissue expression of genes in the duplicated region of the patient in this study. **Table S3–2.** Brain expression from Allen Brain and known related diseases in the OMIM of genes in the duplicated region of the patient in this study. (DOCX 24 kb)

Additional file 2: Figure S3. Pyrosequencing results of mother's genomic DNA showing X-chromosome inactivation patterns (XCIP). (DOC 31 kb)

Additional file 3: Whole exome sequencing (WES) data analysis of the patient. (DOCX 18 kb)

Abbreviations

ADHD: Attention deficit hyperactivity disorder; ADI-R: Autism Diagnostic Interview; ADOS: Autism Diagnostic Observation Schedule; ASD: Autism Spectrum Disorder; CRT: Combined Raven's Test; CSAG1: Chondrosarcoma-associated gene; EEG: Electroencephalogram; Head MRI: Head magnetic resonance imaging; MECP2: Methyl-CpG-binding protein 2 gene; *NSDHL*: NAD(P)H steroid dehydrogenase-like protein; WES: Whole exome sequencing; XLID: X-linked intellectual disability; ZNF18: Zinc finger protein 185

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional file. The identifying/confidential patient data will not be shared.

Authors' contributions

XX, CH and YS conceived the study and participated in its design and coordination. QX, BZ and CYL identified the patients and carried out the clinical characterisations. YS, CH and CXL participated in the molecular genetic studies, and CH and XX wrote the manuscript. All authors revised and approved the final version of this manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Children's Hospital of Fudan University (2011–040). Informed consent to participate was obtained from the patient's parents.

Consent for publication

The father of the patient gave written permission for the publication of the patient's clinical details and images.

Competing interests

The authors declare that they have no competing interests.

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