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ARTICLE

A heterozygous microdeletion of 20q13.13 encompassing *ADNP* gene in a child with Helsmoortel–van der Aa syndrome

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Abstract

Helsmoortel–van der Aa (SWI/SNF autism-related or *ADNP* syndrome) is an autosomal dominant monogenic syndrome caused by de novo variants in the last exon of *ADNP* gene and no deletions have been documented to date. We report the first case of a 3 years and 10 months old boy exhibiting typical features of *ADNP* syndrome, including intellectual disability, autistic traits, facial dysmorphism, hyperlaxity, mood disorder, behavioral problems, and severe chronic constipation. 60K Agilent array-comparative genomic hybridization (CGH) identified a heterozygous interstitial microdeletion at 20q13.13 chromosome region, encompassing *ADNP* and *DPM1*. Taking into account the clinical phenotype of previously reported cases with *ADNP* single-point variants, genotype–phenotype correlation in the proband was established and the diagnosis of Helsmoortel–van der Aa syndrome was made. Our report thus confirms that *ADNP* haploinsufficiency is associated with Helsmoortel–van der Aa syndrome as well as highlights the utility of whole-genome array-CGH for detection of unbalanced submicroscopic chromosomal rearrangements in routine clinical setting in patients with unexplained intellectual disability and/or syndromic autism.

Introduction

Helsmoortel–van der Aa (MIM #615873), a novel recognizable intellectual disability syndrome with autism comorbidity, is a monogenic autosomal dominant disorder caused by de novo frameshift or nonsense sequence alterations in the 3′ end of the last exon of *ADNP* [1]. All individuals with Helsmoortel–van der Aa syndrome had consistent clinical features, including autism spectrum disorders, mild to severe intellectual disability, and distinctive facial dysmorphism [2]. *ADNP* (MIM* 611386) is composed of five exons and encodes an activity-dependent neuroprotective homeobox protein. 3D genome structure analysis allows an overview of regulatory landscape and 3D genome structural units within the *ADNP* locus (Supplemental Figure S1). This Krüppel C2H2-type zinc-finger transcriptional factor is a component of human BAF multiprotein complexes and participates in the regulation of neuronal development through chromatin remodeling [3]. Interstitial deletions of 20q13.13 chromosome region have been rarely documented. To date, only five heterozygous interstitial deletions with different sizes ranging from 313 kb to 3.31 Mb involving (partial) *ADNP* gene have been recorded (Table 1 and Fig. 2a). Moreover, no cases with pure *ADNP* deletions have been reported so far. We report the first case exhibiting the typical features of Helsmoortel–van der Aa syndrome carrying the smallest 63 kb heterozygous interstitial deletion at 20q13.13 chromosome region. Having reviewed previously reported cases with deleterious single-point variants, the genotype–phenotype correlation of the present index-patient was established.

Materials and methods

Blood lymphocytes were cultured in RPMI 1640 supplemented with PHA (Life Technologies Sas, Courtaboeuf,

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Table 1 Clinical features of all previously reported *ADNP* deleted patients

Patient ID	Decipher309	Decipher257542	Decipher265392	Decipher258702	Patient0195-5515 [1]	Cas-index
Deletion (hg19/GRCh37)	Chr20:46,212,807–49519078	Chr20:48,461,499–50,342,355	Chr20:48,412,795–50,002,464	Chr20:48,974,247–50,088,251	Chr20:49,398,527–49,711,161	Chr20:49,508,67–49,571,808
Inheritance	De novo	De novo	De novo	De novo	Parents not available for testing	De novo
Size	3.31 Mb	1.88 Mb	1.59 Mb	1.11 Mb	313 kb	63 kb
Sex	Male	Male	Male	Male	NR	Male
Intellectual disability	NR	Intellectual disability	Intellectual disability	Intellectual disability	Severe intellectual disability	Intellectual disability
Developmental delay	NR	NR	NR	NR	Mild motor delay	Motor delay
Speech	Delayed speech and language development	NR	NR	NR	Severe language delay	Severe language and speech delay
Autistic traits	NR	NR	NR	NR	Autism	Autistic traits
Facial features	Microcephaly, anteverted nares, short nose	Frontal bossing, anteverted nares, short nose, long philtrum	NR	Short philtrum, thin upper lip vermilion	Microdolicocephalic (49.5 cm), small forehead, low-set hairline, high nasal bridge, plat philtrum, full lips, open mouth	Prominent forehead, high anterior hairline, wide palpebral fissures, broad nasal bridge, short nose, long/smooth philtrum, thin upper lip and protruding lower lip
Eye abnormalities	NR	Sclerocornea	NR	NR	Protruding eyes, hypertelorism, right convergent strabismus	Hypertelorism, strabismus
Ear abnormalities	Low-set ears	NR	NR	NR	Small ears	Small ears
Hands/feet	2–3 toes syndactyly, clinodactyly of the fifth finger, osseous finger syndactyly	Talipes equinovarus	NR	NR	Tapering fingers	//
Musculoskeletal abnormalities	NR	NR	NR	NR	Pectus excavatum, kyphoscoliosis	Hyperlaxity of joints
Behavioral problems	NR	NR	NR	NR	Self injury, aggressive behavior	Angry/irritable mood, hetero-aggression
Additional details	Feeding difficulties in infancy, micropenis	Cryptorchidism, multiple renal cysts	NR	NR	Psychotic behavior, poor eye contact, stereotyped movements, generalized muscle hypotonia, deep tendon hyperreflexia, bilateral Babinski sign, anxiety disorder	Hypotonia, recurrent respiratory infections, pervasive developmental disorder, ataxic gait, difficulties in social interaction and communication, poor eye contact, sleep disturbance, severe constipation, premature tooth eruption

Note that our case carrying the smallest 63 kb deletion at 20q13.13 chromosome region

NR not reported

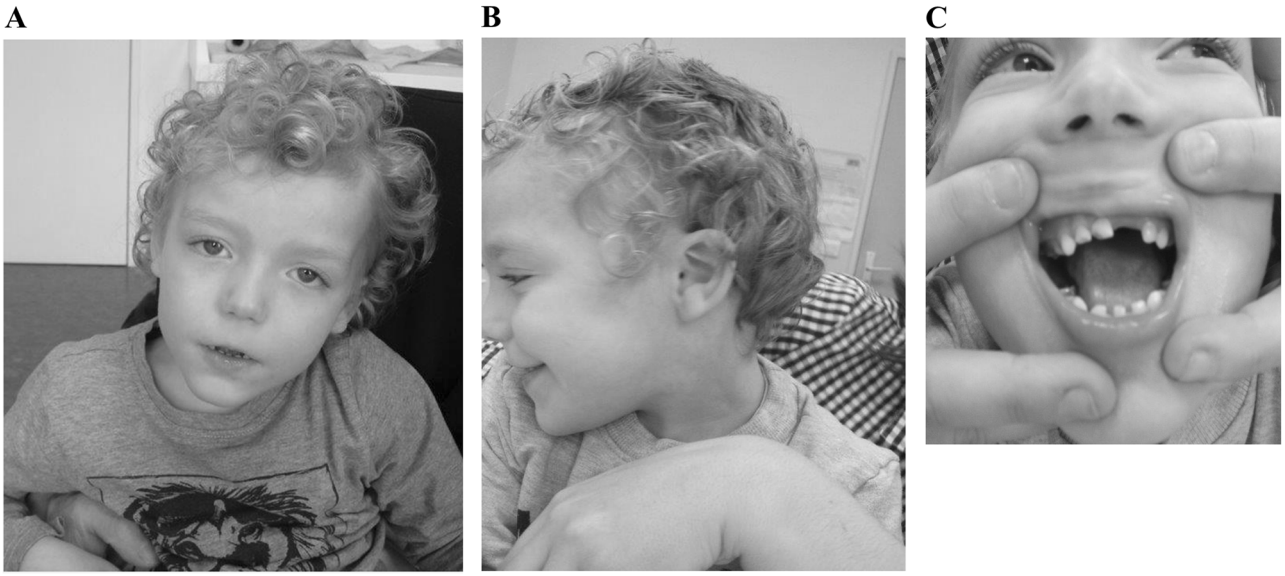

Fig. 1 Photographs of the face (**a**, **c**) and right profile (**b**) of the proband showed typical facial dysmorphic features, including: frontal bossing, high anterior hairline, broad nasal bridge, hypertelorism, wide palpebral fissures, short nose, smooth/long philtrum, small and widely spaced teeth, thin upper lip, and protruding lower lip

France) and chromosome metaphases were harvested with minor modifications according to the standard laboratory protocol as previously described. Fluorescent in situ hybridization (FISH) analysis was performed using two BAC probes: RP5-914P20 (chr19:49,495,091–49,600,424) and RP11-466J8 (chr19:2,840,928–3,027,437) according to the laboratory protocol [4]. 60K array-CGH (Agilent Technologies, Santa Clara, CA, USA) with a median probe spacing of 54 kb was carried out as previously described [5] and required at least three contiguous probes to make a call. Quantitative real-time PCR using SYBR® Green PCR Master Mix (Life Technologies, Woolston Warrington, UK) was performed and all samples were run in triplicate. The dosage of each amplicon relative to control amplicon of *RPPH1* (gene ID85495) and normalized to control DNA using the $2-\Delta\Delta CT$ method as described [6]. Total RNA from blood sample was extracted according to Maxwell® 16 IVD instrument Nucleic Acid Extraction—Protocol Promega. One microgram of total RNA was used for reverse-transcriptase PCR (Thermo Fisher Scientific, Lithuania). Results were normalized with endogenous control gene β-2 microglobulin (*B2MG*) as previously described [7].

Results

Clinical phenotype

The 3 years and 10 months old boy first came to our clinic because of intellectual disability associated with pervasive developmental disorder. The pregnancy was marked with maternal hyperglycemia. He was born at 39 + 6 weeks to a healthy non-consanguineous Caucasian couple. His birth weight, length, and head circumference were, respectively, 3320 g (25th–50th centile), 47 cm (10th–25th centile), and 36 cm (>90th centile). Hypotonia was noted at birth. The family history was unremarkable, the couple already had a healthy 11-year-old child. The proband had recurrent respiratory tract infections, he suffered from congenital stridor and obstructive sleep apnea due to tonsillar hypertrophy, and underwent tonsillectomy at the age of 1 year. His developmental milestones were delayed and with kinesitherapy he could walk unsupported at 24 months. According to clinical evaluation conducted at the age of 55 months, his weight, height, and head circumference were 18 kg (75th centile), 105 cm (50th centile), and 49.8 cm (25th centile), respectively. Clinical physical evaluation showed prominent forehead with high anterior hairline, hypertelorism with inner intercanthal distance of 4 cm (97th centile), strabismus, palpebral fissure length of 30 mm, small ears with an ear length of 4 cm (<3th centile), broad nasal bridge, short nose, long/smooth philtrum, thin upper lip and protruding lower lip, hyperlaxity of the joints, and ataxic gait (Fig. 1). He had severe language delay, spoke the first word at 16 months, and combined words into short sentences at 4 years. Of note, the index-patient displayed premature primary tooth eruption (deciduous tooth eruption at the age of 2 months). He had 12 maxillary teeth, 11 mandibular teeth but lacked 1 mandibular incisor. His four 6-year molars were also prematurely present. He had difficulties in social interaction and communication, poor eye contact, and sleep disturbances. Behavioral problems and the presence of episodic mood disorders such as angry, irritable mood, and hetero-aggression were observed. His parents also

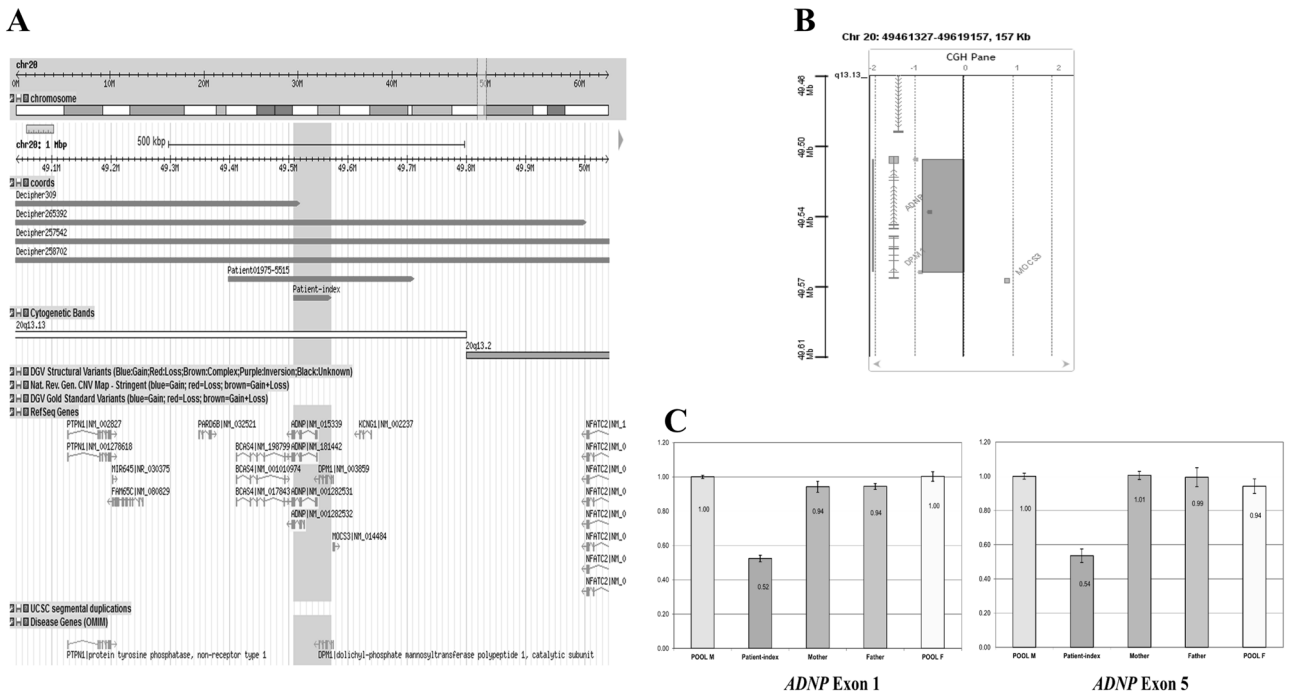

Fig. 2 **a** Overview of 1 Mb at 20q13.13 chromosome region with five reported overlapping deletions in medical literature: Decipher309, Decipher265392, Decipher257542, Decipher258702, Patient01975-5515, and index-patient. Gray shadow highlights the smallest region of overlap (SRO); the proband carried the smallest 63 kb deletion at 20q13.13 chromosome region harboring two genes *ADNP* and *DPM1*. The *ADNP* gene in Helsmoortel–van der Aa syndrome is highlighted in yellow. **b** 60K array-CGH showed a 63 kb interstitial deletion at chromosome 20q13.13 containing *ADNP* and *DPM1*. **c** Quantitative real-time PCR showed that the deletion occurred de novo in the proband with primers in exons 1 and 5 of *ADNP* gene (index-patient: purple color; mother: pink color; father: blue color; pool of male control: light blue color; pool of female control: yellow color)

noticed that he had severe chronic constipation and was treated with laxatives. Echocardiogram, renal ultrasound, chest X-ray, EEG (ElectroEncephaloGram), brain magnetic resonance imaging (MRI), ophthalmologic examination, creatine kinase (CK) dosage, and transferrin isoelectric focusing were normal.

Conventional karyotype and molecular characterization of an interstitial 20q13.13 microdeletion

Conventional cytogenetics showed a normal male karyotype 46,XY. Agilent 60K array-CGH analysis showed a heterozygous interstitial deletion at 20q13.13 chromosome with three probes (A_16_P03530666, A_14_P134776, and A_14_P104282), $\log_2$ratio = −0.85, encompassing two genes: *ADNP* and *DPM1*, chr20:g.(49,457,856_49,508,676)_(49,571,808_49,620,163)del (hg19, NC_000020.10) (NG_034200.1) (Fig. 2b). Quantitative real-time PCR with primers in exons 1 and 5 of *ADNP* gene did not detect the deletion in the parental DNA (Fig. 2c). FISH analysis showed that the deletion occurred de novo in the proband and excluded parental balanced insertional translocation (Supplemental Figure S2). Quantitative reverse-transcriptase PCR showed that *ADNP* expression was decreased approximately 40% in the proband in comparison to his mother and two normal controls (Supplemental Figure S3).

Discussion

Hitherto, 18 patients with Helsmoortel–van der Aa syndrome have been reported [8]. Novel additional clinical features associated with *ADNP* syndrome, including blepharophimosis, craniosynostosis, primary hypothyroidism, hypertension, lack of autistic behaviors, cleft palate, and early deciduous tooth eruption were also recorded [9–11]. All frameshift and nonsense *ADNP* sequence variations were gain-of-function variants except for a stop-codon variant in the fourth exon with predicted loss of *ADNP* function. The index-patient carried a de novo 63 kb heterozygous interstitial deletion at 20q13.13 chromosome region containing two genes: *ADNP* and *DPM1*. The deletion has never been reported in the databases of genomic variants (DGV at http://dgv.tcag.ca/dgv) (Supplemental Figure S4). *DPM1* (MIM* 603503) encodes a key enzyme dolichol phosphate mannose (DPM) synthase which plays an important function in protein glycosylation. Disease-associated sequence variants of *DPM1* cause congenital

disorder of glycosylation type Ie (MIM #608799). To date, nine reported patients had typical features, including: seizure (8/9), postnatal microcephaly (8/9), abnormal MRI (8/9), and increased serum CK levels (7/9) [12]. Based on the detailed analysis of clinical findings, it is worth noting that the clinical features as well as paraclinical examinations of the reported proband are not consistent with the diagnosis of congenital disorder of glycosylation type Ie. Of interest, the proband displayed typical findings of Helsmoortel–van der Aa syndrome. Additionally, he had severe chronic constipation. Only 4 of 18 reported patients had constipation, one carrying a de novo c.2491_2494delTTAA p.Lys831Ilefs*81 variant had intermittent constipation, while other three patients had the same sequence alteration c.2157C>G p.Tyr719* in *ADNP* gene. Up to date, all reported disease-associated variants apparently escaped from nonsense-mediated mRNA decay which showed an increased aberrant *ADNP* mRNA. Unlike dominant-negative mutational mechanism, *ADNP* loss-of-function variants or deletion would produce an amorphic allele leading to gene dosage imbalance hypothesis. Normal BAF complex function requires the physical interaction of distinct subunits which ideally are mutually balanced. The presence of multiple heterozygous variants in genes encoding different components of BAF complex would lead to synergistic effects that could drive the multimer concentration and/or activity below a critical thresold required for a normal phenotype or otherwise, *ADNP* haploinsufficiency associated with hypomorphic alleles of other protein partners in BAF complex could cause a reduced complexes activities below a critical thresold resulting in an abnormal phenotype [13]. Moreover, regarding the probability of loss-of-function intolerance (pLI) score, *ADNP* has a high pLI score of 1.0 which is particularly intolerant to loss-of-function variants, dosage sensitive and predicted to have a high probability of being relevant to haploinsufficiency (pLI ≥ 0.9), while low *DPM1* pLI score of 0 is loss-of-function tolerant. The *ADNP* gene region on 8 × 60K array-CGH platform is inadequately covered and contains only two probes: A_16_P03530666 and A_14_P134776 which could not detect small intragenic *ADNP* rearrangements during routine array-CGH testing. High-resolution techniques should be preferentially performed in patients with unexplained intellectual disability in order to tackle these issues [14]. In summary, we report the first case of *ADNP* haploinsufficiency associated with Helsmoortel–van der Aa syndrome and highlight the importance of high-resolution techniques such as high-density oligonucleotide array-CGH and whole-genome sequencing in clinical diagnostic workup when all available conventional and specific gene tests did not reveal causative abnormalities.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This work is not a clinical research and considered as routine clinical care.

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