BCAP31-related syndrome: The first *de novo* report

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ARTICLE INFO

Keywords:

BCAP31

Xq28

DDCH syndrome

Dystonia

Central hypomyelination

Hearing loss

ABSTRACT

Pathogenic variants in the *BCAP31* gene have recently been associated with a severe congenital neurological phenotype, named DDCH after its key features: deafness, dystonia and central hypomyelination. *BCAP31* is located at the Xq28 chromosomal region and only male individuals are currently known to be affected, the pathogenic variant being usually transmitted by healthy mothers.

Here, we describe a three-year-old male child referred for severe developmental delay, failure to thrive, hearing loss and dyskinetic movements. After a conventional diagnostic workflow, including a normal array-CGH, a tentative diagnosis of dyskinetic cerebral palsy was retained. Clinical exome sequencing in the trio identified a small intragenic deletion in exon 8 of *BCAP31*, c.709_721del (p.Val237Trpfs*69), originated *de novo* and not previously reported. Based on the ACMG variant classification, this variant is predicted to be 'likely pathogenic'. Given the consistent phenotypical overlap with the subjects already ascertained with DDCH, we considered this variant to be clinically relevant for this child and causative of his condition.

1. Introduction

The *BCAP31* gene is located in the chromosomal region Xq28 and encodes BAP31, a chaperone protein localized within the endoplasmic reticulum (ER) membrane and responsible for protein transportation, folding quality control and caspase8-dependant apoptosis (Grimm, 2012; Zhang and Williams, 2006). Due to its key role in ER functioning *BCAP31* is expressed in many tissues, including the central nervous system (Manley and Lennon, 2001).

Loss-of-function variants or small intragenic deletions of *BCAP31* have been identified in seven male individuals from three unrelated families presenting with severe developmental delay, deafness, mild liver dysfunction, dystonia, central hypomyelination and poor prognosis. This phenotype was recapitulated with the acronyms DDCH syndrome (MIM #300475) (Cacciagli et al., 2013). Another male subject with a pathogenic variant in *BCAP31* and consistent phenotype has been recently described, expanding the clinical spectrum to the involvement of basal ganglia and choreoathetosis (Albanyan et al., 2017).

Besides intragenic anomalies, *BCAP31* may be dysregulated by copy number variants as well. *BCAP31* lies between two other OMIM genes, i.e. *SLC6A8* at its 5' end and *ABCD1* at its 3' end, whose disruption is responsible for cerebral creatine deficiency (MIM #300352) and adrenoleukodystrophy (MIM #300100), respectively. For both these

conditions, different authors have reported subjects with a more severe phenotype than usually expected. In all these cases the underlying genetic defect was a microdeletion including at least partially *BCAP31*, in addition to either *SLC6A8* or *ABCD1* (Anselm et al., 2006; Corzo et al., 2002; Iwasa et al., 2013; Osaka et al., 2012); further publications have dissected the genotype-phenotype correlation of this region according to the deletion size (Calhoun and Raymond, 2014; van de Kamp et al., 2015).

This scenario depicts *BCAP31* as a dosage-sensitive gene and associates its impairment to a severe neurological phenotype in males. All the *BCAP31* pathogenic variants already ascertained were transmitted by healthy mothers; only in one case the mother had sensorineural hearing loss, possibly due to her carrier status for *BCAP31* (Albanyan et al., 2017). Here, we present a further individual carrying the first *de novo* causative variant known for *BCAP31*.

2. Clinical report

This male child was born to healthy non-consanguineous parents of Belgian origin with unremarkable family history. During pregnancy ultrasound scans revealed only a slight decrease of growth parameters in the third trimester, when delivery occurred at 32 + 4 weeks of gestation with an urgent C-section due to maternal pre-eclampsia and

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<https://doi.org/10.1016/j.ejmg.2019.103732>

Received 26 October 2018; Received in revised form 2 June 2019; Accepted 17 July 2019

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placental abruption. Birth parameters were: weight 1640 gr, length 42 cm and head circumference 28.8 cm; Apgar scores were 7/1 – 8/V – 8/X. Due to respiratory distress, invasive ventilation was undertaken for the first 28 h then followed by nasal CPAP for 8 days. He also needed surfactant replacement therapy and phototherapy for jaundice. His motor pattern appeared to be chaotic and associated with episodic myoclonus of the face and of the limbs. Neonatal neurosonography and EEG were normal whereas the auditory screening and the brainstem evoked response audiometry were both altered, arguing for bilateral sensorineural hearing loss.

At the age of two months a magnetic resonance (MRI) of the brain showed unspecific T2-weighted hyperintense signal of the periventricular white matter (PVWM), whose interpretation was limited due to his young age; notably, there was no signal alteration of basal ganglia. From the first months failure to thrive coexisted with a significant gastroesophageal reflux (GER). By the age of four months, the eye contact was still poor and neurological examination highlighted gaze-evoked nystagmus, opisthotonus, athetoid wandering movements, axial hypotonia, hypertonia of lower limbs with a positive plantar reflex. At eleven months he underwent positioning of cochlear implant, without clear improvement. At fifteen months his motor functioning level showed an equivalent age of about two months (Peabody Developmental Motor Scale, PDMS-2). Additional normal investigations included a CT scan of petrous temporal bones, ophthalmoscopy, abdominal ultrasound, plasma serology for TORCH complex, plasma amino acids, urinary organic acids and array-CGH (average resolution ~200 kb). Based on clinical presentation and investigations, the diagnosis of dyskinetic cerebral palsy was retained.

His evolution has been characterized by poor psychomotor development with persistence of dyskinetic movements, unsatisfactory growth, difficult feeding and severe GER requiring percutaneous endoscopic gastrostomy (PEG) and Nissen fundoplication respectively, multiple hospitalizations due to recurrent infections (on average one episode every two months). In at least three cases of infection a slight transient increase in transaminases was noticed (AST 70 mU/mL, ALT 109 mU/mL). At 18 months he experienced febrile seizures, then followed by a partial seizure with myoclonus of the right leg and a generalized seizure at the age of 34 months. EEG never revealed epileptic foci but anti-epileptic drugs were prescribed due to his general condition.

His last clinical evaluation was at the age of 28 months. His height was –2.9 SD, his weight –2.4 SD and his head circumference –1.6 SD (it was less than –2 SD till 18 months). He was wheelchair-bound with incomplete control of the head and inconstant eye contact. His motor activity was characterized by intentional and dystonic movements; gaze-evoked nystagmus and bruxism were also noticed. Additional features were: positional brachycephaly, poliosis, bitemporal narrowing, straight eyebrows, deep-set eyes, bluish sclerae, inconstant esotropia, short philtrum, micrognathia. Parents reported hands and feet to be often cold. His therapy includes proton-pump inhibitors, aerosol for bronchial hyperactivity (of family origin), antibiotic prophylaxis and clonazepam. He attends kinesiotherapy and swallowing logopedy.

3. Methods and results

DNA extraction and array-CGH were carried out according to routine protocols (Winters et al., 2017). To assess single nucleotide variants we performed targeted resequencing of OMIM genes in the familial trio. Library preparation for all samples was performed using TruSeq DNA Sample Preparation Kit (Illumina, CA, USA). All exons of the target genes were enriched through hybridization with an in-house developed in-solution Nimblegen SeqCap EZ capture (Roche Nimblegen, WI, USA) and the target-enriched libraries were amplified and sequenced on the HiSeq 2500 (Illumina, CA, USA) by paired-end sequencing. An in-house developed pipeline was used for further

bioinformatics processing of the sequencing data (base-calling, alignment and variant calling were performed with the GATK package). Variant annotation and classification were executed within the Cartagenia BENCHlab NGS module (Cartagenia v4.1, Agilent, CA, USA) according to ACMG guidelines for interpretation of sequence variants. With this approach we identified a total of 66 *de novo* variants, of which 65 showed high frequencies in the general population database or were deep intronic (> 20 bp far from intron-exon boundaries). The only variant predicted to be deleterious and consistent with the phenotype was the hemizygous variant (NC_000023.10:g.152966411ATGGGACC ATCTAC > A; NM_001139441.1:c.709_721del; p. Val237Trpfs*69) in the *BCAP31* gene. This variant is absent in any population or disease database (GnomAD, HGMD, Clinvar) and was confirmed with Sanger sequencing. The hemizygous deletion of 13 base pairs occurs in the last exon of *BCAP31*, positioned 17 base pairs before the stop codon. Its presence disrupts the reading frame, causing readthrough across the position of the normal stop codon. This is predicted to result in a 304 amino acid long protein, the wild type protein being of 246 amino acids. We classified this variant as likely pathogenic (class 4), based on its *de novo* status (ACMG criteria PS2), the absence in control population databases (ACMG criteria PM2), its status of protein length changing variant in haploinsufficiency sensitive gene (ACMG criteria PM4) and the specific overlap with previously reported phenotype (ACMG criteria PP4).

4. Discussion

We describe a 3-year male individual referred with a tentative diagnosis of dyskinetic cerebral palsy. Target resequencing of OMIM genes on the familial trio identified a hemizygous likely pathogenic variant in the *BCAP31* gene not previously reported. Pathogenic variants of this X-linked gene have been recently associated to a severe neurologic condition featuring deafness, dystonic movements and central hypomyelination (Cacciagli et al., 2013). Though all the mutations reported so far were inherited, in our case the segregation analysis revealed the variant to be *de novo*.

Our variant affects the C-terminus of the protein, possibly resulting in a transcript longer than the canonical wild-type one. Interestingly, one of the individuals reported by Cacciagli et al. carried a sub-terminal deletion of *BCAP31*, suggesting that C-terminus disruption might be relevant for the disease. Among the three core signs firstly characterized by Cacciagli et al. our index subject certainly shares the first two, whereas regarding his possible degree of central hypomyelination the cerebral MRI performed at two months of age revealed unspecific T2-weighted hyperintense PVWM, suggestive for delayed myelination (Table 1). The interpretation of this finding must take into account the age-related dependence of the myelination process (Guleria and Kelly, 2014) and the sensitivity of the periventricular region to injuries in preterm infants (Benders et al., 2014). On the other hand, periventricular hypomyelination was also highlighted in two unrelated individuals among those described by Cacciagli et al. aged 5.6 and 11 years at the time of cerebral MRI (for the latter along with both cerebral cortical and cerebellar atrophy). Therefore, though it is not possible to exclude the effect of some confounding factors, MRI findings described in our case are consistent with what already known for DDCH.

Beyond the three main signs, our index subject shares other phenotypical traits with DDCH (Table 1). His facial appearance presents only minor dysmorphic features and strongly recalls the subject described by Albany et al. Severe failure to thrive led auxological parameters to maintain constantly below –2 SD, even when age-matched for prematurity; height growth has been more regular than weight gain, the latter remarkably improving after PEG. His psychomotor development is severely delayed and he is wheelchair-bound, speech is absent and communicative skills are limited to eye contact. Both epilepsy and mild liver dysfunction showed a reduced penetrance in

Table 1
Clinical comparison of individuals with BCAP31-associated syndrome.

	Cacciagli et al. (2013)							Albanyan et al. (2017)	This report
	Family 1		Family 2			Family 3		Singleton	Singleton
	III.2	III.3	III.5	III.6	III.7	IV.1	III.1		
Variant	c.194-2A > G p.Ile64fs*25		Exon 8 deletion				c.97C > T p.Gln 33*	c.533,536dup p.Ser180Alafs*6	c.709,721del p.Val237Trpfs*69
Inheritance	healthy mother		healthy mother			healthy mother	healthy mother	mother with bilateral hearing loss	<i>de novo</i>
Gender	male	male	male	male	male	male	male	male	male
Age of onset	congenital	congenital	congenital	6 w	6 w	congenital	congenital	first weeks	congenital
Age of death	24 y	13 y	7 m	1 y	2 y	living	3 y	living	living
MAIN SIGNS AND SYMPTOMS									
Deafness	+	+	NA	+	+	+	+ / -	+	+
Dystonia	++	++	NA	+++	+++	++	+++	++	++
White matter (performed at)	NP	PVWM (11 y)	NP	NP	NP	PVWM (5.6 y)	diffuse hypomyelination (2.5 y)	NA	PVWM (2 m)
Additional MRI findings		atrophy of cerebral cortex and cerebellum					atrophy of CC, frontal lobe, white matter	bilateral globus pallidus hyperintensity	
Liver function tests	transitory episodes of a 2- to 3-fold increase in 4 out of 7 subjects							+ / + + elevation of AST and ALT during illnesses	+ / + + elevation of AST and ALT during illnesses
Facial dysmorphisms	++	+	NA	+	+	+	+ / -	+	+
Failure to thrive	+++ (IUGR, W -7 SDs, H -8 SDs)	+++ (IUGR, W -6 SDs, H -5 SDs)	NA	+	+	+++	+	4.5 y: H < -2SDs, W normal range (gastrostomy)	28 m: H -2.9 SDs, W -2.4 SDs (gastrostomy)
Microcephaly	+++ (-5 SDs)	+++ (-5 SDs)	NA	NA	NA	+	+	+	+ / -
						(2 m: -3 SDs, 2 y: -2 SDs)	(0-6 m: -2 SDs, > 6 m: -3 SDs)	(< -2 SDs)	(0-18 m: < -2SDs > 18 m: -1.6 SDs)
Motor (acquired at)	none	none	none	none	head (1 y)	head (6 m), sitting (5 y), wheelchair (8 y)	head (1 y, lost at 1.5 y)	none	none
Neurologic	pyramidal signs, quadriplegia	pyramidal signs, quadriplegia	NA	pyramidal signs, quadriplegia	pyramidal signs, quadriplegia	pyramidal signs, paraplegia	pyramidal signs, quadriplegia	choreoathetosis, pyramidal signs, quadriplegia	pyramidal signs, quadriplegia
Seizure (onset)	+	+	NA	none	none	+	none	none	+
	(4 y)					(8 y)			(3 y)
Ocular	Congenital strabismus	Congenital strabismus, abnormal eye movements	NA	optic atrophy	congenital strabismus	congenital strabismus, optic atrophy	congenital strabismus	NA	nystagmus, bluish sclerae, inconstant esotropia

Legend: w = week(s); m = month(s); y = year(s); CC = corpus callosum; PVWM = periventricular white matter hypomyelination; +/- = very mild; + = mild; ++ = moderate; +++ = severe; NA = not available; NP = not performed; IUGR = intra-uterine growth retardation; W = weight; H = height; AST/ALT = hepatic transaminase.

subjects with *BCAP31* haploinsufficiency (Cacciagli et al., 2013). In our case, the child had three isolated episodes of seizure (one of likely febrile origin) not followed by persistent EEG anomalies and his hepatic involvement was restricted to low and transient transaminases increases during febrile episodes. Bruxism and nystagmus were not previously described and might be due to the involvement of basal ganglia, as suggested by the MRI findings from Albanyan et al. and from the association of these two signs with movement disorders (Ella et al., 2017; Jung and Kim, 2019).

BAP31 protein participates on several processes, such as protein fate regulation, ER-Golgi crosstalk and apoptosis. To assess the consequences of BAP31 impairment, Cacciagli et al. investigated its functioning in fibroblasts from affected individuals and identified abnormal ER morphology and disorganization of the Golgi apparatus. The authors suggested that the perturbation of the ER-Golgi interaction might lead

to myelination defect, as already observed for other hypomyelinating disorders (Numata et al., 2013). Some insights about the hepatic involvement have been provided by comparing the phenotype of individuals with Xq28 deletion of different sizes. In fact, only combined deletions of *BCAP31* and *ABCD1* would be responsible for severe hepatic cholestasis, supporting the former playing a role in hepatic homeostasis, possibly through bile metabolism (van de Kamp et al., 2015). However, the molecular mechanisms by which *BCAP31* haploinsufficiency would then result in DDCH remain to be fully elucidated.

In conclusion, we report the ninth unrelated individual presenting with the DDCH phenotype and describe the first pathogenic *de novo* variant in *BCAP31*. Despite the co-occurrence of deafness, dystonia and central hypomyelination is not uncommon in patients with severe congenital neurological conditions, such a clinical presentation may be

enough specific to recall the involvement of *BCAP31*.

Acknowledgments

The authors thank the family for participating in this study.

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