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► To cite this version:

Ivana Dzinovic, Matej Škorvánek, Petra Pavelekova, Chen Zhao, Boris Keren, et al.. Variant recurrence confirms the existence of a FBXO31 -related spastic-dystonic cerebral palsy syndrome. *Annals of Clinical and Translational Neurology*, 2021, 10.1002/acn3.51335 . hal-03163358

HAL Id: hal-03163358

<https://hal.sorbonne-universite.fr/hal-03163358v1>


Submitted on 9 Mar 2021

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BRIEF COMMUNICATION

**Variant recurrence confirms the existence of a
FBXO31-related spastic-dystonic cerebral palsy syndrome**

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Funding information

This work was supported by the Slovak Grant and Development Agency under contract APVV-18-0547 and the Operational Programme Integrated Infrastructure, funded by the ERDF under No. ITMS2014+: 313011V455 to MS. The study was also supported by in-house institutional funding from Helmholtz Center Munich, Munich, Germany, Technical University of Munich, Munich, Germany, and Charles University, Prague, Czech Republic (PROGRES Q27). This study was also funded by the Czech Ministry of Education under grant AZV: NV19-04-00233 and under the frame of EJP RD, the European Joint Programme on Rare Diseases (EJP RD COFUND-EJP No 825575).

Received: 26 January 2021; Revised: 20 February 2021; Accepted: 22 February 2021

Abstract

The role of genetics in the causation of cerebral palsy has become the focus of many studies aiming to unravel the heterogeneous etiology behind this frequent neurodevelopmental disorder. A recent paper reported two unrelated children with a clinical diagnosis of cerebral palsy, who carried the same *de novo* c.1000G > A (p.Asp334Asn) variant in *FBXO31*, encoding a widely studied tumor suppressor not previously implicated in monogenic disease. We now identified a third individual with the recurrent *FBXO31* *de novo* missense variant, featuring a spastic-dystonic phenotype. Our data confirm a link between variant *FBXO31* and an autosomal dominant neurodevelopmental disorder characterized by prominent motor dysfunction.

doi: 10.1002/acn3.51335

Introduction

Cerebral palsy (CP) represents a major neurodevelopmental disorder (NDD) with an estimated prevalence of 2–3 per 1000 live births.^{1,2} The clinical presentation is heterogeneous and the defining features comprise disorders of movement and posture, usually described as non-progressive, but prone to change throughout the course of disease.³ The motor disorder arises in early infancy and persists until the end of life, often accompanied by additional features such as developmental delay, intellectual disability (ID), and speech impairment.³ The etiologic implication of environmental factors has been investigated extensively, whereas genetic contributions to CP have only begun to be elucidated.⁴

Two recent large-scale whole-exome sequencing (WES) studies described patients with CP who harbored causative variants in known NDD-associated genes as well as in genes not previously linked to human Mendelian disorders.^{5,6} Jin and colleagues⁵ identified two unrelated CP-affected individuals with an identical de novo missense variant (c.1000G > A, p. Asp334Asn) in *FBXO31*, encoding F-box only protein 31 (FBXO31), a molecule for which a relationship to disease had only been known from cancer research.⁷ The features shared by both subjects included spasticity, ID, speech deficits, and behavioral abnormalities.⁵ Herein, we report on the identification of the recurrent *FBXO31* c.1000G > A (p. Asp334Asn) variant in a patient with a complex movement-disorder syndrome, providing the first independent replication of the involvement of a specific de novo *FBXO31* mutation in a human NDD. Additionally, we present a comparison of phenotype abnormalities observed in all three *FBXO31*-mutated individuals, contributing information for a better understanding of this newly identified monogenic condition.

Methods

The proband presented here was identified as part of a cohort of ~950 whole-exome-sequenced individuals with various types of dystonic disorders. Clinical evaluation was performed at the Department of Neurology, University Hospital of L. Pasteur, Kosice, Slovakia. Prior to the collection of clinical information and data from genomic testing, written informed consent was obtained from the proband's legal representatives (parents). The case study was carried out in accordance with the Declaration of Helsinki and under protocols approved by respective ethical committees. For the family trio, WES was conducted at the sequencing core of Helmholtz Center Munich using previously published methods.⁶ Briefly, exome capture was

performed using Agilent SureSelect v6 reagents and exome libraries were subjected to paired-end sequencing on an Illumina HiSeq4000. Sequence-data processing was done with a validated in-house pipeline, implementing Burrows-Wheeler-Aligner, SAMtools, ExomeDepth, and custom scripts. An average of 96-fold to 114-fold coverage was obtained for the three exomes, with 97%–98% of the target nucleotides covered ≥ 20 -fold. Variants were annotated with gene, transcript, functional class, population frequency, deleteriousness predictions, and ClinVar entry. The proband's entire WES data set was searched for pathogenic or likely pathogenic variants⁸ in reported disorder-associated genes, as described.⁶ For the identification of de novo alleles, variants were called in all three samples simultaneously, and de novo events were detected by bioinformatics subtraction analysis.^{6,9}

Results

Case presentation

We report the case of a 9-year-old boy demonstrating a complex, non-progressive movement-disorder syndrome dominated by spasticity and dystonia (Table 1). The proband was born at term per caesarian section after an uneventful pregnancy. He was the first child of unrelated, healthy parents of Slovakian descent. Family history was unremarkable. There were no postpartum complications and no congenital anomalies. During the first months of life, he presented with muscular hypotonia and delayed acquisition of developmental milestones. He rolled over at the age of 6 months, but did not come to sit until 9 months. He walked with support at 20 months. He spoke in single words at the age of 12 months, but lost the ability to speak at 18 months. Neuropediatric assessment at the age of 2 years documented a conversion to limb spasticity from hypotonia, more pronounced in the lower extremities. In addition, involuntary abnormal twisting and postural abnormalities affecting all four limbs and occasionally the trunk were noted. A working clinical diagnosis of spastic-dystonic CP was established. Over the following 7 years, there was no significant worsening of tone and movement abnormalities, but the proband required assistance with activities of daily living due to severe physical disability. He could not walk longer distances without bilateral support and he displayed reduced manual dexterity. He was able to follow two-step commands, but had no expressive language. Examined elsewhere, he was diagnosed with an intellectual disability. Because of his motor and speech impairments, he has never been able to engage in active play with his peers. At the latest neurological evaluation (age 9 years), he was alert, interactive with eye contact, but he did not vocalize or

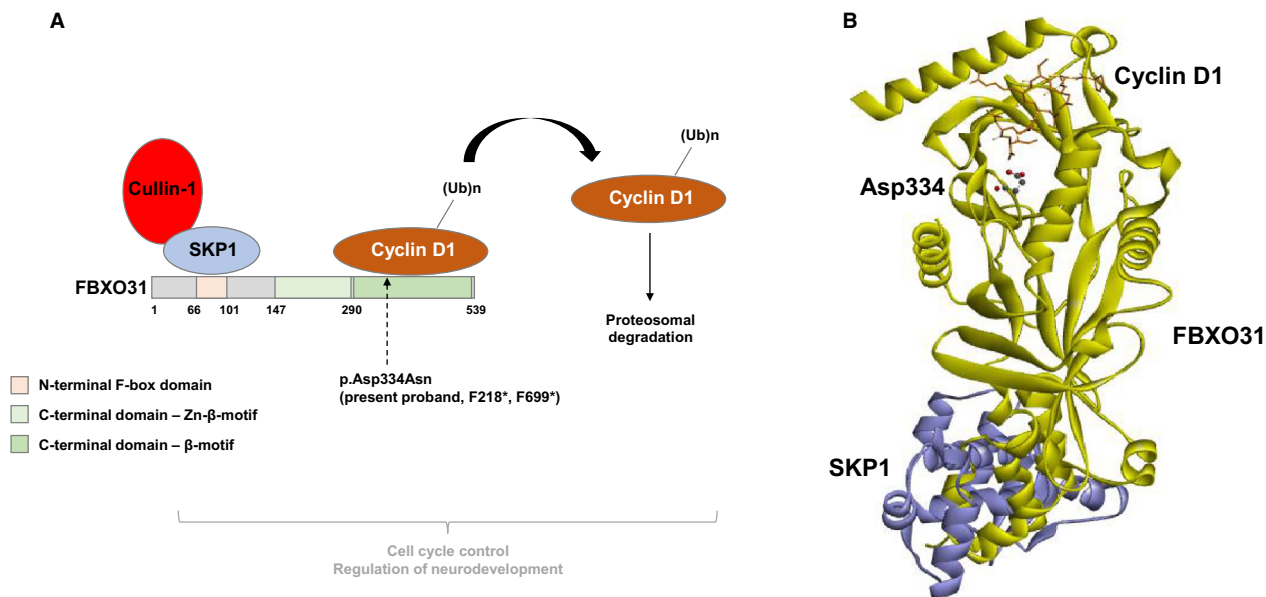


Figure 1. A recurrent *FBXO31* missense variant in patients with neurodevelopmental disease and movement disorder. (A) Linear model of *FBXO31* and schematic representation of the cellular role of *FBXO31* in SCF (SKP1, cullin, F-box protein) complex-mediated regulation of cyclin D1 abundance. The location of the patient-specific recurrent missense variant at amino acid position 334 is indicated. * the previously described cases are labeled as in Jin et al.⁵ (B) Three-dimensional model of the *FBXO31*-SKP1-cyclin D1 complex (PDB: 5VZU). The spatial proximity of the mutated Asp334 residue (stick-and-ball representation) to *FBXO31*'s substrate cyclin D1 (stick representation) is illustrated.

verbalize. He had generalized dystonia involving his arms, legs, and trunk, as well as signs of pyramidal tract dysfunction including spasticity (more severely affecting the legs than the arms), hyperreflexia, and clonus in both legs. Mobility was impaired, with an unsteady spastic-dystonic gait. His intellectual development was estimated as moderately compromised, although formal IQ testing had not been performed. No distinctive craniofacial features or physical defects were documented.

Diagnostic evaluations

Routine laboratory tests were non-diagnostic, although slightly elevated levels of lactate in blood and urine were documented once. A series of metabolic studies were unrevealing. On brain MRI (age 3 years), there was reduced white-matter volume in the parietal and occipital lobes bilaterally, along with an aspect of global white-matter dysmyelination. After targeted molecular investigations had failed to provide an etiologic diagnosis, the proband and his parents underwent trio-WES in a research context. The analysis revealed that the proband was heterozygous for a *FBXO31* c.1000G > A (p. Asp334Asn) missense substitution, which had occurred de novo. This variant, absent from control databases (gnomAD, in-house exomes), has been described recently in two individuals with an NDD diagnosed as spastic diplegic CP.⁵ The variant affected a conserved residue¹⁰ within a

functionally important domain of the encoded protein, involved in the interaction with *FBXO31*'s substrate cyclin-D1 (Fig. 1A and B).^{5,11} According to the guidelines of the American-College-of-Medical-Genetics-and-Genomics,⁸ the variant was classified as "pathogenic". No other potentially disease-relevant variants were observed in the proband's WES data.

Comparative phenotypic analysis

A clinical overview of the proband presented here and the individuals reported by Jin et al.⁵ is given in Table 1. The three cases shared a presentation of motor and/or speech delay, and all had ID that varied from mild to moderate. Additional consistent features included profound abnormalities of speech and muscle tone. The latter comprised spasticity (as seen in all three patients), predominantly affecting the lower limbs and leading to a diagnosis of CP in each subject,⁵ as well as dystonia (observed in the herein reported case). Other neurological and non-neurological manifestations such as congenital malformations, behavioral issues, minor dysmorphia, and neuroanatomical alterations occurred in two or only one of the subjects.

Discussion

FBXO31 encodes a 539 amino-acid protein containing an N-terminal F-box domain and a C-terminal substrate-

Table 1. *FBXO31*-related disease - Genetic findings and clinical features of the present proband and two cases reported by Jin et al.⁵

| | Present proband | F218 ¹ | F699 ¹ |
|--|---|---|---|
| Age at last assessment | 9 years | 9 years | 10 years |
| Gender | Male | Female | Male |
| Genomic variant (GRCh37/hg19) | Chr16:87367889C > T | Chr16:87367889C > T | Chr16:87367889C > T |
| <i>FBXO31</i> cDNA change (NM_024735.5) | c.1000G > A | c.1000G > A | c.1000G > A |
| <i>FBXO31</i> protein change (NP_079011.3) | p. Asp334Asn | p. Asp334Asn | p. Asp334Asn |
| Zygosity | Heterozygous | Heterozygous | Heterozygous |
| Inheritance | de novo | de novo | de novo |
| Family history | Negative | Negative | Negative |
| Prenatal findings | No | No | Decreased fetal movements, mild intrauterine growth restriction |
| Gestational age at birth (weeks) | In term (38) | In term (40) | In term (N/A) |
| Malformations at birth | No | Cleft palate, intestinal malrotation, and midgut volvulus requiring small bowel resection | No |
| Developmental delay | Yes (global) | Yes (global) | Yes (global) |
| Hypotonia | Yes (neonatal) | Yes (neonatal) | Yes (neonatal) |
| Intellectual disability | Yes (moderate) | Yes (mild) | Yes (moderate) |
| Speech impairment | Loss of speech at the age of 1.5 years, receptive language disorder | Dysarthria, mixed receptive/expressive language disorder | Speech not fully acquired, receptive language disorder |
| Behavioral abnormalities | No | Attention deficit hyperactivity disorder | Attention deficit hyperactivity disorder |
| Movement disorder | Generalized dystonia, limb spasticity (lower limbs > upper limbs) | Lower-limb spasticity | Lower-limb spasticity |
| Diagnosis of cerebral palsy (CP) | Yes (spastic-dystonic CP) | Yes (spastic diplegic CP) | Yes (spastic diplegic CP) |
| Loss of independent ambulation | No | No | No |
| Brain magnetic resonance imaging | Bilateral occipital and parietal white-matter volume reduction, irregular white-matter dysmyelination | Normal | Mild ventricular dilatation, thin corpus callosum |
| Dysmorphic signs | No | Esotropia | Strabismus |

N/A, not available.

¹Labeled as in Jin et al.⁵

recognition domain (Fig. 1A).¹¹ *FBXO31* is a component of a SKP1-cullin-F-box (SCF)-type ubiquitin ligase, a ubiquitously expressed complex involved in proteasome-dependent degradation of various regulatory proteins such as cyclin-D1 (Fig. 1A and B).^{11,12} Deregulation of SCF complexes and their subunits has been linked to diverse human pathologies including monogenic disorders.¹³ For instance, within the class of FBXO (F-box-only) proteins, *FBXO11* has been associated with an autosomal dominant NDD (MIM:618089).¹⁴ Moreover, biallelic variants in *FBXO7* cause Parkinson's disease-15 (MIM:260300),^{15,16} linking the occurrence of movement disorder symptoms to FBXO gene perturbation.

A disease-relevant role of *FBXO31* has been initially studied in the context of oncogenesis, where the protein was postulated to act as a potent tumor suppressor.⁷ More recent research suggests that *FBXO31* is also indispensable

for the normal function of the nervous system, impacting neural network formation and synaptic strength.¹⁷ Although the mechanistic basis for how mutation of *FBXO31* leads to an NDD remains an area of investigation, Jin et al.⁵ have shown that c.1000G > A (p. Asp334Asn) produced decreased levels of *FBXO31*'s substrate cyclin-D1 in patient-derived cells, consistent with a gain-of-function effect. We have now identified a third individual with the c.1000G > A (p. Asp334Asn) variant, displaying phenotype characteristics closely resembling those of the originally described cases.⁵ Emerging hallmarks of *FBXO31*-related disease seem to include a delay in psychomotor development, dysfunction of both pyramidal and extrapyramidal systems (as evidenced by the manifestation of spasticity and dystonia), impairments in expressive and receptive language, and ID. Notably, a diagnostic label of CP had been given to all three *FBXO31*-mutated individuals. This

finding supports the recent idea that monogenic defects represent important contributors to the pathogenesis of CP and CP-like syndromes.^{5,6} The proportion of patients diagnosed with CP whose conditions are attributable to *FBXO31* variants remains to be determined. In addition, further studies are required to clarify whether monoallelic *FBXO31* variants other than c.1000G > A (p. Asp334Asn) are disease-causing.

Collectively, this report confirms that a recurrent missense variant in *FBXO31* causes a novel neurodevelopmental syndrome. The occurrence of de novo *FBXO31* mutation should be considered in patients with CP and spastic-dystonic disorders for which no alternative cause is found.

Acknowledgment

The authors are grateful to the family who participated in this study. Open access funding enabled and organized by ProjektDEAL.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

I.D. contributed to data acquisition and analysis and drafted a significant portion of the manuscript. P.P., C.Z., B.K., S.W., S.B., S.C.J., and M.C.K. contributed to data acquisition and analysis. M.S., R.J., and J.W. contributed to design and conception of the study and data acquisition and analysis. M.Z. contributed with design and conception of the study, data acquisition and analysis, supervised the study, and drafted a significant portion of the manuscript. All authors performed a critical review of the manuscript.

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