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

Ivana Dzinovic
Helmholtz Zentrum München

Matej Škorvánek
P.J. Safarik University

Petra Pavelekova
P.J. Safarik University

Chen Zhao
Helmholtz Zentrum München

Boris Keren
Sorbonne Université

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Please let us know how this document benefits you.

Recommended Citation
Dzinovic, Ivana; Škorvánek, Matej; Pavelekova, Petra; Zhao, Chen; Keren, Boris; Whalen, Sandra; Bakhtiari, Somayeh; Chih Jin, Sheng; Kruer, Michael C; Jech, Robert; Winkelmann, Juliane; and Zech, Michael, "Variant recurrence confirms the existence of a FBXO31-related spastic-dystonic cerebral palsy syndrome." Annals of Clinical and Translational Neurology. 8, 4. 951 - 955. (2021).
https://digitalcommons.wustl.edu/open_access_pubs/11292

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Ivana Dzinovic, Matej Škorvánek, Petra Pavelekova, Chen Zhao, Boris Keren, Sandra Whalen, Somayeh Bakhtiari, Sheng Chih Jin, Michael C Kruer, Robert Jech, Juliane Winkelmann, and Michael Zech
Variant recurrence confirms the existence of a FBXO31-related spastic-dystonic cerebral palsy syndrome

Ivana Dzinovic¹, Matej Škovrňák²,³, Petra Pavelekova²,³, Chen Zhao¹, Boris Keren⁴, Sandra Whalen⁵, Somayeh Bakhtiari⁶,⁷, Sheng Chih Jin⁸, Michael C. Krueer⁶,⁷, Robert Jech⁹, Juliane Winkelmann¹,⁴,¹⁰,¹¹,¹² & Michael Zech¹,¹⁰

¹Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany
²Department of Neurology, P.J. Safarik University, Kosice, Slovak Republic
³Department of Neurology, University Hospital of L. Pasteur, Kosice, Slovak Republic
⁴Department of Genetics, Pitié-Salpêtrière Hospital, APHP. Sorbonne Université, Paris, France
⁵UF de Génétique Clinique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, APHP. Sorbonne Université, Hôpital Armand Trousseau, Paris, France
⁶Pediatric Movement Disorders Program, Division of Pediatric Neurology, Barrow Neurological Institute, Phoenix Children’s Hospital, Phoenix, Arizona
⁷Departments of Child Health, Neurology, and Cellular & Molecular Medicine, and Program in Genetics, University of Arizona College of Medicine-Phoenix, Phoenix, Arizona
⁸Department of Genetics, Washington University School of Medicine, St Louis, Missouri
⁹Department of Neurology, Charles University, 1st Faculty of Medicine, General University Hospital, Prague, Czech Republic
¹⁰Institute of Human Genetics, Technical University of Munich, Munich, Germany
¹¹Lehrstuhl für Neurogenetik, Technische Universität München, Munich, Germany
¹²Munich Cluster for Systems Neurology, SyNergy, Munich, Germany

Correspondence
Michael Zech, Institute of Neurogenomics, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany. Tel: 0049-89-3187-1884; Fax: 0049-89-3187-3297; E-mail: michael.zech@mri.tum.de

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.
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 persist 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 colleagues\(^5\) 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 a previous study.\(^6\) Briefly, exome capture was performed at the sequencing core of Helmholtz Center Munich using previously published methods.\(^6\) The proband's left arm 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 2 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 perform

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 \(\geq\)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 variants in reported disorder-associated genes, as described.\(^8\) 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 perform...
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, inhouse 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-
recognition domain (Fig. 1A).\textsuperscript{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).\textsuperscript{11,12} Deregulation of SCF complexes and their subunits has been linked to diverse human pathologies including monogenic disorders.\textsuperscript{13} For instance, within the class of FBXO (F-box-only) proteins,\textsuperscript{14} FBXO11 has been associated with an autosomal dominant NDD (MIM:618089).\textsuperscript{14} Moreover, biallelic variants in FBXO7 cause Parkinson’s disease-15 (MIM:260300),\textsuperscript{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.\textsuperscript{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.\textsuperscript{17} Although the mechanistic basis for how mutation of FBXO31 leads to an NDD remains an area of investigation, Jin et al.\textsuperscript{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.\textsuperscript{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

<table>
<thead>
<tr>
<th>Present proband</th>
<th>F218\textsuperscript{1}</th>
<th>F699\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at last assessment</td>
<td>9 years</td>
<td>9 years</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Genomic variant (GRCh37/hg19)</td>
<td>Chr16:87367889C &gt; T</td>
<td>Chr16:87367889C &gt; T</td>
</tr>
<tr>
<td>FBXO31 cDNA change (NM_024735.5)</td>
<td>c.1000G &gt; A</td>
<td>c.1000G &gt; A</td>
</tr>
<tr>
<td>FBXO31 protein change (NP_079011.3)</td>
<td>p. Asp334Asn</td>
<td>p. Asp334Asn</td>
</tr>
<tr>
<td>Zygosity</td>
<td>Heterozygous</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Inheritance</td>
<td>de novo</td>
<td>de novo</td>
</tr>
<tr>
<td>Family history</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Prenatal findings</td>
<td>No</td>
<td>Decreased fetal movements, mild intrauterine growth restriction</td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>In term (38)</td>
<td>In term (40)</td>
</tr>
<tr>
<td>Malformations at birth</td>
<td>No</td>
<td>Cleft palate, intestinal malrotation, and midgut volvulus requiring small bowel resection</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>Yes (global)</td>
<td>Yes (global)</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>Yes (neonatal)</td>
<td>Yes (neonatal)</td>
</tr>
<tr>
<td>Intellectual disability</td>
<td>Yes (moderate)</td>
<td>Yes (mild)</td>
</tr>
<tr>
<td>Speech impairment</td>
<td>Loss of speech at the age of 1.5 years, receptive language disorder</td>
<td>Dysarthria, mixed receptive/expressive language disorder</td>
</tr>
<tr>
<td>Behavioral abnormalities</td>
<td>No</td>
<td>Attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>Movement disorder</td>
<td>Generalized dystonia, limb spasticity (lower limbs &gt; upper limbs)</td>
<td>Lower-limb spasticity</td>
</tr>
<tr>
<td>Diagnosis of cerebral palsy (CP)</td>
<td>Yes (spastic-dystonic CP)</td>
<td>Yes (spastic diplegic CP)</td>
</tr>
<tr>
<td>Loss of independent ambulation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Brain magnetic resonance imaging</td>
<td>Bilateral occipital and parietal white-matter volume reduction, irregular white-matter dysmyelination</td>
<td>Normal</td>
</tr>
<tr>
<td>Dysmorphic signs</td>
<td>No</td>
<td>Esotropia</td>
</tr>
</tbody>
</table>

N/A, not available.
\textsuperscript{1}Labeled as in Jin et al.\textsuperscript{5}
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.

References