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De novo variants in FBXO11 cause a syndromic form of intellectual disability with behavioral problems and dysmorphisms

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Abstract

Determining pathogenicity of genomic variation identified by next-generation sequencing techniques can be supported by recurrent disruptive variants in the same gene in phenotypically similar individuals. However, interpretation of novel variants in a specific gene in individuals with mild-moderate intellectual disability (ID) without recognizable syndromic features can be challenging and reverse phenotyping is often required. We describe 24 individuals with a de novo disease-causing variant in, or partial deletion of, the F-box only protein 11 gene (FBX011, also known as VIT1 and PRMT9). FBX011 is part of the SCF (SKP1-cullin-F-box) complex, a multi-protein E3 ubiquitin-ligase complex catalyzing the ubiquitination of proteins destined for proteasomal degradation. Twenty-two variants were identified by nextgeneration sequencing, comprising 2 in-frame deletions, 11 missense variants, 1 canonical splice site variant, and 8 nonsense or frameshift variants leading to a truncated protein or degraded transcript. The remaining two variants were identified by array-comparative genomic hybridization and consisted of a partial deletion of FBX011. All individuals had borderline to severe ID and behavioral problems (autism spectrum disorder, attention-deficit/hyperactivity disorder, anxiety, aggression) were observed in most of them. The most relevant common facial features included a thin upper lip and a broad prominent space between the paramedian peaks of the upper lip. Other features were hypotonia and hyperlaxity of the joints. We show that de novo variants in FBX011 cause a syndromic form of ID. The current series show the power of reverse phenotyping in the interpretation of novel genetic variances in individuals who initially did not appear to have a clear recognizable phenotype.

Deceased: Anthonie J. van Essen

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Introduction

Next-generation sequencing (NGS) in neurodevelopmental disorders, especially with regard to intellectual disability (ID), has increased the possibility to identify an underlying genetic defect as the cause of disease to provide a conclusive molecular diagnosis [1–5]. NGS-based techniques such as whole-exome sequencing (WES) and whole-genome sequencing have shown to be efficient for individuals with ID and lead to an overall diagnostic yield of

55–70% [6]. WES is rapidly becoming the first-tier test in individuals with ID due to increased diagnostic yield and reduction of costs [7].

Since the DNA of each individual contains thousands of genic variants, many of which are difficult to interpret [8], different clinical and molecular approaches have been used to prioritize variants [9]. It has, for example, been shown that the identification of recurrently mutated genes in individuals with an overlapping phenotype add to the interpretation of variants. This depends, however, on the rarity and specificity of the clinical feature(s) involved [10]. For instance, a combination of rare congenital abnormalities or dysmorphic features is more specific than non-syndromic ID and hypotonia on its own [10]. Also, the inheritance pattern can be dependent on the severity of the ID and this may help interpretation. Severe ID often occurs sporadic and is caused by de novo variants [6], whereas mild ID can be explained by either a de novo variant, an inherited variant from a more mildly affected parent, or may have a multifactorial origin [11]. Reverse phenotyping, which refers to clinical comparison of individuals after a variant in the same gene has been found [12], can be of great value for interpretation of genetic variation [13–15].

In this report we show the value of reverse phenotyping in multiple individuals with variants in FBX011. FBXO11 (also known as VIT1 and PRMT9) encodes the substrate-recognition component of the SCF (SKP1cullin-F-box) complex, which is responsible for ubiquitination and subsequent degradation of substrates and plays a role in the maintenance of genome stability [16]. In addition, FBXO11 has been reported to be involved in regulating alternative splicing [17]. Downregulation of FBXO11 has previously been associated with vitiligo [18] and somatic variants have been found in diffuse large B cell lymphoma [19]. Germline variants in FBX011 were not reported until recently when a frameshift and missense variant were found in a cohort of individuals with autism spectrum disorder (ASD) [5] and one nonsense variant in a cohort of individuals with ID [20]. Whereas overlap between various neurodevelopmental disorders, such as ID and ASD, has been described before, detailed phenotypic information of additional individuals is needed to specify the phenotypic spectrum associated with FBXO11 variants. In this study, we collected clinical information on 24 individuals with a de novo disease-causing variant in or partial deletion of FBX011 and used reverse phenotyping to establish the related phenotypic spectrum. We show that reverse phenotyping in our patient cohort delineates a novel ID syndrome caused by de novo disease-causing variants in FBX011.

Methods

See Supplementary Information.

Results

Identification of individuals with FBXO11 variants

Individuals 1, 2, 3, 18, and 20 were identified by routine diagnostic WES at the Radboudumc, Nijmegen and Maastricht University Medical Center, Maastricht, and were performed as previously described [21]. Based on the identification of three individuals with a de novo disease-causing variant in *FBXO11*, in combination with FBXO11 function, we considered *FBXO11* an interesting candidate gene for ID, warranting further studies. Hence, we performed trio-based targeted re-sequencing using molecular inversion probes (MIPs [22, 23]) in 1031 ID and/or ASD individuals and their healthy parents: 512 individuals from Antwerp and 519 from Nijmegen. Individuals 4 and 5 were identified by this MIP assay.

In addition, we collected clinical information on 17 individuals (Individuals 6–17, 19, 21–24) with a variant in, or deletion of, *FBXO11* through direct contact with (inter) national colleagues and by using Genematcher [24, 25] and Decipher [26].

Using data from seven laboratories contributing patients to this study, which included 21 individuals with a de novo disease-causing variant in *FBXO11* in a total cohort of 14,088 individuals with ID/ASD, we estimated the frequency of a de novo disease-causing variant in *FBXO11* in an unbiased ID/ASD cohort to be in the range of 1:500 to 1:1000 (Supplemental Table 1). In addition, if we compare the frequency of these de novo disease-causing variants in *FBXO11* in our cohorts to the gene-specific mutation rate [21, 27], we find significantly more such disease-causing variants than expected (p = 1.879082e - 19, Bonferroni correction for multiple testing).

Of note, Individuals 23 and 24 were previously published as part of a large-scale study reporting on de novo disease-causing variants in ASD using the Simons Simplex Collection cohort [5].

All 24 individuals were re-evaluated by reverse phenotyping, and clinical data were collected and compared.

Spectrum of disease-causing variants

Twenty-four individuals carried 23 unique de novo diseasecausing variants in the gene, comprising ten missense variants, one canonical splice site variant, two in-frame single amino acid deletions, two partial gene deletions, two



Fig. 1 *FBXO11* with deletions and variants identified in our cohort. *FBXO11* is expressed in two different isoforms (NM_001190274.1 and NM_0251433.4), which differ by an alternative exon 1, and of which NM_001190274.1 represents the longest transcript. Variants are

nonsense variants, and six frameshift variants (Fig. 1; Supplemental Tables 2 and 3). Two individuals had an identical missense variant (Individuals 8 and 17). Interestingly, 16 of the 21 de novo variants are located in important protein domain structures (Fig. 1; Supplemental Table 2). Nine missense variants are in the carbohydrate-binding/ sugar hydrolysis (CASH) domain, which is characterized by internal repeats of glycines and hydrophobic residues forming the β -helix repeat structures of the protein. Prediction of the pathogenicity of these missense variants using various classification tools, including SIFT (scale-invariant feature transform) and MutationTaster, indicate that these variants are likely pathogenic (Supplemental Table 2 and Supplemental Fig. 1). Three frameshift variants, one nonsense variant, and an in-frame deletion affect a UBR-type zinc-finger domain, which contains an N-degron sequence serving as a degradation signal that can be recognized by a unique class of E3 proteins for ubiquitin-dependent proteolysis [28] (Fig. 1).

In two individuals, a partial deletion of *FBXO11* was identified by array-comparative genomic hybridization: a 21-kb intragenic deletion of exons 2–13 of *FBXO11* (Individual 6), and an ~170-kb deletion disrupting the 3' end of *FBXO11* by deleting exons 12-24 (Individual 7) (Fig. 1).

Summary phenotypes

All 24 individuals had developmental delay ranging from borderline to severe ID. In addition, 16 individuals (67%) had behavioral problems, mostly consisting of ASD (n = 7), but attention-deficit/hyperactivity disorder (ADHD) (n = 5), aggression (n = 3), and anxiety (n = 3) were also observed. More than half of the individuals had hypotonia and a fraction had epilepsy (n = 3). Imaging of the brain was performed in 17 individuals, which showed enlarged ventricles in six of them. Three individuals had additional brain abnormalities (hypoplasia of anterior pituitary; bilateral hippocampal malformations, borderline large cerebellum; Chiari 2 malformation, syringomyelia).

annotated based on NM_0011190274.1. FBXO11 protein domains are depicted in color. Individuals 6 and 7 have a partial deletion of *FBXO11* (gray bars)

Seven individuals were obese, which was not observed in the remaining 17 individuals. Although individuals did have dysmorphic features, there was no consistent facial gestalt (Fig. 2). The most consisted facial features were a high broad forehead (6/17, 35%), long palpebral fissures (7/20, 35%), and a thin upper lip with broad space between the paramedian peeks (15/19, 79%) (Figs. 2 and 3). Twelve individuals had minor hand abnormalities like brachydactyly or tapering fingers and eight individuals had hyperlaxity of the joints. Gastrointestinal problems such as feeding difficulties and vomiting was reported in 11 individuals (52%) and 9/21 (43%) had vision problems. Individuals encountered several other health problems, such as atrial septum defect (n = 1), cardiomyopathy (n = 1), diabetes mellitus (n = 1), and hypertrichosis (n = 2), but these only appeared in a minority. An overview of the most frequent features is shown in Table 1. Detailed clinical information is provided in the Supplemental Table 3 and the Supplemental Case Reports.

Discussion

Here we report on 24 individuals with a de novo diseasecausing variant in or partial deletion of, *FBXO11*. We have provided a detailed clinical description showing that these individuals had borderline to severe ID, various behavioral problems (ASD, ADHD, aggression, anxiety), hypotonia, hyperlaxity of the joints, and some overlapping dysmorphisms (thin upper lip, broad space between the paramedian peaks of the upper lip, high broad forehead, long palpebral fissures). Six individuals had enlarged ventricles, of which three had additional brain abnormalities. A minority of individuals had other health problems, such as atrial septum defect, cardiomyopathy, diabetes mellitus, and hypertrichosis.

The 22 de novo disease-causing variants comprised 21 unique variants, including ten missense variants, two inframe single amino acid deletions, one canonical splice site variant, two nonsense variants, and six frameshift variants.



Fig. 2 Photographs of individuals with a variant in *FBXO11*. The first row shows the individuals at a younger age, and the second row shows the respective individual at an older age. Facial features comprise a broad forehead, long palpebral fissures, and a thin upper lip with a

broad space between the paramedian peaks of the upper lip. Some individuals show coarsening at an older age. The third row shows the hands of the respective individual with brachydactyly and tapering fingers in several cases



Fig. 3 a Detailed photographs of the eyes of individuals with a variant in FBXO11. Eyes show long palpebral fissures. b Detailed photographs of

the lips of individuals with a variant in *FBXO11*. There is a thin upper lip with a broad space between the paramedian peaks of the upper lip

FBXO11 is part of the F-box protein family, consisting of at least 69 different members in humans, divided over three protein subfamilies, which all contain at least one Fbox motif, and, depending on the subfamily, another motif, that is, FBXL (leucine-rich repeats), FBXW (WD repeats), and FBXO (O = only or other motif) [29–32]. Fbox proteins are part of SCF complexes, which are RING domain-containing E3 ubiquitin ligases [16, 30] degrading cellular proteins by ubiquitination during posttranslational modification. This type of protein degradation is a quick and definitive way of (in)activating processes important for genome stability and maintenance [16]. In this complex, the F-box protein functions as a docking site for binding the protein requiring degradation [29, 30].

Of note, ubiquitination defects have previously been reported in ID [33, 34], and dysregulation of other SCF complex members, like Skp1 and Cul1, have also been associated with neurological disorders [35]. Moreover, pathogenic germline variants in other F-box protein family members have been linked to neurodevelopmental and neurodegenerative disorders [36–38].

FBXO11 is expressed in the (fetal) brain (https://gtexporta l.org/home/, accessed date 19 January 2018; https://www. proteomicsdb.org/proteomicsdb/#human/proteinDetails/ Q86XK2/expression, accessed date 19 January). *Fbxo11*

	Number	Percentage
Gender		
Male	18/24	75%
Neurological		
DD/ID	24/24	100%
Behavioral problems	16/24	67%
Hypotonia	12/21	57%
Facial		
High broad forehead	6/17	35%
Long palpebral fissures	7/20	35%
Thin upper lip with broad space between the paramedian peeks	15/19	79%
Skeletal		
Mild hand abnormalities	12/20	60%
Joint hyperlaxity	8/18	44%
Other		
Gastrointestinal problems	11/21	52%
Vision problems	9/21	43%

 Table 1
 Summary of clinical features of individuals with a variant in FBX011

homozygous mutant mice were born with a cleft palate and died during the neonatal period [39, 40], whereas heterozygote mice (Jeff mouse) did not show a cleft palate, but did suffer from otitis media [40]. However, although one individual had a submucosal cleft palate, we did not see a relation between the phenotype in mice and the individuals with a de novo variant in *FBXO11*.

The occurrence of two deletions disrupting the coding sequence of FBX011 suggests that haploinsufficiency is the molecular pathophysiological mechanism. This might also be the case for at least eight of 19 de novo disease-causing variants, including six frameshift variants and two nonsense variants. Whereas two frameshift variants are in the penultimate exon, they occur beyond the 50-55 nucleotides from the last exon-exon junction, thereby still predicted to be degraded by nonsense-mediated decay (NMD). However, two nonsense variants and one frameshift variant are in the ultimate exon, escaping NMD. This might result in a stable, truncated protein with an altered function or an unstable protein with shortened protein half-life. Either way, these variants all lead to a shorter UBR-type zincfinger domain by premature protein termination of FBX011. Since the UBR-type zinc-finger domain has an important function in binding proteins with a N-terminal residue for ubiquitination [41], shortening of this domain results in functional loss of the SCF complex, similar to direct loss-of-function variants.

The nine missense variants located in the CASH domain affect amino acids strongly conserved in orthologs and it could therefore be speculated that these variants severely compromise normal protein function. Interestingly, we observed two recurrent identical de novo variants: c.2145G>C; p.(Lys715Asn) in Individuals 8 and 17, and c.2738_2739del; p.(Tyr913*) in Individual 9 and the Individual in the study of Martínez et al. [20]. At the moment, a clear genotype–phenotype correlation could not be established.

Population constraint metrics indicative for variation tolerance of *FBXO11* as reported in, for instance, ExAC show that *FBXO11* is extremely intolerant for loss-of-function variation (pLi = 1.00) and that there is an intolerance for missense variants (*z*-score = 4.03) [42]. Also, its residual variation intolerance score of -0.69 indicates that *FBXO11* belongs to the 15% most intolerant human protein-coding genes [43] and we found more de novo disease-causing variants in *FBXO11* than expected based on its gene-specific mutation rate [21, 27]. These findings, and the absence of de novo disease-causing variants in *FBXO11* in over 2000 controls [5, 44], corroborate the pathogenicity of these variants.

Whereas these population metrics point towards the importance of de novo disease-causing variants in FBX011 in ID, genotype-phenotype correlation at the individual level may be more complex. That is, 2 of 22 de novo disease-causing variants occurred as a postzygotic event and 5 of 22 individuals carried more than one de novo proteincoding disease-causing variants. Whereas postzygotic disease-causing variants are increasingly recognized as cause for early-onset genetic diseases [45–47], it is currently not clear to what extent the frameshift variant of Individual 10, which occurred in 35% of lymphocytes, and the missense variant in Individual 20, which occurred in 20-40% of lymphocytes, contribute to the phenotype. Unfortunately, other tissues of these individuals were not available for testing. Notably, Individual 10 also has a de novo missense variant in FGR, which is in a conserved amino acid (NM 001042747.1:c.1261C>T; p.(Pro421Ser)). The function of FGR and its relation to human disease has thus far not been reported, but a role for this variant in the individual's phenotype cannot be formally excluded. Individual 20 also had a de novo in-frame deletion of five amino acids (NM_001258282.1:c.1543_1557del; in LINGO2 (p. (Pro515_Thr519del)). LINGO2 deletions have previously been reported in individuals with developmental delay, autistic behavior, and craniofacial abnormalities [48, 49], and a contribution of this variant to the individual's phenotype cannot be excluded.

In Individual 7, the de novo 170-kb deletion not only included *FBXO11*, but also *MSH6*. Whereas heterozygous *MSH6* whole gene deletions have not yet been reported, single-nucleotide variants in *MSH6*, including loss-of-function variants, are a known cause of Lynch syndrome (hereditary non-polyposis colon cancer) and result in an

increased risk of colon cancer and other types of tumors [50–53]. The parents of the individual have been informed about Lynch syndrome as well as the related screening programs.

Given the per generation de novo mutation rate and the size of the exome [54], it is not uncommon that individuals have more than one protein-coding de novo variant. Most of these additional de novo variants are in genes that are unlikely to be related to ID, whereas others are in genes related to autosomal recessive disease, but for which no second mutated allele was present (Supplemental Case Reports). However, dominant disease-causing variants in *ANK3*, *LINGO2*, *NF1*, and *CIC* have previously been described in individuals with DD/ID and might have an additional effect on the phenotype [48, 49, 55–58]. Future (functional) research will resolve the role of each individual variant and further examine the role of *FBXO11* in ID.

The individuals reported here with a de novo diseasecausing variant in FBXO11 share their developmental and behavioral problems, although variable, but differ in other areas like gastrointestinal or vision problems. Although we observe recurrent facial dysmorphisms, a typical facial gestalt could not be established. FBX011-related ID syndrome shows variability and is not highly recognizable. However, variability is seen frequently in many, also established, ID syndromes since we moved from phenotype-first to genotype-first approach. For instance, a broad phenotypic spectrum is also increasingly observed for syndromes such as ARID1B-related ID and ARID1B-related Coffin–Siris syndrome [59]. Future clinical research is needed and might reveal a genotype-phenotype relation, which could not be established now. We therefore founded the website http://www.humandiseasegenes.com/fbxo11 to collect additional clinical data.

In conclusion, we presented 24 cases that either had de novo disease-causing variants in *FBXO11* or de novo disruptive deletions of *FBXO11*. All individuals showed ID with variable severity, behavioral problems, and dysmorphisms. While initial identification of these variants did not lead to immediate conclusive diagnoses, our reverse phenotyping strategy has been successful in determining pathogenicity of *FBXO11* genomic variation and identifying a novel ID syndrome with a broad clinical spectrum.

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Compliance with ethical standards

Conflict of interest M.J.G.S., K.G.M., and R.E.S. are employees of GeneDx Inc. H.R. is an employee of Impact Genetics Inc. The other authors declare that they have no conflict of interest.

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