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FBXO28 causes developmental and epileptic encephalopathy with profound intellectual disability

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Summary

Chromosome 1q41-q42 deletion syndrome is a rare cause of intellectual disability, seizures, dysmorphology and multiple anomalies. Two genes in the 1q41-q42 microdeletion, *WDR26* and *FBXO28*, have been implicated in monogenic disease. Patients with *WDR26*-encephalopathy overlap clinically with those with 1q41-q42 deletion syndrome, whilst only one patient with *FBXO28*-encephalopathy has been described. Seizures are a prominent feature of 1q41-q42 deletion syndrome, therefore we hypothesised that pathogenic *FBXO28* variants cause developmental and epileptic encephalopathies (DEEs). We describe nine new patients with *FBXO28* pathogenic variants (4 missense, including 1 recurrent, 3 nonsense and 1 frameshift) and analyse all 10 known cases to delineate the phenotypic spectrum. All patients had epilepsy and 9/10 had DEE, including infantile spasms (3) and a progressive myoclonic epilepsy (1). Median age at seizure onset was 22.5 months (range 8 months–5 years). 9/10 patients had intellectual disability, which was profound in 6/9 and severe in 3/9. Movement disorders occurred in 8/10 patients, 6/10 had hypotonia, 4/10 had acquired microcephaly and 5/10 had dysmorphic features, albeit different to those typically seen in 1q41-q42 deletion syndrome and *WDR26*-encephalopathy. We distinguish *FBXO28*-encephalopathy from both of these disorders with more severe intellectual impairment, drug-resistant epilepsy and hyperkinetic movement disorders.

Key words: *FBXO28*, developmental and epileptic encephalopathy, profound intellectual disability, movement disorder

Introduction

Copy number variants have been the key to gene identification in many human genetic diseases. Copy number variants, such as microdeletions or microduplications, may span from no genes at all to hundreds of genes. As microdeletion syndromes have complex clinical features and the causative microdeletions often contain many genes, it can be challenging to disentangle which features arise due to which gene defects.

Chromosome 1q41-q42 deletion syndrome (OMIM 612530) includes developmental delay, seizures and dysmorphic features[1-3]. Two of the six genes in the common microdeletion, *WDR26* and *FBXO28*, have been implicated in causing monogenic disease[4,5]. There is significant overlap in the clinical features of patients with pathogenic sequence variants in *WDR26* and those of patients with 1q41-q42 deletion syndrome; therefore, *WDR26* has been postulated as the main genetic driver of the 1q41-q42 deletion phenotype[5]. Only one patient with a *de novo FBXO28* pathogenic variant has been described with profound intellectual disability, intractable epilepsy and dysmorphic features[4]. When considering patients with *de novo* 1q41-q42 deletions, Balak and colleagues found that 30/37 patients had deletions spanning *FBXO28*[4]. Of the patients older than two years, 76% (16/21) had seizures, compared to 25% of patients with deletions that did not encompass *FBXO28*[4]. Thus, *FBXO28* may be an important contributor to the 1q41-q42 deletion syndrome phenotype.

Developmental and epileptic encephalopathies (DEEs) are characterised by frequent epileptiform activity that contributes to developmental regression or plateauing[6]. Onset of seizures is usually in infancy or childhood and patients show developmental slowing when the epileptiform activity or seizures are frequent. DEEs are genetically heterogeneous with many genes and mutation

types implicated, from single nucleotide variants to larger copy number variants[7,8].

Given that seizures are a prominent feature of 1q41-q42 deletion syndrome and the reported patient with a *de novo FBXO28* variant has epilepsy, we hypothesised that pathogenic *FBXO28* variants may cause DEEs. Here, we characterise nine new patients with *FBXO28* pathogenic variants and analyse all 10 known cases to delineate the phenotypic spectrum.

Methods

Patients with pathogenic and likely pathogenic variants in *FBXO28* were identified through international collaboration with research and diagnostic genetic testing laboratories[9]. Our patients were identified via different cohorts; 1/41 patients with moderate to severe intellectual disability (patient 4)[10], 1/111 patients with neurodevelopmental disoders (patient 6), 1/250 patients with DEEs or neurodevelopmental disoders (patient 7) and 1/579 patients with DEEs (patient 10)[7]. Patients 6 and 10 were tested using custom gene panels[7], whilst the remaining seven patients underwent exome sequencing of either the proband or proband-parent trio[10]. Parental mosaicism was identified using single molecule molecular inversion probes in patient 10 and exome sequencing in patient 5.

The study was approved by the Austin Human Research Ethics Committee and institutional review boards at each participating site. Parents or legal guardians provided written informed consent for all participants.

Medical history information was gathered for each patient from treating paediatric neurologists and paediatricians, medical record data together with parental interview, and included developmental and seizure data, results of brain imaging and EEG investigations. Intellectual disability was classified as

profound in patients who were non-verbal, non-ambulatory and fully dependent and severe in patients who could walk or communicate with non-verbal signs or follow simple commands. Our phenotypic analysis incorporated additional information on the published patient (patient 3)[4]. ILAE classification criteria was used to categorise seizure types and epilepsy syndromes[6].

Results

We identified nine patients (four male) with pathogenic or likely pathogenic *FBXO28* variants (NM_015176), including four missense, three nonsense and one frameshift variants (Figure 1A, Table S1). In terms of inheritance, 7/9 variants arose *de novo* and 2/9 were inherited from a parent who was mosaic for the variant in blood-derived DNA; p.Lys360X from a mother with 6% mosaicism and p.Arg348Leu from a father with 2.5% mosaicism. Both parents were clinically unaffected.

Of the four missense variants, two were located within the F-box domain (Figure 1A): p.Leu64Arg had pathogenic *in silico* prediction scores, and p.Ala66Pro had mixed *in silico* prediction scores, both were absent in gnomAD[11] and arose *de novo*. We identified a recurrent missense variant in two unrelated patients (p.Arg348Leu), which also involved the same amino acid position as patient 4 (p.Arg348Gly). The variants are located outside the F-box domain and had mixed *in silico* prediction scores but were absent from gnomAD[11].

No other likely pathogenic variants were identified in the 7 patients who had exome sequencing, apart from patient 2 who also had a rare *de novo ADGRV1* missense variant (p.Gly4171Cys). Likely pathogenic missense variants in *ADGRV1* may contribute to myoclonic epilepsy with a complex inheritance pattern [Myers et al. 2018], however, *ADGRV1* is tolerant to missense variation

with an observed/expected ratio of 1 (0.97-1.03) and Z-score of 0.07 in gnomAD[11].

The cohort of 10 patients had a median age of 9.5 years (range 11 months to 25 years), including patients 8 and 5 who died at 15 years due to pneumonia and 25 years due to progressive neurological disease, respectively. Of the 9/10 (90%) patients who had a DEE; three had infantile spasms, patient 2 had a progressive myoclonic epilepsy (Video S1), and the remaining four cases did not have a classifiable epilepsy syndrome. Patient 6 had intellectual disability and epilepsy. Seizure onset occurred at median age 22.5 months (range 8 months to 5 years). The three patients with infantile spasms had onset at 8-9 months.

All patients had multiple seizure types including; myoclonic (5), tonic (4), focal (4), tonic-clonic (3), febrile seizures (2), startle (1), focal to bilateral tonic-clonic (1), absence (1) and status epilepticus (1). All had developmental delay prior to seizure onset, and 6/10 showed developmental regression. 6/10 patients had profound intellectual disability, 3/10 severe intellectual disability, whilst patient 2 with progressive myoclonic epilepsy was not testable due to her myoclonic activity, speech decline and ataxia.

Movement disorders occurred in 8/10 patients including; myoclonus (4), hyperkinetic movements (2), choreoathetosis (2), stereotypies (2), ataxia (2), dyskinesia (1) and dystonia (2) (Video S1 and Video S2). Six patients had hypotonia, three contractures, one scoliosis, one kyphosis and one kyphoscoliosis. Four patients had drooling, three gastroesophageal reflux disease, two dysphagia and six patients had gastrostomies. Four patients had recurrent apnoea, 2/4 required oxygen. 4/10 patients had acquired microcephaly, with borderline microcephaly in an additional two patients. 5/10 patients had dysmorphic features (Figure 1B, Table 1, Table S1)[4].

EEG was abnormal in 9/10 patients with available data: multifocal epileptiform discharges (5), background slowing (5), hypsarrhythmia (2) or modified hypsarrhythmia (1), continuous spike-wave in sleep (2), generalised paroxysmal fast activity (1), and photo-paroxysmal response (1). Brain MRI was reported as abnormal in 9/10 patients showing prominent findings of atrophy (6) and delayed/abnormal myelination (2). Brain malformations included a simplified gyral pattern (2), pachygyria (1), cortical dysplasia (1) and polymicrogyria (1, Figure 1C).

Discussion

FBXO28-encephalopathy typically presents with a DEE associated with profound developmental impairment, hyperkinetic movement disorders, microcephaly, hypotonia, contractures, and kyphoscoliosis. Our cohort considerably expands the phenotypic spectrum of *FBXO28*-encephalopathy and distinguishes it from the 1q41-q42 deletion syndrome[1-3,12-15] and *WDR26*-encephalopathy[5] (Table 1). In particular, our cohort did not share the distinctive congenital anomalies seen in both of these disorders, such as cardiac, skeletal and genito-urinary anomalies. The one previously reported patient with *FBXO28*-encephalopathy had dysmorphic features involving facies and limbs, without multisystem involvement. The characteristic multisystem features of patients with 1q41-q42 deletion syndrome are more frequently seen with *WDR26* single nucleotide pathogenic variants than with *FBXO28* variants[5].

FBXO28-encephalopathy causes a more severe epilepsy phenotype than that seen in 1q41-q42 deletion syndrome and *WDR26*-encephalopathy. 9/10 patients with *FBXO28*-encephalopathy had DEEs, including infantile spasms and one case with a progressive myoclonus epilepsy, reinforcing the overlap between genes causing DEEs and early onset progressive myoclonus epilepsies[16]. All 15 reported patients with *WDR26* sequence variants had

seizures yet, in contrast, only 8/15 had severe enough epilepsy to require anti-epileptic drugs (AEDs), and all but two were well-controlled on a single AED[5]. 18/26 patients with 1q41-q42 deletion syndrome had seizures: two had only 1-2 febrile seizures[15] and of just four patients who took AEDs, three were well-controlled on 1-2 drugs[1,2]. However, ascertainment bias could be responsible for the severe phenotype we are describing, given our interest in the DEEs and that the full phenoptyic spectrum of *FBXO28*-encephalopathy is yet to be realised.

The degree of intellectual impairment also distinguishes *FBX028*-encephalopathy from these other diseases. We found that 9/10 patients had profound (6) or severe (3) intellectual impairment, whilst no patients with *WDR26*-encephalopathy had profound intellectual impairment, 9/15 had severe and 5/15 moderate intellectual impairment[5]. Only 10/26 patients with 1q41-q42 deletion syndrome had enough clinical information provided to classify their degree of intellectual impairment; 6/10 had severe intellectual impairment and 1/10 moderate-severe, whilst 3/10 had mild to moderate intellectual impairment[1,2,15].

A genotype-phenotype correlation in patients with *FBXO28*-encephalopathy emerged with truncating variants causing a more severe phenotype than missense variants (Table S2). The three patients with infantile spasms had a strikingly similar phenotype with onset at 8-9 months, profound intellectual disability and movement disorders; their nonsense mutations occurred within 5 amino acids of each other. The two patients with frameshift variants also had DEEs with profound impairment and movement disorders with later seizure onset at 21 months and 2 years. Conversely, the four patients with missense variants showed a milder phenotype; three had later seizure onset at 4-5 years with tonic-clonic seizures, which were not seen in patients with truncating variants. One had severe intellectual disability and epilepsy, without an epileptic encephalopathy.

All five *FBXO28* truncating variants and 2/4 missense variants, found in 8 patients, are located close to each other (positions 325-360) in the last exon of the protein, which finishes at amino acid 368 (Figure 1A). While 1q41-q42 deletion syndrome and *WDR26*-encephalopathy are postulated to be haploinsufficiency syndromes[1,5], it is possible that *FBXO28*-encephalopathy is due to a different mechanism. *FBXO28* encodes F-box only protein 28, a ubiquitin ligase that promotes ubiquitination and degradation of phosphorylated proteins. These terminal pathogenic variants could be less susceptible to nonsense-mediated decay, as recently demonstrated in progressive myoclonic epilepsy due to truncating variants in the last exon of *SEMA6B*.[17] Thus, a similar mechanism could be invoked in *FBXO28*-encephalopathy as all but two of the pathogenic variants are located in the final exon. Interestingly, the remaining two missense variants are just two base pairs apart, clustering in the F-box domain, implicating a second pathogenic region.

We report a cohort of patients with *FBXO28*-encephalopathy with a severe DEE in most, characterised by profound impairment, drug-resistant epilepsy, movement disorders and many concomitant comorbidities. *FBXO28*-encephalopathy is distinguished by a more severe phenotype than typically seen in the haploinsufficiency syndromes, 1q41-q42 deletion syndrome and *WDR26*-encephalopathy[1,5]. Truncating *FBXO28* variants were associated with a more severe phenotype than missense variants. The localisation of 8/10 pathogenic variants to the last exon raises the tantalising question of an alternative mechanism to haploinsufficiency.

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Disclosure of conflicts of interest

I.E.S serves/has served on the editorial boards of the Annals of Neurology, Neurology, Epileptic Disorders and Epilepsy Currents; may accrue future revenue on pending patent WO2009/086591: Diagnostic And Therapeutic Methods For EFMR (Epilepsy And Mental Retardation Limited To Females); has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies (WO/2006/133508); she has a patent for a molecular diagnostic/therapeutic target for benign familial infantile epilepsy (BFIE) [PRRT2] WO/2013/059884 with royalties paid. She has served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia, Rogcon and Xenon Pharmaceuticals; has received speaker honoraria from GlaxoSmithKline, UCB, BioMarin, Biocodex and Eisai; has received funding for travel from UCB, Biocodex, GlaxoSmithKline, Biomarin and Eisai; has served as an investigator for Zogenix, Zynerba, Ultragenyx, GW Pharma, UCB, Eisai, Anavex Life Sciences and Marinus; and has consulted for Zynerba Pharmaceuticals, Atheneum Partners, Ovid Therapeutics and UCB. MSP serves on the editorial board of Epilepsy Currents; has served on advisory boards or as a consultant for Encoded Therapeutics, Stoke Therapeutics, Biocodex, Greenwich Biosciences, and Zogenix. He has received speaker honoraria from

Biocodex and served as an investigator for Zogenix, Greenwich Biosciences, Stoke Therapeutics, Ovid, and Marinus. The remaining authors have no conflicts of interest to disclose.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Table 1: Phenotypic features of patients with pathogenic sequence variants in *FBXO28* or *WDR26* and 1q41-q42 deletions

	FBXO28 (n=10)		*WDR26* (n=15)		1q41-q42 deletion syndrome (n=26)	
Gender	6 female : 4 male		10 female : 5 male		10 female : 16 male	
Median age (range)	9.5 years (11 months – 25 years)		6.5 years (24 months – 34 years)		5 years (10 months – 17 years)	
Deceased (age at death)	2 (15 and 25 years)		Nil		1 (1 hour)	
Seizures	10/10		15/15		18/26	
Median age at seizure onset (range)	22.5 months (8 months – 5 years)		24 months (newborn – 7 years)		14.5 months (8-21 months)	
Seizure types	Myoclonic (5); Tonic (4); Focal (4); Infantile spasms (3); Tonic-clonic (3); Startle (2); FBTCS (1); Absence (1); Febrile (1); Status epilepticus (1)		Febrile (9); Absence (2); Tonic-clonic (1); Focal rolandic (1)		Febrile (4); Tonic-clonic (3); Generalised (2); Absence (1); Hypoglycaemic (1)	
Epilepsy outcome	10/10 on AEDs with ongoing seizures		8/15 on AEDs; 6/8 well-controlled on 1 AED		Well-controlled on 1-2 AEDs (3); 1-2 febrile seizures only (2); drug-resistant (1)	
Intellectual disability	9/10	Severe (3); Profound (6); Not testable (1)	15/15	Moderate (5); Severe (9); Delayed (1)	25/25	Severe (6); Moderate-severe (1); Mild-moderate (2); Mild (1)
Regression	6/10		Nil		Nil	
Hypotonia	6/10		9/12		10/25	
Movement disorder/ stereotypies/autistic/ repetitive behaviours	8/10	Myoclonus (4); Hyperkinetic movements (2); Choreoathetosis (2); Stereotypies (2); Ataxia (2); Dystonia (2); Dyskinesia (1)	6/9	Rocking (2); Hand flapping (1); Repetitive hand movements (4); Tics (1)	2/25	Autistic features (1); Repetitive behaviours and ASD (1); Ataxia (1)
Dysmorphic features	5/10	High palate (3); Short palpebral fissures (3); Hypertelorism (2); Broad and flat/depressed nasal bridge/root (2); Full lips (2); Gingival hyperplasia (2); Micrognathia (2); Opaque, sparse, and brittle hair (1); Lateral flaring of eyebrows (1); Low set ears (1); Large and mildly dysmorphic ears (1); Prominent cheeks (1); Toenail and fingernail hypoplasia (1); Additional features in patient 3 as previously described[4]	13/15	Prominent maxilla and protruding upper lip (13); Widely spaced teeth (13); Coarse facial features (12); Full nasal tip (11); Flattened/decreased cupid's bow (11); Full cheeks as a child (11); Large irises with rounded palpebral fissures (10); Wide mouth (10); Abnormal gums (9); Anteverted nares (8); Sparse lateral eyebrows (6); Depressed nasal root (5); Micrognathia (5); Cleft palate (1)	25/26	Coarse facial features (14); Full +/- thick lips (11); Cleft palate (11); Depressed or flat nasal bridge or base (11); Hypertelorism (10); Prominent brow/frontal bossing (8); Broad/wide nasal bridge/root (7); Broad nasal tip/bulbous nose (7); Congenital diaphragmatic hernia (7); Nail hypoplasia (7); Upslanting palpebral fissures (6); Anteverted nares (5); Wide/broad mouth (5); Narrow face (4); Low set ears (4); Gingival hyperplasia (4); Dolicocephaly (3); Deep-set eyes (3); Epicanthal folds (3); Short philtrum (3); High palate (3); Widely spaced teeth (3); Short neck (3); Micrognathia (2); Prominent mandible/maxilla (2)*
Microcephaly	4/10		1/15		9/26	

Skeletal abnormalities	3/10	Scoliosis (1); Kyphosis (1); Kyphoscoliosis (1); Long digits (1)	10/15	Clinodactyly (4); Short stature (3); Pectus excavatum/carinatum (2); Pes cavus (2); Short fingers (2); Osteopathia striata of distal femurs (1); Moderate forefoot varus (1); Mild left hip dysplasia (1); Scoliosis (1); Pes planus (1); Wideley spaced 1st and 2nd toes (1)	14/26	Club foot (7); Short stature (6); Clinodactyly (5); Wideley spaced 1st and 2nd toes (5); Scoliosis (1); Pes planovalgus (1)
Contractures	3/10		2/15		3/25	
Cardiac abnormalities	Nil		2/15		10/26	
Genitourinary abnormalities	Nil		1/15	Cryptorchidism and inguinal hernia (1)	7/26	
Ophthalmologic abnormalities	2/10	Strabismus (1); Nystagmus (1)	9/14	Strabismus +/- amblyopia (9); Marcus Gunn jaw winking (1)	9/25	
Drooling/dysphagia	4/10		2/15		1/25	
Feeding issues	5/10	G-tube fed (5); GORD (3); PEG fed (1)	7/15	GORD (5); G-tube fed (3); Failure to thrive (3)	7/25	GORD (2); G-tube fed (2); Failure to thrive (2)
Apnoea	4/10		Nil		2/25	
Brain abnormalities	9/10		10/14		15/25	

Abbreviations: AED, anti-epileptic drug; FBTCS, focal to bilateral tonic-clonic seizure; GI, gastrointestinal; GORD, gastro-oesophageal reflux disorder; G-tube, gastrostomy tube; PEG, percutaneous endoscopic gastrostomy. *Features reported if present in at least 2 published patients.

Figure 1. A: Distribution of pathogenic variants in *FBXO28* (NM_015176) and corresponding patient numbers (ptX) with the recurrently mutated amino acid in red and gene domains in green. **B:** Photos of patients with pathogenic *FBXO28* variants; (a-b) patient 1 aged 5 years; (c) patient 3 aged 20 months[4]; (d) patient 2 aged 6 years; (e-f) patient 8 aged 10 years; (g) patient 10 aged 20 months; (h-j) patient 6. **C:** T1 weighted axial and sagittal images of patient 1 aged 15 months.

Supplementary files

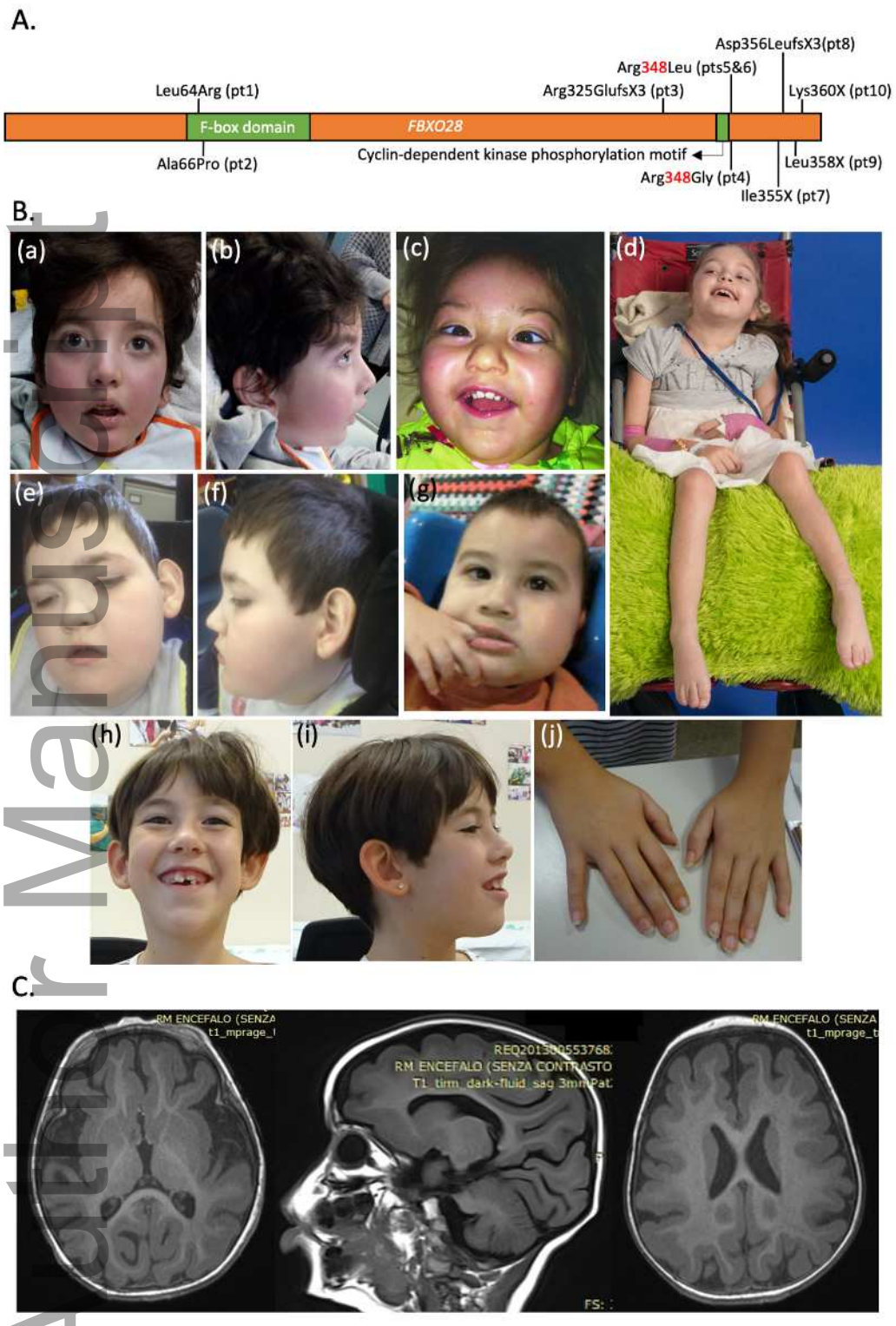
Table S1: Phenotypic features of patients with pathogenic variants in *FBXO28*

Table S2: Comparison of phenotypic features of patients with truncating and missense *FBXO28* variants

Video S1: Video of patient 2 aged 6 years with progressive myoclonus epilepsy and *FBXO28* p.Ala66Pro

Video S2: Videos of patient 10 with infantile spasms and *FBXO28* p.Lys360X demonstrating tongue protrusion at age 6 months and 9 years

Short summary



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