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De novo missense variants in *HECW2* are associated with neurodevelopmental delay and hypotonia

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Abstract

Background—The causes of intellectual disability (ID) are diverse and de novo mutations are increasingly recognised to account for a significant proportion of ID.

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Contributors Study concept and design: ERB and WKC. Recruitment of patients and collection of clinical information: MTC, CE, YS, DAS, JW, NHR, FB, SD, PM, CGB, MB, CM, LAW and CJT. Acquisition of data: ERB, MTC, CE, YS, DAS, JW, NHR, FB, SD, PM, CGB, MB, CM, LAW, CJT and WKC. Analysis and interpretation of data: ERB, MTC, YS, KR, FM and WKC. Drafting of the manuscript: ERB. Critical revision of the manuscript: ERB, MTC, CE, YS, DAS, JW, NHR, FB, SD, PM, CGB, MB, CM, LAW, CJT, YS, KR, FM and WKC. Study supervision: WKC.

Competing interests MTC, KR and FM are employees of GeneDx. WKC was previously an employee of GeneDx.

Patient consent Parental/guardian consent obtained.

Ethics approval Columbia University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The variants reported in this study have been deposited in ClinVar.

Methods and results—In this study, we performed whole exome sequencing on a large cohort of patients with ID or neurodevelopmental delay and identified four novel de novo predicted deleterious missense variants in *HECW2* in six probands with ID/developmental delay and hypotonia. Other common features include seizures, strabismus, nystagmus, cortical visual impairment and dysmorphic facial features. HECW2 is an ubiquitin ligase that stabilises p73, a crucial mediator of neurodevelopment and neurogenesis.

Conclusion—This study implicates pathogenic genetic variants in *HECW2* as potential causes of neurodevelopmental disorders in humans.

INTRODUCTION

Intellectual disability (ID) is a common and aetiologically heterogeneous disorder, affecting 1% of the general population.[1] A significant fraction of profound ID is caused by genomic alterations, with cytogenetically detectable anomalies found in up to 15% of cases and copy number variants in up to 15%–20% of individuals.[2,3] Recent progress in whole exome sequencing (WES) have rapidly advanced our identification of genetic causes of ID, establishing de novo mutations as an important and common cause of ID[4–6] in 16%–55% of cases.[7–10] Many novel candidates genes have been identified through WES and have advanced our understanding of potential molecular mechanisms implicated in neurodevelopmental processes.[11–13]

In this study, we identified novel de novo missense variants in *HECW2* in six independent probands of 3309 total patients with neurodevelopmental delays or ID referred for clinical WES. HECW2, also known as NEDL2, is one of the nine members of the Nedd4 family of HECT domain E3 ubiquitin ligases.[14] E3 ligases control specificity of the ubiquitin modification of proteins targeted for degradation. HECW2 acts on a diverse group of proteins including p73, a member of the p53 family with specific neurodevelopmental expression.[15] We identified multiple and recurrent de novo, novel variants in *HECW2* in six unrelated probands with neurodevelopmental disorders, supporting its role as a potential cause of developmental delay and ID.

MATERIALS AND METHODS

Informed consent was obtained from all participants' parents included in this study. This study was approved by the Institutional Review Board of Columbia University.

Whole exome sequencing

Genomic DNA was extracted from whole blood from affected children and their parents. Exome sequencing was performed on exon targets captured using the Agilent SureSelect Human All Exon (V.4) (50 Mb) kit or the Clinical Research Exome kit (Agilent Technologies, Santa Clara, California, USA). Whole exome sequencing data for all sequenced family members were analysed using GeneDx's XomeAnalyzer (a variant annotation, filtering and viewing interface for WES data), which includes nucleotide and amino acid annotations, population frequencies (NHLBI Exome Variant Server and 1000 Genomes databases), in silico prediction tools, amino acid conservation scores and mutation

references. Variants were filtered based on inheritance patterns, variant type, population frequencies and gene lists of interest in relation to the patient's major phenotypic features, as appropriate. The full sequencing methodology and variant interpretation protocol has been previously described.[11] The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/). As part of our analysis, individuals with de novo variants occurring in the same gene are examined for overlapping clinical features and were Sanger-sequenced to confirm that the variant is present in the proband and neither parent and is de novo.

RESULTS

Exome sequencing was performed in 3309 probands with neurodevelopmental disorders sequenced in a single clinical laboratory; most of the probands had previous non-diagnostic genetic testing including chromosome microarrays. WES of the six probands identified with a *HECW2* de novo variant produced an average of 11.5 GB of sequence per sample. Among the 3309 probands with neurodevelopmental disorders, there were six probands with four unique de novo variants in *HECW2*. All variants were confirmed with Sanger sequencing in the proband and the parents. The false discovery rate corrected q-value of the observing six de novo missense variants in this population is 6.20e–07, using the Transmission and De novo Association algorithm,[16] and the default parameters for missense variation in neurodevelopmental cases are γ=4.7 and β=1, with a missense mutation rate of 4.85e–05 for *HECW2*.[17]

All variants are novel and none have been described in the Exome Aggregation Consortium,[18] although the Exome Variant Server reports a synonymous variant at codon 1193.[19] The variants were also absent in our own internal database consisting of 9651 adult unaffected controls. Residual Variation Intolerance score for this gene is −2.47 (0.98%), indicating that the gene is intolerant to variation,[20] with a misZ of 3.11 (3-sigma conservation) and the pLI or probability of being intolerant to a loss-of-function allele is 1.[21] Eight deletions that encompass *HECW2* are reported in ClinVar, with six of those individuals demonstrating a phenotype of developmental delay and/or significant developmental morphological phenotypes. The four de novo missense variants are all predicted to be deleterious and damaging to protein function by Polyphen, Provean, SIFT and other prediction algorithms[22–24] (table 1). One of the missense variants, R1330W, lies within the HECT domain of the protein (figure 1).

Age of patients with de novo *HECW2* missense variants ranges from 18 months to 11 years (table 2). All patients have developmental delay and hypotonia and are all severely neurologically impaired. Common clinical features include seizures (five probands), cortical visual impairment (three probands), autism (two probands), dysmorphic features (five probands) (figure 2) and problems with feeding requiring gastrostomy tube (three probands).

The two unrelated probands (patients 1 and 2) with the same de novo p.Arg1191Gln variant have autism and similar dysmorphic features with midface retrusion. Patient 2 had an abnormal EEG as an infant, with excessive slowing throughout and abnormal burst discharges. Patient 1 had absence and generalised tonic clonic seizures starting at age 4

years with an abnormal EEG (slow for age showing runs of rhythmic theta activity with intermixed spikes). She also has absent speech, a broad-based ataxic gait, extremely high pain tolerance, aggressive and self-injurious behaviour and strabismus. She was clinically felt to have a phenotype similar to Angelman syndrome and had negative Angelman molecular testing.

The p.Phe1193Val variant was identified once in a set of monozygotic twins (patients 3 and 4) with generalised hypotonia with severe kyphosis, strabismus, nystagmus and mildly dysmorphic features (highly arched palate, prominent forehead and deep set eyes). MRI showed non-specific cortical atrophy, with resultant ex-vacuo dilatation of ventricles and increased extra-axial fluid. One twin (patient 3) had intrauterine growth restriction and neonatal hypoglycaemia and the other twin (patient 4) had hypothermia after birth. Both twins were able to be breast-fed for 7 months but had feeding regression which led to all feeds being given through gastrostomy tube. At approximately 30 months of age, they both began having intractable tonic clonic seizures.

Two unrelated patients (patients 5 and 6) carry the same p. Arg1330Trp variant. Both have cortical visual impairment. Patient 5 has abnormal behaviours with repetitive movements and self-injurious behaviours. Patient 6 had a history of infantile spasms and now at 3 years of age has intractable tonic seizures as well as feeding difficulties requiring a gastrostomy tube. MRI demonstrated loss of cerebral hemispheric white matter under-operculisation and thin corpus callosum. Patient 6 also had hyperglutaminaemia and on chromosome microarray analysis had a 439 Mb 15q11.2 duplication that contains four genes (arr 15q11.2(22,770,421–23,209,654)x3) and a de novo p.G184E *SLC7A7* variant. *SLC17A7* encodes a vesicular glutamate transporter expressed in the brain that plays a role in synaptic transmission. To our knowledge, no mutations in *SLC17A7* have been associated with a specific human disease. This variant was not observed in approximately 115 000 alleles in the ExAC dataset. We cannot exclude the possibility that this variant contributes to the phenotype in this patient.

The p.Glu1445Gly variant was also identified once in patient 7 with hypotonia, epileptic encephalopathy and cortical visual impairment. This patient also has choreiform movements. MRI showed generalised volume loss with non-specific white matter changes. The patient has gastroparesis and constipation and has a gastrostomy tube and also has cardiomyopathy. A heterozygous variant in the *EIF2B2* gene was also found in this patient. Typically, *EIF2B2*-related disorders are inherited in an autosomal recessive manner and this patient was not found to have a second variant in the gene.

DISCUSSION

In our series of 3309 patients with neurodevelopmental delays, we identified six probands, each with one of four de novo, novel missense variants in *HECW2* in highly conserved regions of the gene. Two of the variants are recurrent. The clinical characteristics shared by all the patients include severe developmental delay and hypotonia. Seizures, cortical visual impairment, autism, stereotypical and repetitive behaviours and difficulty with feeding are common associated phenotypes observed in the majority of the patients. MRIs in some

patients are largely normal and showed only non-specific cortical atrophy with ex-vacuo ventriculomegaly.

HECW2 is an E3 ubiquitin ligase and contains a N-terminal C2 domain, two internal WW domains that bind to PY motifs of substrate proteins and a C-terminal HECT domain (figure 1). *HECW2* is expressed largely in brain, lung and heart tissue.[25]

HECW2 is extremely intolerant of haploinsufficiency and the patients with microdeletions that encompass *HECW2* have overlapping phenotypes, suggesting that the missense variants have a functional consequence. However, functional studies will be necessary to assess the missense alleles for loss or gain of function or dominant negative mechanism.

The amino acids identified in this study in individuals with neurodevelopmental deficits are highly conserved, with alterations predicted to be extremely deleterious (figure 1). The protein prediction algorithms consistently predict that these missense variants are harmful to protein structure and function.

The main known function of HECW2 is to stabilise and enhance transcriptional activity of p73.[25] p73 is a member of the p53 family of tumour suppressors that promotes cell cycle arrest and apoptosis and also has an essential role in neurodevelopment.[15] Loss of HECW2 activity could lead to decreased p73 activity.

Mouse knockout models of p73 show profound central nervous system abnormalities with hippocampal dysgenesis, cortical thinning, progressive communicating hydrocephalus and small olfactory bulbs and subventricular zones.[2627] The brain pathology is progressive, with worsening cortical loss as the mice age.[28] p73 is specifically involved in embryonic neurogenesis and maintenance of adult neural stem cells (NSCs). Neurospheres derived from p73−/− cells are smaller than wild type, demonstrating decreased proliferation and survival of neural progenitors and have decreased capacity for self-renewal.[29] p73-deficient animals are born with decreased pool of neural progenitors and continue to display defects in adult neurogenesis and long-term maintenance of NSCs.[273031] Additionally, neurons and oligodendrocytes that differentiate from p73−/− progenitors are abnormal, showing deficiencies in both quality and quantity. Neurons have defects in neurite outgrowth and synaptic connectivity.[2732]

p73 is an essential regulator of embryonic neurogenesis, neuronal differentiation and organisation and maintenance of adult NSCs.[33] HECW2 stabilises and enhances p73 transcriptional activity and aberrant p73 activity could at least partially explain the severe neurological phenotype we observed.

HECW2 also interacts with other regulators of cell growth and differentiation. HECW2 has recently been shown to co-precipitate with the APC/C-Cdh1 complex that mediates the transition from metaphase to anaphase. Overexpression or underexpression of *HECW2* leads to early or delayed activation of the complex, respectively, and resultant cell cycle instability.[34] In addition to its role in cell cycle regulation, the APC/C-Cdh1 complex is highly expressed in brain, specifically in post-mitotic neurons.[35] APC/C-Cdh1 is involved in neuronal patterning and regulating axon growth in the developing and mature brain. In vivo

knockdown of *Cdh1* in developing animal models caused aberrant axonal growth trajectories and fibre defasciculation.[36] Further work is necessary to determine if this functional role of the APC/CDH1 complex requires HECW2 interaction and regulation.

SUMMARY

Four novel de novo predicted deleterious missense variants in *HECW2* were identified in six probands with severe developmental delay, hypotonia and dysmorphic features and was frequently associated with seizures, cortical visual impairment, autism, stereotypical and repetitive behaviours and difficulty with feeding. Two of the missense variants were recurrent and *HECW2* is intolerant of haploinsufficiency. The phenotype of these patients overlaps with patients with contiguous gene deletions of *HECW2* and additional functional data are necessary to evaluate the E3 ubiquitin ligase activity of these alleles and their effects on downstream proteins such as p73 and APC/C-Cdh1 in neurogenesis and neuronal differentiation.

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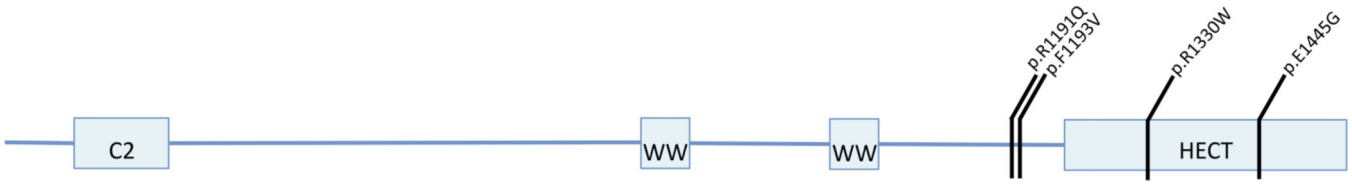
A



B

HUMAN	1158	PSYCQSPRGSPVSSPQNSPGTQRANARAPAPYKRDFEAKLRNFYRKLETKGYGQGPGKLKLIIRRDHLLEDAFNQIMGYS	1237
CHIMPANZEE	1158	PSYCQSPRGSPVSSPQNSPGTQRANARAPAPYKRDFEAKLRNFYRKLETKGYGQGPGKLKLIIRRDHLLEDAFNQIMGYS	1237
COW	929	PSYCQSPRGSPVSSPQNSPGTQRANARAPAPYKRDFEAKLRNFYRKLETKGYGQGPGKLKLIIRRDHLLEDAFNQIMGYS	1008
MOUSE	1164	PSYCQSPRGSPVSSPQNSPGTQRANARAPAPYKRDFEAKLRNFYRKLETKGYGQGPGKLKLIIRRDHLLEDAFNQIMGYS	1243
ZEBRAFISH	1058	PSYCQSPRGSPVSSPQNSPGTQRANARAPAPYKRDFEAKLRNFYRKLETKGYGQGPGKVKLIIRRDHLLEDAFNQIMCYS	1137
YEAST	423	----------SLDQNVP----------QYKRDFRRKVIYFRSQPALRIL---PGQCHIKVRRKNIFEDAYQEIMRQT	476
HUMAN	1318	LIHQYLLDAFFTRPFYKALLRILCDLSDLEYLDEEFHQSLQWMKDNDIHDILDLTFTVNEEVFGQITERELKPGGANIPV	1397
CHIMPANZEE	1318	LIHQYLLDAFFTRPFYKALLRILCDLSDLEYLDEEFHQSLQWMKDNDIHDILDLTFTVNEEVFGQITERELKPGGANIPV	1397
COW	1089	LIHQYLLDAFFTRPFYKALLRILCDLSDLEYLDEEFHQSLQWMKDNDIHDILDLTFTVNEEVFGQITERELKPGGANIPV	1168
MOUSE	1324	LIHQYLLDAFFTRPFYKALLRILCDLSDLEYLDEEFHQSLQWMKDNDIHDILDLTFTVNEEVFGQITERELKPGGANIPV	1403
ZEBRAFISH	1218	LIHQYLLDAFFTRPFYKGLLRIPCDLSDLEYLDEEFHQSLQWMKDNDIEDMLDLTFTVNEEVFGQITERELKPGGANIPV	1297
YEAST	556	VFHRRFLDAFFVGALYKMMLRKKVVLQDMEGVDAEVYNSLNWMLENSIDGVLDLTFSADDERFGEVVTVDLKPDGRNIEV	635
HUMAN	1398	TEKNKKEYIERMVKWRIERGVVQQTESLVRGFYEVVDARLVSVFDARELELVIAGTAEIDLSDWRNNTEYRGGYHDNHIV	1477
CHIMPANZEE	1398	TEKNKKEYIERMVKWRIERGVVQQTESLVRGFYEVVDARLVSVFDARELELVIAGTAEIDLSDWRNNTEYRGGYHDNHIV	1477
COW	1169	TEKNKKEYIERMVKWRIERGVVQQTESLVRGFYEVVDARLVSVFDARELELVIAGTAEIDLSDWRNNTEYRGGYHDNHIV	1248
MOUSE	1404	TEKNKKEYIERMVKWRIERGVVQQTESLVRGFYEVVDARLVSVFDARELELVIAGTAEIDLNDWRNNTEYRGGYHDNHIV	1483
ZEBRAFISH	1298	SEKNKKEYIERMVKWRIERGVVQQTESLVRGFYEVVDARLVSVFDARELELVIAGTAEIDLGDWRNNTEYRGGYHDNHIV	1377
YEAST	636	TDGNKKEYVELYTQWRIVDRVQEQFKAFMDGFNELIPEDLVTVFDERELELLIGGIAEIDIEDWKKHTDYRG-YQESDEV	714

Figure 1.

(A) Location of *de novo* variants in *HECW2* and (B) conservation of these amino acids across species are shown.

Figure 2.

Photographs showing the facial features of probands with variants in *HECW2*. (A) Photograph showing patient 1 with midface retrusion and thin vermillion upper lip. (B) Photograph showing patient 2 with features including flat nasal bridge, mild epicanthal folds, telecanthus, thick eyebrows, synophrys, short, upturned nose with bulbous nasal tip, widely spaced teeth and deep set eyes. (C and D) Photographs showing patients 3 and 4, respectively, with deep set eyes, ptosis and a prominent forehead. (E and F) Photographs showing patient 5 with slightly large ears and an upturned nose with bulbous nasal tip. (G

and H) Photographs showing patient 6 with sparse eyebrows, slightly depressed nasal bridge and upturned nasal tip.

Table 1

Predictions of pathogenicity for the four novel, de novo missense variants in *HECW2*

Amino acid alternation	Patients	Nucleotide change	Protein domain	SIFT prediction	Polyphen prediction	Likelihood ratio test	Mutation taster	Meta-SVM	MetaLR	Provean
p.Arg1191Gln	1 and 2	c.3572 G>A	Inter-domain	Deleterious	Probably damaging	Deleterious	Disease causing	Deleterious	Deleterious	Deleterious
p.Phe1193Val	3 and 4 (twins)	c.3577 T>G	Inter-domain	Deleterious	Probably damaging	Deleterious	Disease causing	Deleterious	Deleterious	Deleterious
p.Arg1330Trp	5 and 6	c.3988 C>T	HECT	Deleterious	Probably damaging	Deleterious	Disease causing	Deleterious	Deleterious	Deleterious
p.Glu1445Gly	7	c.4334 A>G	HECT	Deleterious	Probably damaging	Deleterious	Disease causing	Deleterious	Deleterious	Deleterious

LR, logistic regression; SVM, support vector machine.

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Table 2

Phenotypic characteristics of patients with de novo HECW2 missense variants

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age	11 yo	9 yo	33 mos	33 mos	18 mos	3 yo	6 yo
Gender	Female	Male	Female	Female	Female	Male	Female
Mutation	c.3572G>A: p.Arg1191Gln	c.3572G>A: p.Arg1191Gln	c.3577T>G: p.Phe1193Val	c.3577T>G: p.Phe1193Val	c.3988C>T: p.Arg1330Trp	c.3988C>T: p.Arg1330Trp	c.4334A>G p.Glu1445Gly
Birth weight	3175 g	3941 g	2412 g	2155 g	3544 g	4196 g	2353 g
Current growth parameters	At 10 yo Wt=34.56 kg (36%ile) Ht=141 cm (35%ile)	Wt=25.4 kg (21%ile) Ht=131 cm (32%ile) OFC=52.8 cm (50%ile)	Wt=13.8 kg (60%ile) Ht=90 cm (23%ile) OFC=48 cm (39%ile)	Wt=12.8 kg (34%ile) Ht=87.9 cm (10%ile) OFC=50 cm (86%ile)	At 15 mos Wt=10.22 kg (59% ile) Ht=79.2 cm (88%ile) OFC=46 cm (65% ile)	At 23 mos Wt=12.56 kg (69%ile) Ht=90 cm (87%ile) OFC=48 cm (48%ile)	Wt=28.8 kg (<3%ile), Ht=104 cm (<3%ile), OFC=49.5 cm (<3%ile)
DD	+	+	+	+	+	+	+
ID	+ (Moderate)	+ (IQ=55)	NA	NA	NA	+	+
Autism	+	+	NA	NA	NA	NA	−
Age at sitting	9 mos	11–12 mos	Unable	Unable	Unable	Unable	Unable
Age at walking	3 yo	3 yo with braces	Unable	Unable	Unable	Unable	Unable
Age at talking	Non-verbal	2 yo; currently has ~15 words	Non-verbal	Non-verbal	One word at 17 mos	Non-verbal	Non-verbal
Hypotonia	+	+	+	+	+	+	+
Seizures	Generalised tonic clonic seizures at 5 yo (now seizure free)	EEG showed excessive slowing with abnormal burst discharges	Currently intractable tonic seizures	Currently intractable tonic seizures	−	Infantile spasms starting ~5 mos; has been a few months seizure free	EEG showed multifocal and generalised epileptiform and slow spike-wave discharges with diffuse background slowing
Abnormal behaviours	Hand flapping, self-injurious behaviours, aggressive behaviour, inappropriate laughter	Self-stimulatory behaviour	Rocking and flapping hand behaviours	Rocking and flapping hand behaviours	Repetitive hand movements, self-injurious behaviours	−	Self-stimulatory rocking, sucking on fingers
Other neurological problems	Wide-based gait, exercise intolerance	−	−	−	−	−	Choreiform movements

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Visual problems	Strabismus	None	Strabismus, nystagmus with fevers	Strabismus, nystagmus with fevers	CVI	CVI	Optic atrophy and CVI
Brain MRI	Normal	Normal	Mild ventriculomegaly and increased extra-axial fluid	Mild ventriculomegaly and increased extra-axial fluid	Normal	Cerebral atrophy, thin corpus callosum	Progressive cerebral atrophy with mild cerebellar loss, atrophy of visual pathways, several small arachnoid cysts
GI problems	GERD, constipation	None	G-tube fed	G-tube fed	Requires burping regularly	G-tube fed	G-tube, gastroparesis, constipation
Dysmorphic features	Midface retrusion, thin vermillion upper lip	Flat nasal bridge, mild epicanthal folds, telecanthus, thick eyebrows, synophrys, short, upturned nose with bulbous nasal tip, midface hypoplasia, full lower lip, widely spaced teeth, tongue protrusion, thick supraorbital ridge, deep set eyes, mouth is wide and down turned, prominent central incisors	Deep set eyes, ptosis, prominent forehead	Deep set eyes, ptosis, prominent forehead	Slightly large ears, upturned nose with bulbous nasal tip	Sparse eyebrows, slightly depressed nasal bridge, upturned nasal tip	Hypotonic facies, highly arched palate
Other	−	Joint laxity in knees, ankles, pain insensitivity	Osteopenia, frequent waking at night	Osteopenia, frequent waking at night	−	Hyperglutaminaemia, scrotal hypoplasia, pectus excavatum	Gracile bones, mild osteopenia throughout with no definite dysplasia. Cardiomyopathy started prenatally, heart block, prolonged QT interval
Other genetic findings	−	−	−	−	−	439 Mb 15q11.2 duplication; *de novo* p. G184E SLC7A7 variant	Heterozygous G200V variant in *EIF2B2* gene

%ile, percentile; −, absent; +, present; CVI, cortical visual impairment; GERD, gastro-oesophageal reflux disease; GI, gastrointestinal; G-tube, gastrostomy tube; Ht, height; ID, intellectual disability; mos, months old; NA, not available; OFC, occipital frontal circumference; Wt, weight; yo, years old.