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ZMYND11-related syndromic intellectual disability: 16 patients delineating and expanding the phenotypic spectrum

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Abstract

Pathogenic variants in *ZMYND11*, which acts as a transcriptional repressor, have been associated with intellectual disability, behavioural abnormalities and seizures. Only 11 affected individuals have been reported to-date, and the phenotype associated with pathogenic variants in this gene have not been fully defined.

Here, we present 16 additional patients with predicted pathogenic heterozygous variants in *ZMYND11,* including four individuals from the same family*,* to further delineate and expand the genotypic and phenotypic spectrum of *ZMYND11*-related syndromic intellectual disability. The associated phenotype includes developmental delay, particularly affecting speech, mild-moderate intellectual disability, significant behavioural abnormalities, seizures, and hypotonia. There are subtle shared dysmorphic features, including prominent eyelashes and eyebrows, depressed nasal bridge with bulbous nasal tip, anteverted nares, thin vermilion of the upper lip and wide mouth. Novel features include brachydactyly and tooth enamel hypoplasia.

Most identified variants are likely to result in premature truncation and/or nonsense mediated decay. Two *ZMYND11* variants located in the final exon - p.(Gln586*) (likely escaping nonsense-mediated decay) and p.(Cys574Arg) - are predicted to

disrupt the MYND-type zinc finger motif and likely interfere with binding to its interaction partners. Hence, the homogeneous phenotype likely results from a common mechanism of loss-of-function.

Keywords: Gene Expression Regulation, Intellectual Disability, Seizures, Zinc Fingers, Behavioral Symptoms

Introduction

The chromosome 10p15.3 microdeletion syndrome is characterised by developmental delay (DD) and intellectual disability (ID), craniofacial dysmorphism, behavioural abnormalities, hypotonia, and seizures (DeScipio et al., 2013). Haploinsufficiency of *ZMYND11* (NCBI Gene ID: 10771) is believed to account for many of the features associated with chromosome 10p15.3 microdeletion (Tumiene et al., 2017). ZMYND11 has been shown to act as a transcriptional repressor by inhibiting the elongation phase of RNA Polymerase II by recognizing histone modification present in transcribed regions, specifically H3K36 trimethylation (Wen et al., 2014).

In support of the critical role of *ZMYND11* in the chromosome 10p15.3 microdeletion syndrome, patients with *de novo* truncating variants in *ZMYND11* have a similar phenotype, including ID, seizures, and behavioural issues (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Popp et al., 2017). In addition, missense variants in this gene have also been associated with ID and seizures, although there is a more severe phenotype in patients with specific variants, which may be related to a gain-of-function mechanism (Cobben et al., 2014; Moskowitz et al., 2016). A splice site variant has also been reported in a child with autism spectrum disorder (Iossifov et al., 2012). In total, 11 patients with pathogenic variants in *ZMYND11* (MIM# 616083) have been reported to date (Aoi et al., 2019; Cobben et al., 2014; Coe, Witherspoon,

Rosenfeld, Van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017).

Here, we present 16 previously unreported individuals with pathogenic variants in *ZMYND11*, including four from the same family. We further delineate and expand the genotype-phenotype correlations and phenotypic spectrum of *ZMYND11*-related intellectual disability.

Methods

All patients were ascertained after routine referral to their local Clinical Genetics service. Patients 1, 3, 5 and 8 were gathered through international collaboration using GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2016). Patients 2, 6, 7, 9, 11 and 12 were identified through the Wellcome Trust Deciphering Developmental Disorders study (Wright et al., 2015). Patients 13-15 were identified as affected relatives of patient 12. Patients 4, 10 and 16 were identified through personal communication. Exome sequencing was performed on all probands, with a trio approach on patients 1, 3, 5, 6, 9-12, and 16; a duo approach on patients 2, 4, 7, and 8, as DNA samples were only available from one parent. Sanger sequencing only was used to ascertain the presence of the familial variant in patients 13-15, and all other patients had their *ZMYND11* variant confirmed using this method. All sequence variants were described with reference to *ZMYND11* transcript NM_006624.5. All variants were classified according to the American College of Medical Genetics (ACMG) guidelines (Richards et al., 2015). Further information is available in the supplemental data. Patient variants have been uploaded to either ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Global Variome shared LOVD http://www.lovd.nl, or DECIPHER (https://decipher.sanger.ac.uk).

Results

Molecular results

16 individuals (including 13 probands and two additional children of one affected mother) had a predicted pathogenic variant in *ZMYND11*. Of these, eight were *de novo*, one was inherited by three sibs from their affected mother, one was paternally-inherited, and three were of unknown inheritance. Ten variants were predicted to result in protein truncation, two were missense, and one affected a splice site (Table 1). None of the variants in this series were present in the gnomAD database (v2.1.1) (Karczewski et al., 2019). Of the two missense variants, one was located in a zinc finger domain (c.1720T>C; p.(Cys574Arg)), and the other was not in a known functional domain (c.1246G>A; p.(Glu416Lys)). Further information is available in the supplemental data.

Patient phenotypes

Phenotypic information for all patients is shown in Table 1 In-depth patient summaries are available in the supplemental data (Supp. Patient Summaries). Prominent phenotypic features are detailed below. The denominators refer to the number of patients for whom the specific information is available.

Birth weight was at or above the 98th centile in three patients (3/14; 21%). Feeding problems (e.g. excess vomiting after feeds, bottle feeding requiring more than one hour), were present in 6/13 patients (46%). Most patients had normal growth parameters and head circumference.

Development was delayed in all patients (14/14; 100%). The median age at independent walking was 24 months (with age range of 17 months to four years).

Three patients remained unable to walk at the ages of two-and-a-half (for two individuals) and four years, respectively. Speech delay was prominent, with 14/14 (100%) affected. First words were achieved at a median age of two-and-a-half years (with age range of two years to four years). Two patients were non-ambulatory, and had not achieved speech at two-and-a-half and four years age respectively (2/14; 14%). All patients had mild to moderate intellectual disability (13/13; 100%).

Almost all patients had behavioural issues (14/16; 88%). These include attention deficit, hyperactivity and impulsivity (8/16; 50%), aggressive behaviour (8/16; 50%), and autism spectrum disorder or autistic traits (3/15; 20%). Neurological abnormalities were detected in 10/16 (63%); mostly hypotonia (5/16; 31%) and epilepsy (5/16; 31%).

Photographs of patients in this series are shown in Fig. 1. Dysmorphic facial features were judged to be present in 11/16 (69%). There were a number of shared facial features, including thick eyebrows, prominent eyelashes, depressed nasal bridge with bulbous nose, anteverted nares, thin vermilion of the upper lip and wide mouth.

Patients 12-14 in this series inherited their *ZMYND11* variant from their mother (patient 15). All individuals in this family had special educational needs; two of the siblings are now in employment. The *ZMYND11* variant found in Patient 9 was paternally inherited. Detailed phenotypic information is not available for the father.

Discussion

Here, we present 16 new individuals with predicted pathogenic variants in *ZMYND11*. Comparison with all previously published patients allows further delineation of the phenotypic spectrum associated with mutations in this gene (Table 2) (Supp. Table

S1) (Aoi et al., 2019; Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017).

All patients (including our series) had developmental delay, particularly affecting speech, and ID. The severity of ID in this series is mild to moderate, but four patients have previously been described with severe ID (Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Moskowitz et al., 2016; Popp et al., 2017). Behavioural issues are also a prominent feature both in our series and in those previously published (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Popp et al., 2017), including aggression, attention deficit/hyperactivity, and autism/autistic traits. Therefore, this series provides further evidence that behavioural abnormalities are a significant part of the *ZMYND11*-associated phenotype. These behavioural problems may pose a substantial psychosocial burden, especially if the intellectual disability is mild. Hypotonia and epilepsy affect 48% and 39% of all patients, respectively (including our series). This enables us to indisputably establish hypotonia and epilepsy as part of the phenotype associated with this syndrome.

Dysmorphic features, particularly thick eyebrows, prominent eyelashes and a bulbous nose, are present in the majority of patients (Fig. 1). These are in line with the patients reported by Coe et al., (2014). These dysmorphisms may prove useful with regard to reverse phenotyping. Feeding difficulties were present in 59% of all patients (including our series), although only three patients required supplementary feeding.

Brachydactyly, seen in two patients in our series, is a possible novel feature. Interestingly, tooth enamel hypoplasia, present in one patient in our series, has previously been reported in another patient with a *ZMYND11* variant (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014), indicating this may be a rare and/or

overlooked phenotypic feature, although formal dental assessment has not been documented for most patients.

In this series, three individuals inherited a predicted pathogenic *ZMYND11* variant from their affected mother; another patient inherited the pathogenic variant from his father on whom detailed phenotypic information was lacking. Inheritance of a pathogenic *ZMYND11* variant from an affected parent has been previously reported (Coe et al. 2014). Familial inheritance should therefore be considered in variant filtering and interpretation and reproductive counselling.

The majority of patients, including those in our series, have truncating variants, which are likely subject to nonsense-mediated decay and hence, result in haploinsufficiency (Fig. 2). Of note, the p.(Gln586*) variant in our series is located in the last exon and therefore may escape nonsense-mediated decay. The p.(Cys574Arg) variant is similarly located in the last exon. These variants may be expected to have a deleterious effect through disruption of the MNYD-type zinc finger motif. This motif interacts with a number of intracellular partners, for example the NCoR transcriptional corepressor (Masselink & Bernards, 2000), and amino acid variation within this motif has been shown to disrupt binding of these partners, resulting in reduced efficacy of *ZMYND11*-mediated transcriptional repression (Kateb et al., 2013; Masselink & Bernards, 2000). We suspect, therefore, that the two variants affecting the MYND-type zinc finger motif domain in our series will at least result in a reduced function of the protein. The phenotype of these patients and a previously reported individual (Coe et al. 2014) with a p.(Gln587del) variant in this motif is not notably different to those patients harbouring variants causing haploinsufficiency, supporting a loss-of-function mechanism. The p.(Glu416Lys) variant in this series is

not in a functional domain. It has been classified as likely pathogenic given that it is *de novo* and not present in the gnomAD database; however further research is required to determine the effect of this variant.

In contrast, two missense pathogenic variants have been reported in patients with notably different phenotypes to those in this series. The p.(Ser421Asn) variant resulted in a severe Angelman-like phenotype, and the p.(Arg600Trp) variant caused distinct facial dysmorphism, moderate to severe intellectual disability, and short stature (Cobben et al., 2014; Moskowitz et al., 2016). Given these distinct phenotypes, it is possible that other mechanisms, including a gain-of-function, may be at play, but further research is required to characterise the effects of these specific variants.

Conclusions

We present a series of 16 patients with predicted pathogenic *ZMYND11* variants, predicted to result in haploinsufficiency or reduced protein function, together with a review of the published literature, allowing further delineation of the associated phenotype. Developmental delay and ID, usually mild to moderate, are universally present. Behavioural issues are frequent, and hypotonia and seizures are common. Feeding difficulties occur, but are usually mild. Subtle dysmorphism includes prominent eyelashes and eyebrows. Novel features include brachydactyly and tooth enamel hypoplasia. Our data will contribute to successful reverse phenotyping following genomic sequencing.

Data Availability Statement

Patient variants have been uploaded to either ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Global Variome shared LOVD http://www.lovd.nl, or DECIPHER (https://decipher.sanger.ac.uk). The accession

numbers are: RCV000627377.1 (Clinvar), SGS 306759, SGS 307296, CAR 279594, SMB 307553, BWH 264849, GSH 282655 (DECIPHER) and via https://databases.lovd.nl/shared/genes/ZMYND11 (LOVD).

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Conflict of Interest

The authors do not have any conflict of interest to disclose.

Consent

Informed consent was obtained for all subjects for inclusion in this study.

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Figures

Figure 1.

Photographs of patients in this series. Patient ages are as follows (y- years; mo – months): 1 – 3y, 2 – 8y, 3 – 5y 10mo, 4 – 8y, 6 – 4y, 7 – 13y 8mo, 9 – 8y, 10 – 2y 7mo, 11 – 15y, 12 – 17y, 13 – 22y, 14 – 20y, 15 – 47y, 16 – 2.5y. Note shared dysmorphic features (particularly in patients 1, 3-14 and 16) including prominent eyelashes and flattened nasal bridge with bulbous nasal tip.

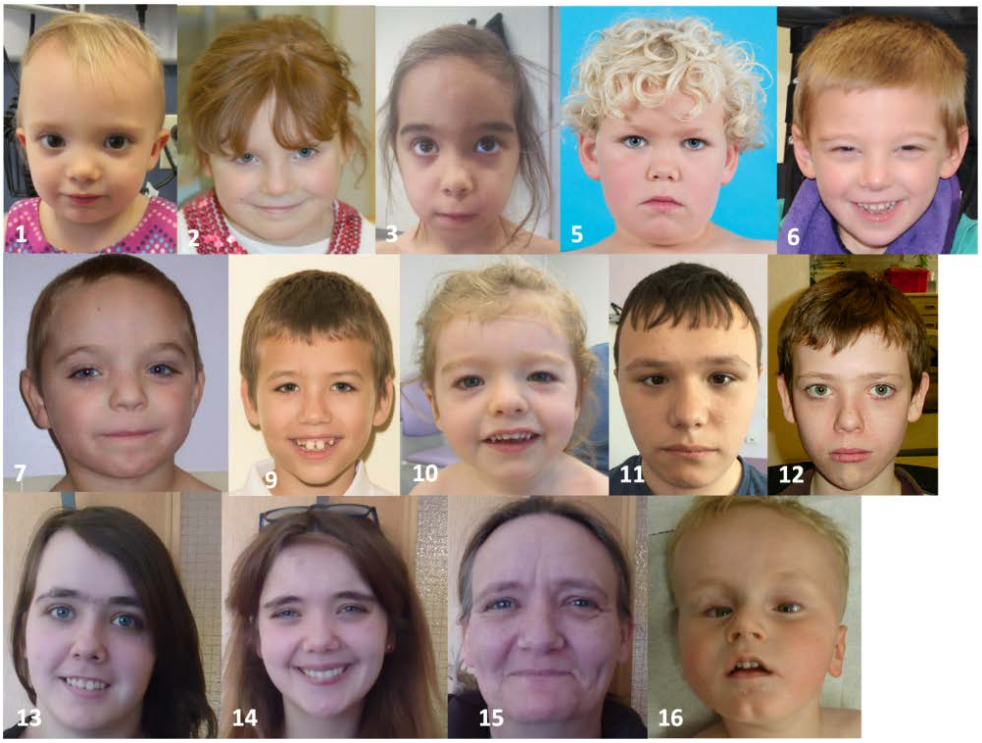

Figure 2.

ZMYND11 protein showing pathogenic variants in this series (below protein) and previously reported (above protein) (Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017) (transcript NM_006624.5, Human Genome Build GRCh37.p13). Functional domains are labelled according to their location in the protein. The tandem PWWP (Pro-Trp-Trp-Pro)-Bromo domains function in recognising H3K36 trimethylation.

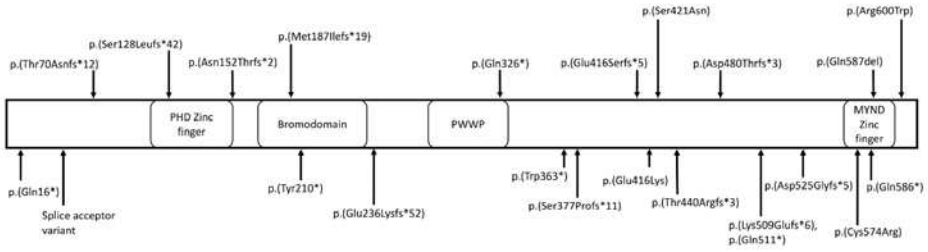

Table 1. Genotypic and phenotypic data for patients in this series, ordered according to likely pathogenic mechanism. Totals include only those patients for whom the presence or absence of the feature is reported. Mutation nomenclature according to Human Genome Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). All variants were analysed according to transcript NM_006624.5. American College of Medical Genetics and Genomics sequence interpretation criteria according to Richards et al., 2015.

Proposed pathogenic mechanism	**Haploinsufficiency: NMD**										
Patient no	1	2	3	4	5	6	7	8	9	10	**Total**
DECIPHER ID		SGS306759				SGS307296	CAR279594		SMB307553		
ZMYND11 variant	c.46C>T	c.117-2A>T	c.630C>G	c.705_708delTGAG	c.1089G>A	c.1129del	c.1315_1318del	c.1525_1526del	c.1531C>T	c.1572dup	
Predicted effect on	p.(Gln16*	Splice acce	p.(Tyr210*	p.(Glu236Ly	p.(Trp363*	p.(Ser377Profs*11	p.(Thr440Ar	p.(Lys509Gl	p.(Gln51	p.(Asp525Gl	

protein	)	ptor vari ant	)	sfs*52)	)	)	gfs*3)	ufs*6)	1*)	yfs*5)	
Inheritance	de nov o	unk now n	de nov o	de novo	de nov o	de novo	unkno wn	unkno wn	pat	de novo	
Pathogenicity (ACMG criteria)	Pat hog enic (PV S1, PS2 , PM 2, PP3)	Path oge nic (PV S1, PM 2, PP3)	Pat hog enic (PV S1, PS2 , PM 2, PP3)	Pathog enic (PVS1, PS2, PM2)	Pat hog enic (PV S1, PS2 , PM 2, PP3)	Pathog enic (PVS1 , PS2, PM2, PP3)	Likely pathog enic (PVS1 , PM2)	Likely pathog enic (PVS1 , PM2)	Path oge nic (PV S1, PM 2, PP3)	Likely Pathog enic (PM2, PVS1 _S, PS2_ M)	
Age reported	3y	8y	5y 10 mo	8y	8y	4y	13y 8mo	18y	8y	2y 7mo	
Gender	F	F	F	M	M	M	M	M	M	F	
Feeding problems	Yes	No	nd	Yes (NG supple mentat ion)	nd	No	Yes (NG supple mentat ion)	Yes	Yes	No	5/8 (63 %)
Dysmorphic	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	6/1 0 (60 %)
Delayed development	Yes	nd	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/9 (10 0%)
Gross motor delay	2y	17 mo	2y	2y	2y	Canno t walk unaide d 4y	22 mo	18 mo	2- 2.5y	Not yet achiev ed	8/1 0 (80 %)

Speech delay	Limited vocabulary, difficult to understand	nd	Short sentences at 4y	2y	First words 3y; 2-word phrases 3.5y	Not yet achieved	2y; difficult to understand until 3y	4y	2-2.5y	2y	9/9 (100%)
ID	nd	Mild, mainstream school with extra help	Mild	Mild	Mild	Mild	Moderate	nd	Mild, mainstream school with extra help. Dyspraxic.	Moderate	8/8 (100%)
Behavioural difficulties	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	9/10 (90%)
Attention deficit/ hyperactivity/ impulsivity	Yes	Yes	No	Yes	No	No	Yes	Yes	No	No	5/10 (50%)
Aggression/ anger	No	No	Yes	No	Yes	No	Yes	No	Yes	No	4/10(40%)
Autism/ autistic traits	No	No	Yes	No	No	No	No	No	Yes	No	2/10 (20%)
Hypotonia	Yes	No	No	Yes	No	No	No	Yes	Yes	No	4/10

											(40 %)
Epilepsy	No	Yes	Yes	No	No	No	No	No	No	No	2/10 (20 %)

Proposed pathogenic mechanism	**Predicted to disrupt MYND zinc-finger domain**						**Missense**	**Overall Total**
Patient no	11	12	13	14	15	**Total**	16	
DECIPHER ID	BWH 264849	GSH 282655						
ZMYND11 variant	c.1720T>C	c.1756C>T	c.1756C>T	c.1756C>T	c.1756C>T		c.1246G>A	
Predicted effect on protein	p.(Cys574Arg)	p.(Gln586*)	p.(Gln586*)	p.(Gln586*)	p.(Gln586*)		p.(Glu416Lys)	
Inheritance	de novo	mat	mat	mat	unknown		de novo	
Pathogenicity (ACMG criteria)	Likely pathogenic (PS2, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)		Likely pathogenic (PS2, PM2)	
Age reported	15y	17y	22y	20y	47y		2y 5 mo	
Gender	M	M	F	F	F		M	
Feeding problems	No	No	No	No	nd	0/4 (0%)	Yes	5/13 (38%)
Dysmorphic	Yes	Yes	Yes	Yes	No	4/5 (80%)	Yes	11/16 (69%)

Delayed development	Yes	Yes	Yes	Yes	nd	4/4 (100 %)	Yes	14/14 (100%)
Gross motor delay	13 mo	2.5 y	18 mo	4y	nd	2/4 (50%)	Not yet achieved	11/15 (73%)
Speech delay	2-2.5y	4-5y	First words around 4y	First words around 4y	nd	4/4 (100 %)	Not yet achieved	13/13 (100%)
ID	Moderate ID, attends special school	Yes, attended special school, now in simple employment	Yes, attended special school, not able to take GCSE, volunteering activities in school, currently in college	Yes, attended special school, not able to take GCSE but currently working in retail	Yes, attended special school	5/5 (100 %)	nd	13/13 (100%)
Behavioural difficulties	Yes	Yes	Yes	Yes	No	4/5 (80%)	Yes	14/15 (93%)
Attention deficit/ hyperactivity/ impulsivity	Yes	Yes	Yes	No	No	3/5 (60%)	No	8/16 (50%)
Aggression/ anger	Yes	Yes	Yes	Yes	No	4/5 (80%)	No	8/16 (50%)
Autism/ autistic traits	Yes	No	No	No	No	1/5 (20%)	nd	3/15 (20%)
Hypotonia	No	No	No	No	No	0/5 (0%)	Yes	5/16 (31%)

Epilepsy	Yes	No	Yes	No	Yes (as child)	3/5 (50%)	No	5/15 (33%)

Table 2. Genotypic and phenotypic data for all previously reported patients with *ZMYND11* variants, ordered according to likely pathogenic mechanism, with summary total including this series. Totals include only those patients for whom the presence or absence of the feature is reported. Mutation nomenclature according to Human Genome Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). All variants were analysed according to transcript NM_006624.5. American College of Medical Genetics and Genomics sequence interpretation criteria according to Richards et al., 2015.

Proposed pathogenic mechanism	**Haploinsufficiency: NMD**								**Disruption of MYND zinc-finger domain**		**Possible gain-of function**		**Total in all patients (including this series)**
Patient no	Iossifov et al.	Coe et al. ***Nijmegen DNA05-04370***	Coe et al. ***Adelaide 3553***	Coe et al. **Nijmegen DNA-017151**	Coe et al. **Nijmegen DNA-000224**	Coe et al. **Nijmegen DNA-013587**	Popp et al.	Aoi et al.	Coe et al. ***Adelaide 20124***	**Total (where documented)**	Cobben et al.	Moskowitz et al.	
ZMYND11 variant	c.1159-1G>A	c.1246_1247del	c.454_455insC	c.206dup	c.976C>T	c.561del	c.383del	c.1438del	c.1759_1761del		c.1798C>T	c.1262G>A	

Predicted effect on protein	Splice variant	p.(Glu416Serfs*5)	p.(Asn152Thrfs*26)	p.(Thr70Asnfs*12)	p.(Gln326*)	p.(Met187Ilefs*19)	p.(Ser128Leufs*42)	p.(Asp480Thrfs*3)	p.(Gln587del)		p.(Arg600Trp)	p.(Ser421Asn)	
Type of predicted variant effect	Splice variant	Frameshift	Frameshift	Frameshift	Nonsense	Frameshift	Frameshift	Frameshift	In-frame		Missense	Missense	
Feeding problems	nd	nd	nd	nd	nd	nd	Yes	nd	Yes	2/2 (100%)	Yes (NG supplementation)	Yes	10/17 (59%)
Dysmorphic	nd	Yes	No	Yes	Yes	Yes	nd	Yes	Yes	6/7 (86%)	Yes	Yes	19/25 (66%)
Gross motor delay	nd	nd	Yes	Yes	nd	Yes	nd	nd	nd	3/3 (100%)	Yes	Yes	16/20 (80%)
Speech delay	nd	Nonverbal	Yes	Yes	Yes	Yes	nd	nd	Yes	6/6 (100%)	Yes	Nonverbal	21/21 (100%)
ID	nd	Severe	nd	Mild	Mild	Mild	Severe	Yes	Mild	7/7 (100%)	Severe	Severe	21/21 (100%)
Behavioural difficulties	nd	nd	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7/7 (100%)	nd	No	21/24 (88%)

Attention deficit/ hyperactivity/ impulsivity	nd	nd	nd	No	No	Yes	nd	No	nd	1/4 (25%)	nd	Yes	10/21 (48%)
Aggression/ anger	nd	nd	nd	No	nd	Yes	Yes	No	nd	2/4 (50%)	nd	No	10/21 (48%)
Autism / autistic traits	Yes	Yes	nd	Yes	nd	No	nd	No	No	3/6 (50%)	nd	No	6/22 (27%)
Neurological abnormality	nd	Yes	Yes	No	nd	No	Yes	nd	Yes	4/6 (67%)	Yes	Yes	16/24 (67%)
Hypotonia	nd	Yes	No	Yes	nd	nd	Yes	nd	Yes	4/5 (80%)	Yes	Yes	11/23 (48%)
Epilepsy	nd	Yes	Yes	No	nd	No	Yes	nd	No	3/6 (50%)	No	Yes	9/24 (38%)