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CNTNAP1-encephalopathy: six novel patients surviving the neonatal period

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Abstract

CNTNAP1 encodes CASPR1, involved in the paranodal junction. Thirty-three patients, with *CNTNAP1* biallelic mutations have been described previously. Most of them had a very severe neurological impairment and passed away in the first months of life. We identified four patients, from two unrelated families, who survived over the neonatal period. Exome sequencing showed compound heterozygous or homozygous variants. Severe hypotonia was a constant feature. When compared to previous reports, the most important clinical differences observed in our patients were the absence of antenatal problems and, in two of them, the lack of respiratory distress. Less commonly reported characteristics such as epileptic seizures, dystonia, and impaired communication skills were also observed. MRIs revealed hypomyelination or abnormal white matter signal, cerebral or cerebellar atrophy. The present observations support a wider than initially reported clinical spectrum, including survival after the neonatal period and additional neurological features. They contribute to better delineate the phenotype–genotype correlations for *CNTNAP1*.

In addition, we report one more family with two sibs [who carry a missense variant of uncertain significance which we propose could be associated with a milder phenotype.](#)

Key words: contactin-associated protein, *CNTNAP1*, *CASPR1*, hypotonia, dystonia, hypomyelination

1. Introduction

CNTNAP1 (OMIM 602346) encodes CASPR1, a contactin associated protein, involved in the formation and stability of myelinated axons and the generation of action potential. It may also mediate the timing of neuron and astrocytes differentiation (1, 2).

Pathogenic variants of *CNTNAP1* gene were first reported in 2014 in four patients of a 31 patients cohort with non-syndromic foetal hypomobility/arthrogryposis multiplex congenital syndrome (3,4). All of them passed away before 40 days of life. Following these publications, *CNTNAP1* mutations have been described in patients with Congenital Hypomyelinating Neuropathy (OMIM 618186), that consists in severe hypotonia with very slow nerve conduction velocities (5,6) and in patients with lethal congenital contracture syndrome type 7 (OMIM 616286). Before 2018, 22 cases with pathologic *CNTNAP1* variants were described. In most of the reported cases, the main phenotypic characteristic was early death. In 2018, Low et al (4) described seven patients, some of them surviving in infancy and in childhood. Conant et al. in 2018 (6) and Sabbagh et al. in 2020 (7) increased the number of reported patients with *CNTNAP1* mutation to 33, and confirmed that a range of phenotypes can be associated with these mutations.

We present six additional patients with *CNTNAP1* novel genetic variants, who survived after the neonatal period and further expand the phenotypic range.

2. Patients and methods

2.1. Patients:

All six patients (Table 1), belonging to three unrelated families, are followed at the department of Neuropaediatrics, at the University Hospitals of Lyon (HCL). They all suffered from a congenital encephalopathy. Informed written consent for video-EEG monitoring was obtained (Clinical Epileptology and Neurophysiology Dpt., HCL) and the Ethics committee of the HCL approved this observational study.

2.2. DNA isolation

Extraction of genomic DNA was performed from blood, using NucleoSpin® Blood XL kit (Macherey-Nalgel, Düren, Germany), according to the manufacturer's instructions.

2.3. Exome sequencing

Libraries were built using SeqCap EZ MedExome kit (Roche, Basel, Switzerland) and paired-end sequencing 2x150bp was performed on a Nextseq 500 or a HiSeq 4000 Sequencing System (Illumina, San Diego, CA, USA).

2.4. Variant annotation, filtering and prioritization

Genomic alignment against the hg19/GRCh37 assembly and variant calling were, respectively, done with BWA-MEM, GATK HaplotypeCaller (Broad Institute, Boston, MA, USA) and Picard Tools (<http://picard.sourceforge.net>).

For patients 1, 2, 5, and 6:

Only highly confident variants were kept for analysis (total depth >9; alternative allele depth >4; no strand bias; mosaicism >10%). Rare variants were considered as having a frequency <1% in gnomAD v2.1 control database.

For patients 3 and 4:

Single-nucleotide variants and indels were subsequently called by the SAMtools suite (mpileup, bcftools, vcfutil). All calls with a read coverage $\leq 5x$ and a Phred-scaled SNP quality of ≤ 20 were filtered out. Substitution and variation calls were made with the SAMtools pipeline (mpileup). Variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensemble database (release 67).

2.5. Sanger sequencing

CNTNAPI variants were confirmed by Sanger sequencing. Sequencing of PCR amplified fragments was performed on an ABI 3100 or a 3500DX Genetic Analyzer (Applied Biosystems Foster City, CA, USA) with the Big Dye Sequencing Kit according to the manufacturer's specifications (Thermo Fischer Scientific, Waltham, MA, USA).

3. Results

The clinical features of our six patients and *CNTNAPI* variants, belonging to three unrelated families (A to C) are detailed in Table 1.

3.1. Prenatal features

No significant event was reported during pregnancy for all patients (see Table 1 for pregnancy follow-up details).

3.2. Clinical description

Family A (Patients 1 and 2): No abnormalities were reported at birth and during the first month of life. Developmental delay was noticed by the age of six months. Both patients showed signs of spasticity and stiffness, at one month (patient 2) and nine months of age (patient 1), without extrapyramidal signs. At six months of age, patient 1 was still unable to sit. Currently, aged fourteen and eleven years respectively, they present with central hypotonia and a pyramidal syndrome. A spastic stiffness of all four limbs, knee flexum, and ankle valgus is observed. The hands of patient 2 are spontaneously fixed on flexion, without any resistance on passive mobilisation. Rough palm digital gripping is observed. Limb movements are slow. Facial hypotonia is more pronounced in patient 2. They have a relatively good interaction and understand simple questions but present with a quite severe speech delay. Patient 1 pronounced only a few words at the age of four years, then a few sentences when eight years old. Patient 2 only vocalizes and has stereotypic movements (hand flapping) with erratic laugh.

Family B (Patients 3 and 4; video): The first clinical signs appeared at three months of age in patient 3 and at birth in patient 4, characterized by secondary respiratory distress and central hypotonia. A pyramidal syndrome and knee flexum were observed in both patients. Patient 3 can now walk a few steps with help and can sit. At the age of seven months, patient 4 could not hold his head or sit. He can only roll on the floor. Both present with slow movements, no cog-wheel sign, and patient 3 shows abnormal posture of the hands (flexion and abduction). He is a sociable boy, communicates with some words and sign language but he is dysarthric.

Family C (Patients 5 and 6): Contrary to the two families described above, patients 5 and 6 presented with respiratory distress and global hypotonia at birth, that persists, with no head control at the age of five and three years, respectively. Later in life they experienced spasticity. At the age of five years, patient 5 still needs oxygen support during sleep.

Epileptic seizures appeared in the first days of life for patient 6 and at the age of two months for patient 5. Patient 5 experienced episodes of oculogyric crisis, epileptic spasms and tonic seizures. Patient 6 initially had tonic seizures with cyanosis. Currently, both suffer from a drug-resistant epilepsy.

Communication is limited to facial expression and smiles. Oral nutrition is not possible due to major swallowing issues.

3.3.MRI features:

MRI of patients 1 and 2 (Family A) show no abnormality of white matter but subcortico-cortical and infra-tentorial atrophy for patient 2. Neuroimaging in patients 3, 4 and 5 was consistent with delayed myelination of the white matter (Fig 1). Besides global atrophy, patient 5 also has infra-tentorial abnormalities (Fig 1). A previous brain MRI of patient 6 was normal at ten days of life.

3.4.Genetic analyses:

All six patients, from the three families were carrying missense variants of *CNTNAP1* (NM_003632). One of the variants (p.Cys585Tyr) was already reported in another family. The others are novel. They were either absent or with a very low occurrence in the gnomAD database for control individuals (gnomAD_V2.1). All data,

including in silico predictions are displayed in Table 2. All variants were located in regions of the protein intolerant to amino acid changes (Fig 2). According to the ACMG classification, three of them were considered as likely pathogenic, whereas p.(Glu1247Lys) was scored as a variant of unknown significance. No pathogenic or likely pathogenic variant of other genes was found in the three families.

4. Discussion

CNTNAP1 is now a recognized aetiology of severe neonatal encephalopathy. Sabbagh et al (7) reported 31 patients and Lesmana et al. (8) two others, bringing to 33 the number of cases with pathogenic variants of *CNTNAP1*.

One of the most prevalent clinical signs described was hypotonia, reported, according to Sabbagh et al.(7), in 87% of the patients. However, such a finding may be underestimated since tonicidity cannot be tested in cases of severe neonatal distress followed by death in the first hours of life. Neonatal respiratory distress is reported in 97% of the children (Table 3). Sabbagh et al. (7) reported that 58% of the children had facial diplegia and 23% associated myopathic facial features. Among the 33 reported children, 76% had facial diplegia, myopathic facies, or weak facial expression (Table 3). Moreover, 85% of the children described in literature have swallowing or orobulbar dysfunction.

In the present study, we report six additional patients with *CNTNAP1* variants. Families B and C, harboured missense variants fulfilling the criteria for pathogenicity. The p.(Cys585Tyr) missense variant, found in family B, has already been described by Lesmana et al (8). These four children presented with severe hypotonia, which is one of the most frequent clinical signs. Swallowing problems of patients from family C and respiratory distress concerning patients 4, 5 and 6, are also well described in literature.

Finally, these clinical signs are supported by MRI findings. Myelination abnormalities in patients 3, 4 and 5 concur with previous studies, as detailed below.

The four patients with pathogenic variants reported in our study, highlight some new or less common characteristics. Firstly, all were still alive at ages three to nine years, which confirms the recent observation made by Low et al. (4) that infancy survival can occur. We also provide a detailed description of epilepsy and Video-EEG characteristics (patients 5 and 6), described rather briefly in six previously reported patients (6–9) (Table3). The age of onset of epilepsy was only specified for one of the latter (ten weeks old). Seizure spectrum was large with tonic, clonic, and myoclonic seizures. Our Patients 5 and 6 presented with epileptic spasms, which was only described once (8).

Vanderver et al. (6) recall the role of CASPR/contactin complex to maintain voltage gated potassium channels in the juxtaparanodal region suggesting that seizures are a consequence of dysfunction of these channels. To better delineate the epilepsy phenotype related to CNTNAP1 encephalopathy the detailed description of more patients is still necessary. Only five of the children previously reported had EEG recordings, including two who did not experience seizures. One EEG was normal whereas four patients showed abnormal background patterns. EEGs of our patients 5 and 6 showed an hypsarythmic pattern, whereas the EEG of the patient with infantile spasms from Lesmanas et al. (8) showed moderate background slowing and multifocal spike-waves.

The third novel input from this study is the description of dystonia in our patients 3 and 4. Dystonia was reported in two cases (4) (Table 3). It is defined as a syndrome of involuntary sustained or intermittent muscle contractions leading to

twisting or repetitive movements or abnormal postures.(10) Although the classical view localizes the origin of dystonia to dysfunction of the basal ganglia circuits, recent network models involve the cerebellum (11,12). Caspr1 expression is marked throughout most of the brain with the strongest expression in the dentate gyrus of the hippocampus (pyramidal layer of hippocampus and granular layer of the dentate gyrus), prefrontal cortex and within the cerebellum (5). Thus, it may explain, at least partly, that dystonia was observed in some patients with this syndrome.

Other clinical differences were observed in our patients. Concerning the perinatal period, no polyhydramnios was present, whereas polyhydramnios is described in 88% of cases reported in literature (Table 3). Besides, the children of families B and C of our study were born at term whereas, according to Sabbagh et al.(7), 58% of the previously reported patients were born preterm. This last percentage may be questioned. Indeed, [among the 33 children described in literature, fourteen were born preterm and data was missing for fifteen. Thus, the resulting percentage of preterm children is 42%.](#) Sabbagh et al [may have misinterpreted](#) the four gestational ages of AMC (Arthrogryposis Multiplex Congenita) diagnosis given by Laquérière et al. (3) with the gestational ages of birth. This would explain why they numbered eighteen preterms among 31 children, giving a percentage of 58%. Also, two of the four children from Families B and C did not experience symptoms of respiratory distress at birth, contrary to 32 of the 33 children reported in literature (3-6, 8, 9, 13, 14).

Developmental delay and intellectual disability were reported to be severe in previous studies. Fourteen patients were still alive at the time of the publication. However, developmental outcomes were very limited, reported as: “non-verbal, non-ambulatory” (8); “artificial ventilation, no independent motor skills”, “lethargy” (6);

“vegetative” (5); “profound intellectual delay” (4). Only Low et al. (4) described two patients with some communication skills. One had a “social smile at the age of twelve weeks and was attempting single words at the age of two”. The other, who was the oldest of the cohort, was described to “communicate some preferences through noise and body language” and as “enjoying certain television programmes”. The communication and motor skills of our patient 3, were more developed than what reported.

Since CASPR is crucial in myelination process, it may not be surprising to find myelin abnormalities on MRI, nerve biopsies, or ENMG of patients with pathogenic *CNTNAP1* variants. Due to premature death, only seventeen of the 33 patients benefited from an MRI investigation. Two of them were described as normal, at two months of age (13,15). Consistent with the observation of Lesmana et al. (8) our patient 6 illustrates the fact that brain MRI may be initially reported normal and later abnormal. Twelve MRIs (among the seventeen reported in the literature) reveal signs of hypomyelination or abnormal white matter signal (4–6,9,14,16). Seven MRIs show cerebral and/or cerebellar atrophy. This is consistent with the MRIs of our patients 3, 4, 5 and 6.

CASPR1, belonging to the neurexin superfamily, is a 190kDa neuronal transmembrane protein highly concentrated at paranodes (1). CASPR1 is composed of an extracellular, helical (transmembrane), and cytoplasmic regions (5). The functional domains of CASPR1 include an F5/8 type C, four laminin G-like domains, two EGF-like domains, a fibrinogen C-terminal domain, and a SH3-binding motif.

Variants of our patients 3 and 4 involve different protein domains than before (<https://uniprot.org/uniprot/P78357>). Indeed, our patients 3 and 4 have a unique association of a pathogenic variant involving the Fibrinogen C-terminal domain with another involving the Laminin G-like 2 domain (Fig 3), and they are the only two patients of this study who present dystonia.

In family A, we also report a novel homozygous missense p.(Glu1247Cys) variant of *CNTNAPI*. These patients shared the core features of severe global hypotonia, revealed before six months of age and facial hypotonia, with the other *CNTNAPI* patients reported so far. The two sibs had no perinatal signs such as polyhydramnios or respiratory distress. Patient 2 also had swallowing problems and white matter abnormalities on brain MRI, which have also been reported in the literature. Patient 1 stands out as, at the age of fourteen years, his MRI shows neither signs of hypomyelination nor cerebral atrophy. In addition, EMG performed at the age of thirteen did not show axonal or demyelinating neuropathy, as compared to the eight EMGs described in previous studies. A nerve biopsy was not performed to any of our patients. Paranodal abnormalities, along with significant homogeneous axonal loss, significant decrease of large myelinated, and thin myelin sheaths, have been reported previously (7).

In silico predictions of the variant p.(Glu1247Cys) are in favour of a deleterious effect. [The CADD \(Combined Annotation Dependent Depletion\) score predicts deleteriousness of single nucleotide variants in the human genome, by integrating multiple annotations into one metric. The score ranges from 1 to 99. Below 20, the variants were classified as benign and otherwise harmful. Higher values indicate more deleterious cases. CADD value of patients 1 and 2 is 39, which is the highest of our](#)

[series](#). Moreover, the variant is located in a region rather intolerant to amino acid changes but outside any known functional domain. No homozygous variants are reported in the GnomAD database of control individuals. No other variant was found in this family that could explain the clinical phenotype, [inspite of agnostic approach with trio WES](#). This variant is the only one located in Laminin G-like 4 domain (Fig 3). We used the tool HOPE, to analyse the structural effects of a point mutation in the protein sequence (17). There is no structural information known for this protein. Therefore, HOPE uses annotations from the UniProt-database and predictions from the Reprof software for mutational analysis. This variant is predicted to have a severe effect on the 3D-structure of the protein, could cause destabilization of the structure and probably damaging the protein. Nevertheless, due to the location of the variant in a region in which no pathogenic variant has been previously reported and to the unusual clinical phenotype, this variant was considered of unknown significance. [In the future, the use of techniques such as methylation signatures, if they become possible for CNTNAP1, might provide clarity about this variant of unkown significance.](#)

In 2018, Low et al(4) suggested a possible genotype-phenotype correlation and made the hypothesis that “hypomorphic missense variants modify the deleterious effect on peripheral/central myelination and paranodal junction”. Therefore, the missense variants we report on lead to different amino-acids changes located in different domains compared to those from previous studies. These differences might explain the more moderate phenotype observed in our study. Family A might strengthen this hypothesis, as they have better communication skills, normal myelination on brain MRI and a

variant located in a brand-new domain. However, this hypothesis must be taken with caution as the pathogenicity of the missense variants is not formally proven.

Linking variants to the phenotypic differences reported here, would require a better knowledge of the CASPR1 structure and of the role of each domain on the functions of the protein, which is not currently the case.

5. Conclusion

This study supports *CNTNAP1* mutation as a novel aetiology for severe hypotonia in newborns and infants. It confirms and enlarges the full clinical spectrum and, in contrast to previous reports, the possibility of surviving after the neonatal period. Our study also highlights the possible association of dystonia, which has been rarely reported so far. [Repeat MRI at a later stage could be considered where clinically appropriate if an early MRI is normal. Indeed, our study provides evidence that this can progress in some patients and thus provide useful phenotypic information. Finally,](#) the lack of signs of hypomyelination on brain MRI, linked with some localisation of the variant, is a new hypothesis that needs further cases to be confirmed.

Conflicts of interest

None

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TABLES AND FIGURES:

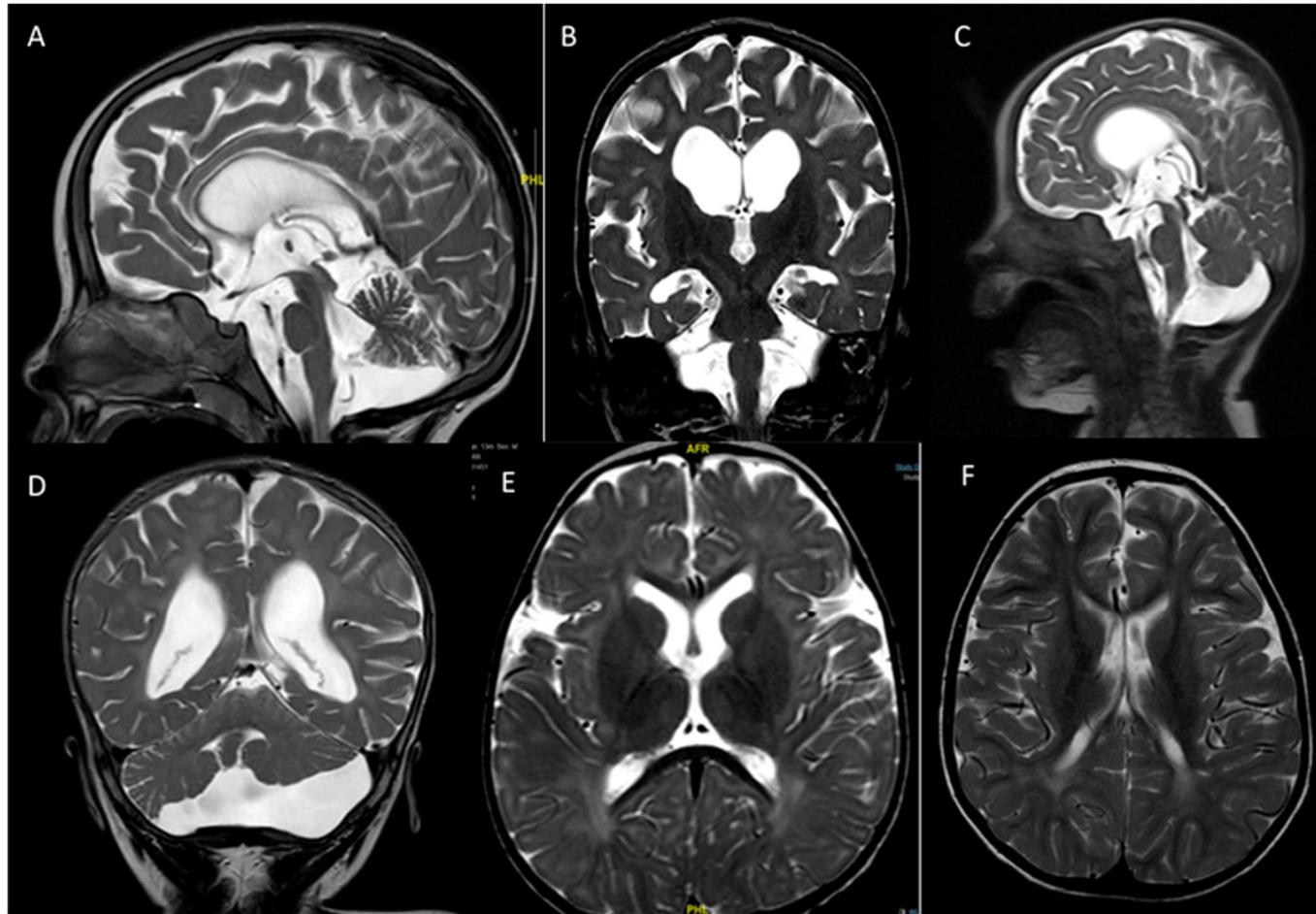


Figure 1: Brain MRI findings. (A)(B)(C)(D): Extra-axial spaces consistent with brain atrophy, thin corpus callosum, T2 hypersignal of white matter consistent with delayed myelination. (D) Cerebellar hypoplasia. (E)(F) Reduced white matter with T2 hypersignal consistent with myelination retardation. (A)(C) Sagittal, (B)(D) coronal images. (A)(B) Images of Patient 6. (C)(D) Images of Patient 5. (E) (F) Transversal images of Patients 4 and 3.

Table 1: Clinical features of the patients included in this study

Patient	1	2	3	4	5	6
Age (years)	14	11	9	4	5	3
Sex	M	M	M	M	M	F
Prenatal/ Birth						
Gestation (weeks+days)	41	38	41	40	37+6	39
Birthweight (g) [z-score]	4450 [1,81]	4430 [2,54]	3000 [-1,53]	3020 [-1,22]	3460 [0,69]	3570 [0,85]
Head circumference (cm) [z-score]	38 [1,99]	36 [1,20]	34,5 [-0,60]	36 [0,69]	37,5 [2,27]	36,8 [2,026]
Birth hight (cm) [z-score]	54 [1,62]	51 [0,97]	50,5 [-0,32]	50,5 [-0,04]	52 [1,49]	49 [-0,07]
Pregnancy follow-up*	Level II maternity – physiologic pregnancy	Level II maternity – physiologic pregnancy	Level I maternity – physiologic pregnancy	Level I maternity – physiologic pregnancy	Level III maternity – physiologic pregnancy	Level III maternity – physiologic pregnancy
Fetal akinesia	-	-	-	-	-	-
Polyhydramnios	-	-	-	-	-	-
APGAR	10.10	10.10	10.10	10.10	9.9	6.8.9.9
Neonatal respiratory distress	-	-	-	Secondary respiratory distress, Optiflow 8 days	+	PPV 2 days then NHF 25 days
Others	Hypoglycemia, 4days enteral nutrition	Obstructive rhinitis	10 days enteral nutrition	Enteral nutrition one week	Tracheostomy nineteenth to fifth month of life/ enteral nutrition	Enteral nutrition
Clinical presentation						
Age at first signs (months)	6	4,5	3	Birth	Birth	Birth
Intellectual deficiency	+	+	+	+	+	+
Central hypotonia	+	+	+	+	+	+
Pyramidal Syndrome	+	+	+	+	-	-
Shift	Crawl	-	Walk with help	Roll on the floor	-	-
Motor skills	Palm-digital prehension	Palm-digital prehension	Eat with no help	Catch things	-	-
Movement disorder	-	Stereotypies (hand flapping) with erratic laugh	Dystonia, slow movement	Dystonia, slow movement	-	-

Seizure	Eyes revulsion, cyanosis (3 and 4 years old)	-	-	-	+	+
Orobulbar dysfunction	Dysarthria, sialorrhea, no deglutition problem	Only vocalize, no sialorrhea, some deglutition problem	No deglutition problem, dysarthria	No deglutition problem	+	+
Facies	Hypomimia	Mouth open	Hypomimia, fixed smile	Hypomimia		
Scoliosis	+	+	-	-	+	-
Hip subluxation/luxation	-	-	-	-	Bilateral luxation (non-congenital)	Excentric femoral heads (non-congenital)
Visual impairment	+	+	-	NA	NA	-
Hearing	-	NA	NA	NA	Right cophosis	Bilateral conductive deafness
Others	Leg oedema			Convergent strabism	Nissen surgery, gastrostomy oxygenotherapy at night	Gastrostomy
Investigation Features						
MRI	Light enlargement of sub-arachnoid space. Foramen magnum stenosis and narrow cervical canal.	Rarefaction of white matter. Cerebral atrophy.	Light rarefaction of white matter and delayed myelination	Aspecific discrete hypersignalT2 on the two pallidum, rarefaction of white matter and periventricular delayed myelination	Cerebellar hypoplasia, brainstem and corpus callosum atrophy, global atrophy	White matter atrophy, mainly supra-tentorial, lack of myelination, thin corpus callosum
EMG	Limited interpretation (non-cooperating child). No point for axonal or demyelinating neuropathy	Microvolted activity right sural muscle. No impairment conduction velocity	NA	NA	NA	NA
Other exams	EEG, ERG, VER: normal (2 years old).	EEG: normal (4 years old)	NA	NA	EEG: Abnormal, hysarythmic pattern	EEG: Abnormal, hysarythmic pattern

*Level 1: physiologic pregnancies, expected to lead to uncomplicated birth. Obstetrical unit.

Level 2: low- to moderate-risk pregnancies. Obstetrical unit and neonatal unit.

Level 3: high-risk pregnancies. Obstetrical unit, neonatal unit and neonatal intensive care unit.

Table 2: Genetic variants of CNTNAP1 found in this study (NM_003632)

Patient	1	2	3	4	5	6
Consanguinity	Yes	Yes	No	No	Yes	Yes
Zygozity	Homozygous	Homozygous	Compound heterozygous	Compound heterozygous	Homozygous	Homozygous
Genomic	Chr17:g.40849742G>A	Chr17:g.40849742G>A	Chr17:g.[40842124G>A]; [40840944T>C]	Chr17:g.[40842124G>A]; [40840944T>C]	Chr17:g.40839879G>A	Chr17:g.40839879G>A
cDNA	c.3739G>A	c.3739G>A	c.[1754G>A];[1507T>C]	c.[1754G>A];[1507T>C]	c.1186G>A	c.1186G>A
Protein	p.(Glu1247Lys)	p.(Glu1247Lys)	p.[(Cys585Tyr)]; [(Phe503Leu)]	p.[(Cys585Tyr)]; [(Phe503Leu)]	p.(Gly396Arg)	p.(Gly396Arg)
CADD	39	39	28.9 / 26.6	28.9 / 26.6	27.3	27.3
SIFT	0	0	0 / 0	0 / 0	0	0
Mutation Taster	1	1	1 / 1	1 / 1	1	1
GnomAD allele count (heterozygous)	12	12	3 / 4	3 / 4	0	0
Functional domain	Lamin G-like 4	Lamin G-like 4	Fibrinogen C-terminal / Lamin G-like 2	Fibrinogen C-terminal / Lamin G-like 2	Lamin G-like 1	Lamin G-like 1
ACMG classification	3 (PM2, PP2, PP3)	3 (PM2, PP2, PP3)	4 (PM1, PP2, PP3, PM2) / 4 (PM1, PP2, PP3, PM2)	4 (PM1, PP2, PP3, PM2) / 4 (PM1, PP2, PP3, PM2)	4 (PM1, PP2, PP3, PM2)	4 (PM1, PP2, PP3, PM2)
Conclusion	Unknown significance	Unknown significance	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic
First appearance in publication	This study	This study	Lesmana et al. 2018	Lesmana et al. 2018	This study	This study
			this study	this study		

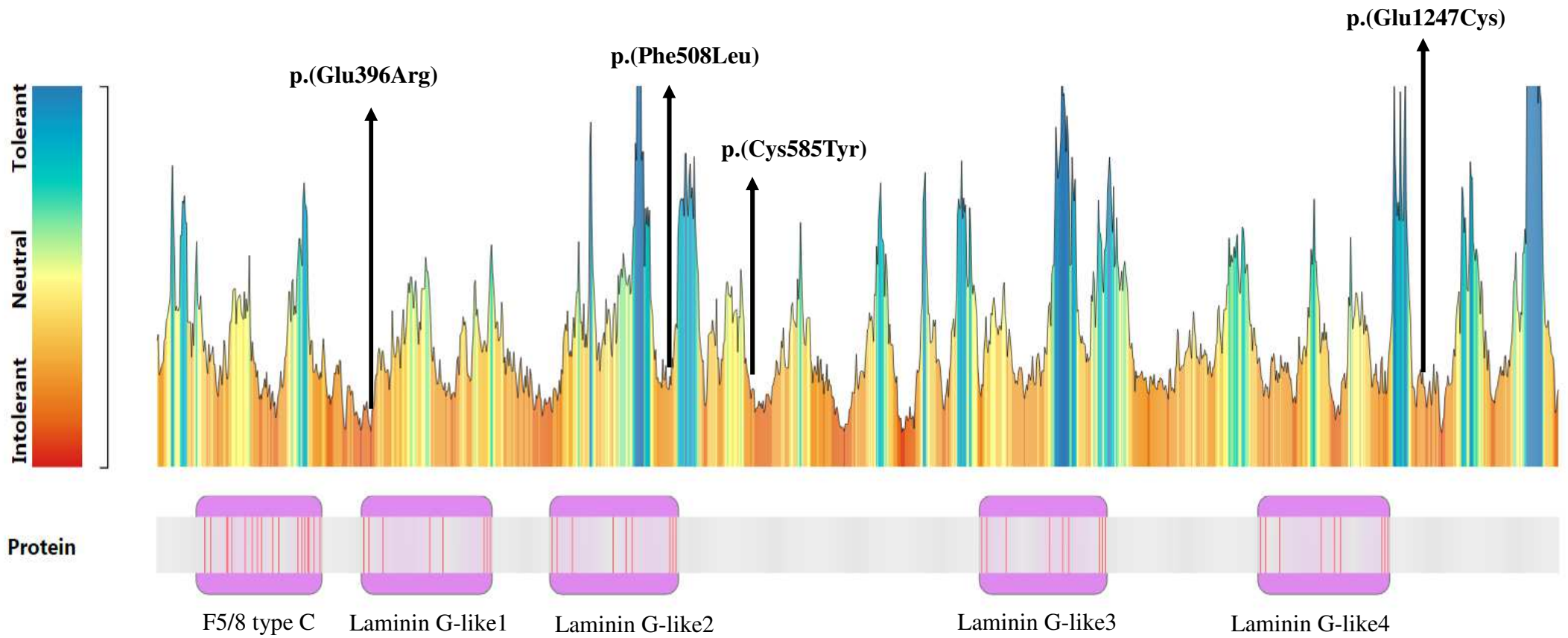


Figure 2: Representation of estimated functional impact of genetic variants of CNTNAP1

<https://stuart.radboudumc.nl/metadome/dashboard>

CNTNAP1 (GENCODE: ENST00000264638.4, RefSeq: NM_003632.2, UniProt: P78357)

Table 3: Comparison of clinical features associated with *CNTNAP1* variants, between the 33 children described in the literature and four children of the present study, with pathogenic variants.

Clinical feature		Number (Percentage) of patients	
		Literature	This study*
Prenatal manifestations	Preterm	14 (42%)	0 (0%)
	Polyhydramnios	29 (88%)	0 (0%)
Respiratory	Respiratory distress	32 (97%)	3 (75%)
Neurologic	Hypotonia	28 (90%)	4 (100%)
	Seizures	6 (19%)	2 (50%)
	Dystonia	2 (6%)	2 (50%)
Neurodevelopment	Language skills: isolated words, vocalization	2 (6%)	1 (25%)
	Ability to move around	0 (0%)	1 (25%)
	Intellectual disability	8 (24%)	6 (100%)
	Vegetative state, or non verbal and non ambulatory state	4(12%)	0 (0%)
Others	Swallowing or orobulbar dysfunction.	28 (90%)	2 (50%)
	Diplegia and/or myopathic facies or weak facial expression	25 (78%)	2 (50%)
Death		19 (59%)	0 (0%)

*Patients 3, 4, 5 and 6 with pathogenic variants. Family A was not included in the calculation.

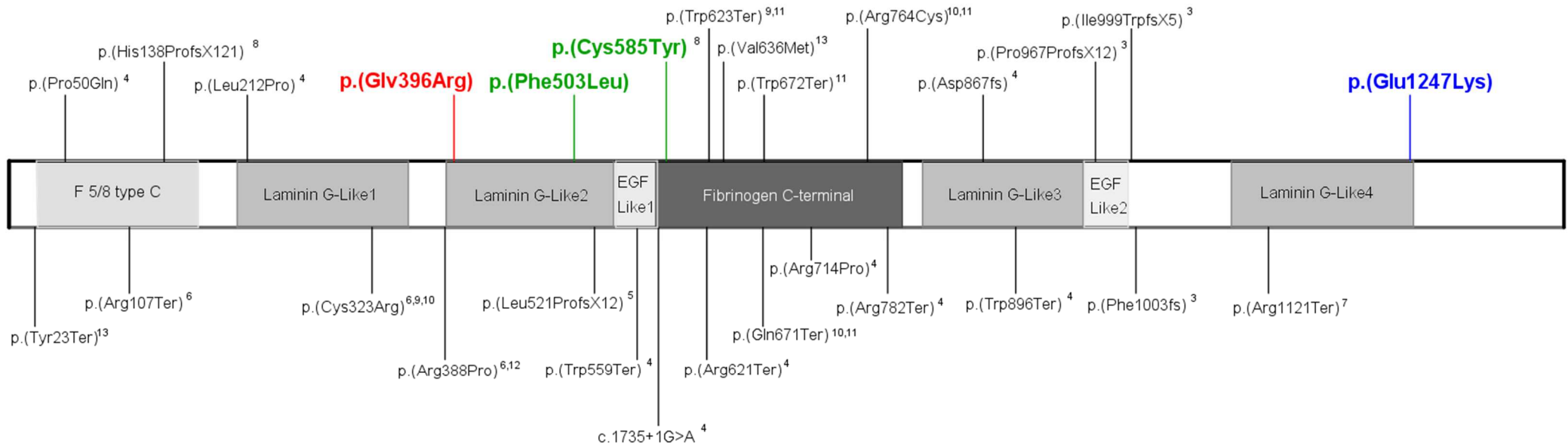


Figure 3: Representation of the Caspr1 protein showing all pathogenic variants reported in literature and in this article (blue writing: variant of children 1 and 2, green writing: variants of children 3 and 4, red writing: variant of children 5 and 6). This representation was derived from Low's article and is completed with the new pathologic variants.