SHORT REPORT

Two novel variants in *CNTNAP1* in two siblings presenting with congenital hypotonia and hypomyelinating neuropathy

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Homozygous frameshift variants in *CNTNAP1* have recently been reported in patients with arthrogryposis and abnormal axon myelination. In two brothers with severe congenital hypotonia and foot deformities, we identified compound heterozygous variants in *CNTNAP1*, reporting the first causative missense variant, p.(Cys323Arg). Motor nerve conductions were markedly decreased. Nerve microscopical lesions confirmed a severe hypomyelinating process and showed loss of attachment sites of the myelin loops on the axons, which could be a characteristic of Caspr loss-of-function. We discuss the pathophysiology of the myelination process and we propose to consider this disorder as a congenital hypomyelinating neuropathy.

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INTRODUCTION

Congenital hypotonia is a non-specific feature of a large range of genetic disorders. Given this strong pathophysiology heterogeneity, a molecular diagnosis is often lacking.¹ In early and severe cases, fetal akinesia can manifest by arthrogryposis. Defects in motor neuron function, neuromuscular transmission or muscle contraction may be involved. In rare cases, constitutive defects in myelin constituents impair the myelination process.

A few congenital hypomyelinating neuropathies (CHN) have been reported so far (MIM#605253). Variants in *EGR2* or *MPZ* genes (MIM159440, MIM129010) can lead to early onset of hypotonia, areflexia, distal muscle weakness, very slow nerve conduction velocities and hypomyelination process.^{2,3} *MPZ* (Myelin Protein Zero) is the major structural protein of peripheral nerve myelin. The *Egr2* transcription factor is critical for expression of myelin proteins and lipids.⁴ A homozygous deletion of an *EGR2* enhancer was also reported in a patient with congenital amyelinating neuropathy.⁵ Recently, variants in *GPR126* were reported in patients with lethal congenital contracture syndrome and lack of myelin basic protein.⁶

Homozygous frameshift variants in *CNTNAP1* (Contactin-associated protein 1) have been identified in seven patients with arthrogryposis and abnormal axon myelination, considered as lethal congenital contracture syndrome (MIM#616286).⁷ *CNTNAP1* encodes Caspr that is essential to the paranodal junctions.⁸ We report on two brothers affected with hypotonia and CHN caused by compound heterozygous *CNTNAP1* variants.

MATERIALS AND METHODS

Clinical assessment

Patient 1 was the first male child of non-consanguineous French parents. He was born eutrophic at 34 WG (weeks of gestation) after a pregnancy complicated by polyhydramnios, treated by amniocentesis. A severe and generalized hypotonia was immediately reported and was associated with respiratory distress requiring intubation, absence of swallowing, foot varus deformity, low gesticulation, weak facial expression and poor eye contact. Brain MRI was normal. Auditory and visual evoked potentials were altered. Electroneuromyogram revealed a severe decrease of sensorimotor nerve conduction velocities at 1 week, suggestive of CHN. Death occurred at 2 months of age.

Patient 2 was the third male child to same couple. Polyhydramnios with decreased fetal movements was noticed at 22 WG, necessitating an amniocentesis. He was born eutrophic at 36 WG, showing no respiratory movements. He died a few minutes after birth, with palliative care procedure. Mild retrolabial and bilateral clubfeet were observed. Muscle biopsy revealed early muscle innervation disorder.

Electron microscopy

Sural nerve biopsy was performed for both children. Samples were fixed for 3 h in 2.5% glutaraldehyde in Scerensen buffer and osmicated for 1 h in 1% OsO₄. Afterwards, they were rinsed in Scerensen buffer, dehydrated in graded acetone and embedded in Epon. Semi-thin sections (1 µm) were stained with toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate. The sections were observed through a JEOL electron microscope.

DNA analysis

Written informed parent consents were obtained for whole-exome sequencing (WES). DNA was extracted from muscle tissue according to standard

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procedure. WES was performed with the Clinical Research Exome kit (Agilent, Santa Clara, CA, USA) on Illumina HiSeq2500 (paired-end sequencing, 2×75 bp). Reads were aligned to the human reference genome sequence (NCBI build 37.3, hg19). Bioinformatics analyses were performed according to the best practices of GATK (v3.4). Variants were annotated using ANNOVAR and filtered with in-house scripts to remove synonymous variants, non-coding variants and variants with an allele frequency above 0.5% in ExAC (<http://exac.broadinstitute.org/>) and EVS (<http://evs.gs.washington.edu/EVS/>) and >1 in a local database of 120 exomes. The possible functional impact of amino-acid changes was predicted by SIFT (Sorting Intolerant from Tolerant), PolyPhen-2 hvar and CADD score (Combined Annotation Dependent Depletion). The Alamut software (Interactive biosoftware) was used to study retained variant sites among different species. Segregation analysis by direct sequencing was then performed in the family to confirm candidate variants. The variants were submitted in ClinVar database.

RESULTS

Nerve biopsy

The same lesions were observed on both biopsies. On sural nerve biopsy semi-thin sections of patient 1, there was a significant loss of myelinated fibers, which was homogeneous between nerve fascicles. Most of the remaining myelinated fibers presented too thin myelin sheaths. Electron microscopic examination showed that a few axons had no myelin and several hypomyelinated fibers were surrounded by proliferations of basal membrane laminae, like onion bulbs (Figure 1). Myelin debris in the cytoplasm of some Schwann cells, in a few

macrophages and in some endothelial cells were also seen. On longitudinal sections, frequent and marked widenings of the nodes of Ranvier were observed. Moreover, most of the paranodal regions were characterized by loss of attachment sites of the myelin loops, which seemed to be associated to Schwann cell processes penetrating between the axon and the loops.

DNA analysis

WES was performed in patient 2 with a 70× mean coverage. After filtering, we identified two heterozygous variants in *CNTNAP1* (NM_003632.2, exons are numbered like in NG_042091.1): c.[967T>C];[1869G>A] (dbSNP rs768554986 with ID 237757 and dbSNP rs878853221 with ID 237756). Missense variant c.[967T>C], p.(Cys323Arg) was found only twice over 120 018 alleles in ExAC and was absent from EVS and 1000genome databases. It was predicted deleterious by SIFT, PolyPhen-2 hvar and CADD (phred score: 25.8). It substitutes a highly conserved cysteine predicted to be involved in a disulfide bond (Uniprot) in a laminin G domain, possibly resulting in protein misfolding. Nonsense variant c.[1869G>A], p.(Trp623*) in exon 13 likely results in the degradation of mRNA by nonsense mediated decay. Both variants were confirmed by Sanger sequencing in the proband (Figure 2). Segregation analysis showed that the variants were inherited from father and mother respectively, not found in healthy sister and both present in affected brother.

Figure 1 sural nerve (case 1) transverse section, electron microscopy. (a) Significant rarefaction of myelinated fibers; some are hypomyelinated (thin arrows). One of them is surrounded by a concentric proliferation of basal laminae (thick arrow). (b) Age-matched control of sural nerve.

Figure 2 (a) Segregation analyses for *CNTNAP1* variants c.[967T>C];[1869G>A], p.(Cys323Arg) and p.(Trp623*) in the two affected brothers, their healthy parents and their healthy sister. (b) Orthologous alignment for p.(Cys323Arg) substitution: cysteine in position 323 is highly conserved across species. Source: UniProt.

DISCUSSION

We report two affected siblings with novel variants in *CNTNAP1*. Previously, seven patients with *CNTNAP1* frameshift variants from four consanguineous families were reported.⁷ All nine patients shared common features namely polyhydramnios, fetal akinesia, severe neonatal hypotonia, facial diplegia, absence of swallowing and of spontaneous breathing and arthrogryposis. The most striking feature in our patients was generalized hypotonia. However, foot deformities were noticed and can be considered as a minor sign of arthrogryposis. Motor nerve conductions were markedly decreased in all patients. The nerve microscopical lesions of our patients showed a severe hypomyelinating process as reported by Laquerrière *et al*. We also observed unusual lesions of the paranodal regions characterized by loss of attachment sites of the myelin loops on the axons.

Frameshift *CNTNAP1* variants previously reported are located in exons 18 and 19. Our patients carry a missense variant in exon 7, probably resulting in non-functional protein and a nonsense variant inducing a premature stop codon in exon 13. *CNTNAP1* encodes a contactin-associated transmembrane receptor (Caspr), with high expression level in central and peripheral nervous system.⁹ Caspr is localized to the paranodal axoglial junction of myelinated axons.¹⁰ The specific organization of Ranvier nodes and paranodal regions is necessary to action potential saltatory conduction.¹¹ These domains are the result of interactions between axons and myelinating glial cells such as Schwann cells in the peripheral nervous system.¹² Caspr interacts with Contactin-1, which is a glycosyl phosphatidyl inositol-anchored neural cell adhesion molecule, to form an extracellular complex with neurofascin resulting to axoglial junction.^{8,13} The missense variant identified in our family is located in a disulfide bond within a laminin G domain, in the extracellular region of Caspr, which seems to be involved in molecular interaction between Caspr and Contactin-1. This variant likely interferes with adhesion process at axoglial junction and explains the abnormal myelin organization. In ExAC database, although 635 missense variants were expected in *CNTNAP1*, 404 were observed with a z-score of 4.5, suggesting that, despite being responsible for a recessive condition, *CNTNAP1* may be intolerant to missense variants.¹⁴

Mice lacking Caspr displayed growth failure with severe neurological defects, including cerebellar syndrome, generalized motor paresis and premature death.⁸ Absence of Caspr resulted in altered distribution of juxtaparanodal components, including Kv1 channels and contactin-1.¹⁵ Finally, dissociations of the myelin loops from the axons were described in optic nerves of *Cntn1*-KO mice.¹⁶ Given that similar lesions were observed in our patients in sural nerve biopsy, such unusual anomalies could be a hallmark of Caspr/Contactin-1 complex loss of function.

In conclusion, our data extend the phenotype associated with *CNTNAP1* variants and confirm Caspr critical role in human

myelination. Considering the suggestive electroneuromyogram pattern, nerve conduction velocities should be performed when severe congenital hypotonia remains unexplained, even in absence of complete arthrogryposis sequence. Hence, we propose to reclassify this phenotype into the congenital hypomyelinating neuropathy group. Finally, our report suggest that abnormal interaction between Caspr and Contactin-1 is the main pathophysiological hypothesis for this rare and severe disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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