J Neuropathol Exp Neurol
Vol. 0, No. 0, 2016, pp. 1–5
doi: 10.1093/jnen/nlw093

ORIGINAL ARTICLE

Contactin-Associated Protein 1 (*CNTNAP1*) Mutations Induce Characteristic Lesions of the Paranodal Region

Jean-Michel Vallat, MD, Mathilde Nizon, MD, Alex Magee, MD, Bertrand Isidor, MD, Laurent Magy, MD, PhD, Yann Péréon, MD, PhD, Laurence Richard, PhD, Robert Ouvrier, MD, Benjamin Cogné, MD, Jérôme Devaux, PhD, Stephan Zuchner, MD, PhD, and Stéphane Mathis, MD, PhD

Abstract

Congenital hypomyelinating neuropathy is a rare neonatal syndrome responsible for hypotonia and weakness. Nerve microscopic examination shows amyelination or hypomyelination. Recently, mutations in *CNTNAP1* have been described in a few patients. *CNTNAP1* encodes contactin-associated protein 1 (caspr-1), which is an essential component of the paranodal junctions of the peripheral and central nervous systems, and is necessary for the establishment of transverse bands that stabilize paranodal axo-glial junctions. We present the results of nerve biopsy studies of three patients from two unrelated, non-consanguineous families with compound heterozygous *CNTNAP1* mutations. The lesions were identical, characterized by a hypomyelinating process; on electron microscopy, we detected, in all nodes of Ranvier, subtle lesions that have never been previously described in human nerves. Transverse bands of the myelin loops were absent, with a loss of attachment between myelin and the axolemma; elongated Schwann cell processes sometimes dissociated the Schwann cell and axon membranes that bound the space between them. These lesions were observed in the area where caspr-1 is located and are reminiscent of the lesions reported in sciatic nerves of caspr-1 null mice. *CNTNAP1* mutations appear to induce characteristic ultrastructural lesions of the paranodal region.

Key Words: CNTNAP1, Contactin, Nerve biopsy, Node of Ranvier.

INTRODUCTION

Congenital hypomyelinating neuropathy ([CHN]; MIM 605253) is a rare syndrome responsible for severe hypotonia and very slow nerve conduction velocities that are usually diagnosed at birth; some cases present with arthrogryposis multiplex (1). Heterozygous mutations in genes required for myelination such as *EGR2* or *MPZ* genes (MIM159440; MIM129010) can induce CHN (2, 3), and a homozygous deletion of an *EGR2* enhancer has also been reported in a patient with a congenital amyelinating neuropathy (4). In this context, nerve biopsy examination may show amyelination or hypomyelination induced by a congenital impairment of myelin formation resulting from anomalies of some axonal or myelin proteins encoded by the mutated genes. *CNTNAP1* encodes contactin-associated protein 1 (caspr-1), an essential component of the paranodal junctions; its mutations have recently been implicated in CHN, sometimes associated with arthrogryposis (1, 5).

We present characteristic, unique, and identical modifications of the paranodal junctions on identified by electron microscopic (EM) examination of nerve biopsies from 3 patients belonging to 2 families; detailed structural peripheral nerve lesions induced by *CNTNAP1* mutations have never previously been reported in human genetic neuropathies.

MATERIALS AND METHODS

The 3 patients were from non-consanguineous parents (Fig. 1A). Polyhydramnios and reduced fetal movements were diagnosed around the 25th week. At birth, they were hypotonic and required ventilatory support. No arthrogryposis was noted. Spinal amyotrophy due to *SMN1* mutations, Prader Willi syndrome, myotonic dystrophy, mitochondrial disorders, and *PMP22*, *GJB1*, and *SLC52A3* point mutations were ruled out. Comparative genomic hybridization array showed no anomaly. Metabolic investigations were normal. A sural nerve biopsy was performed in all patients (immediately postmortem for patient 3).

Written informed parent consents were obtained for whole-exome sequencing. DNA was extracted from leukocytes according to standard procedures. Whole-exome se-

From the Department of Neurology and 'Centre de Référence des neuropathies rares', University Hospital (CHU) Limoges, Limoges, France (JMV, LM and LR); Department of Medical Genetics, University Hospital (CHU) Nantes, Nantes, France (MN, BI and BC); Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast, Northern Ireland (AM); Centre de Référence des Maladies Neuromusculaires, Hôtel Dieu Hospital, Nantes, France (YP); The Institute for Neuroscience and Muscle Research, The Children's Hospital at Westmead, Sydney, New South Wales, Australia (RO); CNRS, CRN2M-UMR 7286, Aix-Marseille University, Marseille, France (JD); Department of Human Genetics, Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida (SZ); Department of Neurology, Nerve-Muscle Unit, University Hospital (CHU) Pellegrin, Bordeaux, France (SM).

Send correspondence to: Prof. Jean-Michel Vallat, MD, Department of Neurology and 'Centre de Référence des neuropathies rares', University Hospital (CHU) Limoges, 2 Avenue Martin Luther King, 87042, Limoges, France; E-mail: jean-michel.vallat@unilim.fr

The authors have no duality or conflicts of interest to declare.

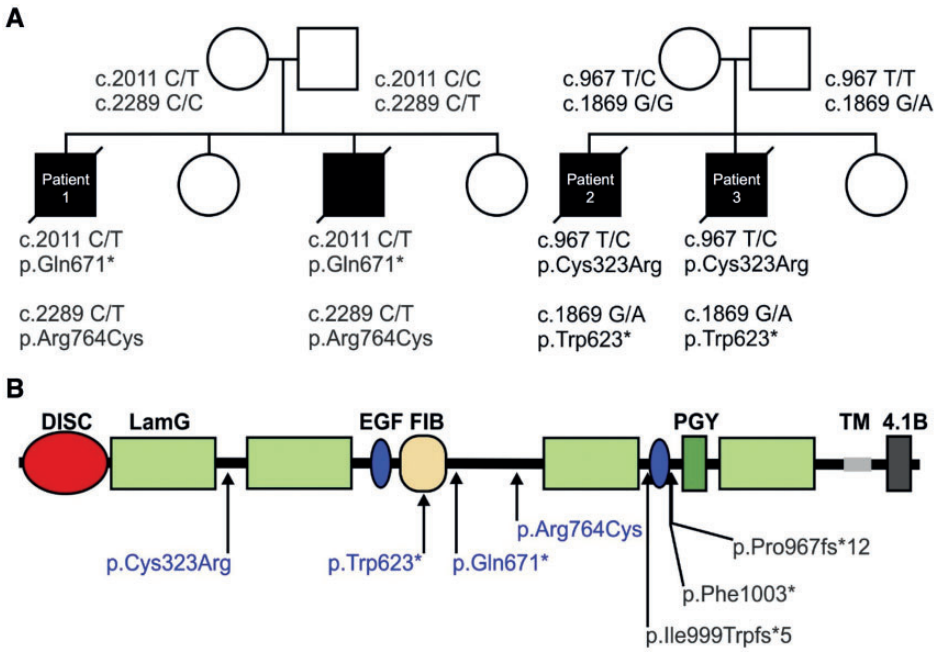

FIGURE 1. Mutations identified in *CNTNAP1*. **(A)** Pedigrees for the 2 families are shown. The patients carry compound heterozygous mutations. The nucleotide and amino acid changes indicated are based on NM_003632.2 and NP_003623.1 reference sequences, respectively (open symbols: unaffected; filled symbols: affected). **(B)** Scheme of the putative domains of caspr-1. The mutations described in this study are shown in blue, those described in (1) are indicated in black (DISC: discoidin-like domain; EGF: epidermal growth factor; FIB: fibrinogen-like domain; LamG: laminin G; PGY: glue domain containing PGY repeats; TM: transmembrane domain; 4.1B: protein 4.1B binding domain).

quencing analysis was performed on patients 1 and 3 (patients 2 and 3 were brothers) and on their 2 sets of parents.

Sural nerve biopsies were performed in the 3 patients and processed as described elsewhere (6). One fragment was fixed in 10% formaldehyde, then embedded in paraffin; another fragment was fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide and embedded in epoxy resin. Cross-sections (1-µm thick) were stained with toluidine blue and examined under the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined using a JEOL (1011) electron microscope at 80 keV. Numerous longitudinal sections of each biopsy were cut to study the node of Ranvier areas in detail.

RESULTS

Clinical Presentations

Patient 1 was noted to have absent swallowing, facial diplegia, complete ptosis, dolicocephaly, epicanthic folds, thickened nares, thickened lips, narrow ridged palate, thickened gums with a notch in the upper midline, and partial bilateral 2–3 toe syndactylies. He was areflexic. Some rare voluntary limb movements were observed. His brother also presented with polyhydramnios and reduced fetal movements. At birth, he was unable to breathe. He had no swallow reflex and was areflexic. He died at 4 hours of age. No nerve biopsy was performed.

At birth, Patient 2 was noted to have absence of swallowing, foot varus deformity, low gesticulation, weak facial expression, and poor eye contact. Brain MRI and electroencephalography were normal. Electroneuromyography revealed a severe and homogeneous reduction (~10 m/s on average) of motor nerve conduction velocities; Sensory nerve action potentials were not obtainable. He died at 2 months of age.

Patient 3 was the brother of Patient 2 and the third male child of the same couple. He was born with absence of respiratory movements and died a few minutes after birth. Mild retrognathism and bilateral clubfeet were observed. No electroneuromyography was done. There were no other similar cases in these 2 families. The parents were clinically normal and there was no consanguinity.

DNA Analysis

The 3 patients were compound heterozygous in *CNTNAP1* gene (Fig. 1). In Patient 1, we identified a paternal missense mutation c.2289C > T (p.Arg764Cys) and a maternal nonsense mutation c.2011C > T (p.Gln671*). Patients 2 and 3 carried the missense mutation c.967T > C (p.Cys323Arg) and the nonsense mutation c.1869G > A (p.Trp623*). Segregation was confirmed in the parents.

Nerve Biopsies

The pathological results were essentially the same for the 3 patients. In semithin sections, myelinated fibers were significantly and homogeneously reduced within nerve fascicles. Most of the myelinated fibers showed abnormally thin myelin

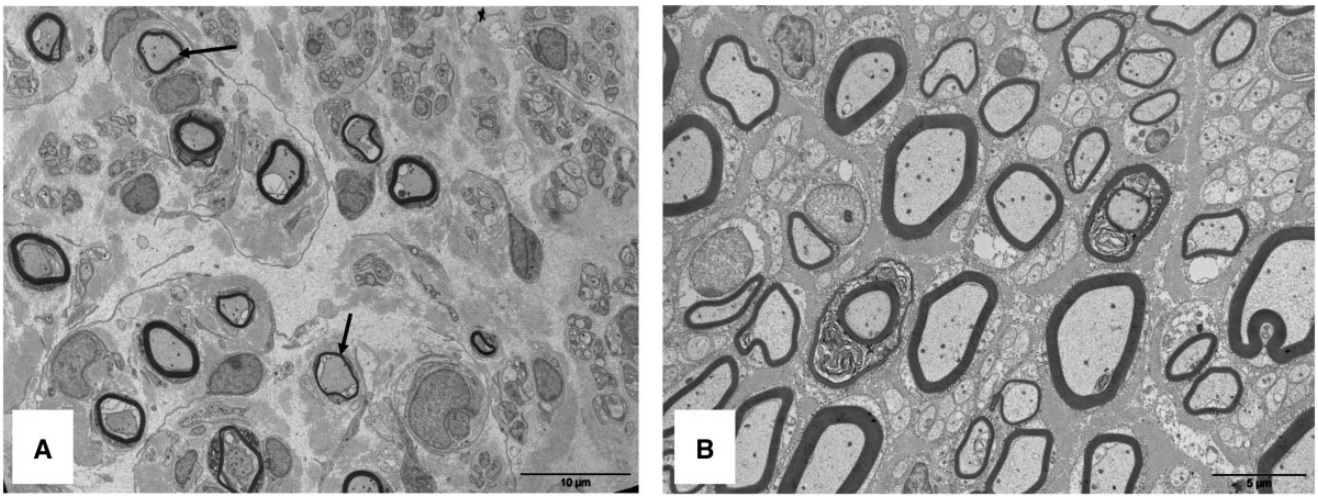

FIGURE 2. Sural nerve biopsy (electron microscopy, transverse section). **(A)** Patient 1. There is moderate rarefaction of myelinated fibers; some have myelin sheaths that are too thin (arrows), which is characteristic of hypomyelinating processes. **(B)** Normal control subject: the thickness of myelin sheaths is more homogeneous and in normal proportion with diameters of axons.

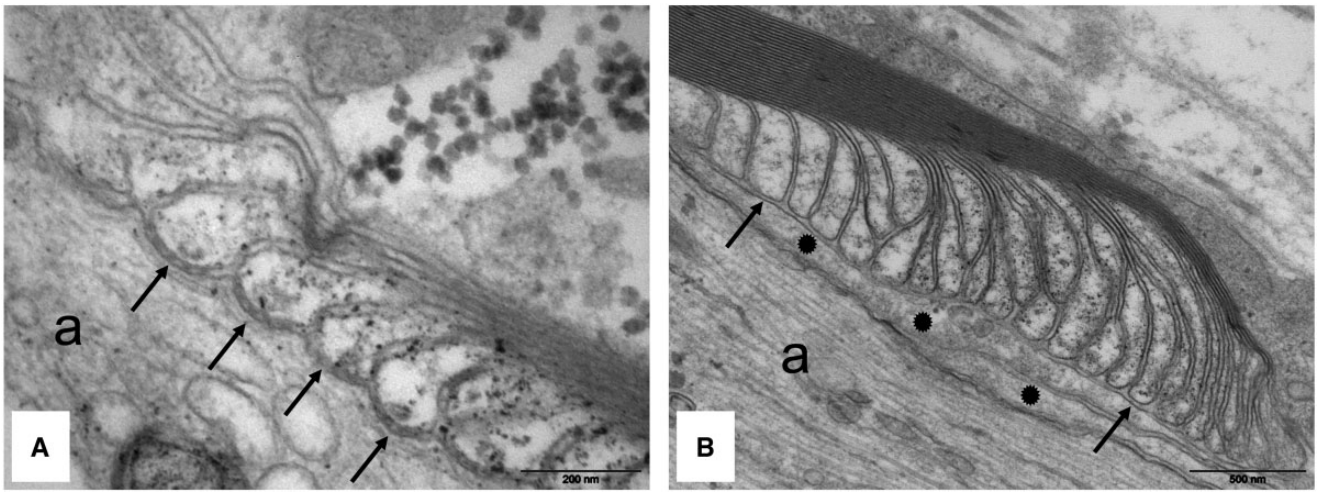

FIGURE 3. Sural nerve biopsy (electron microscopy, longitudinal section). **(A)** Paranodal region of a normal nerve at the level of myelin loops with transverse bands (corresponding to arrows) and axon (a). **(B)** Absence of transverse bands in Patient 1 (arrows); elongated processes of cytoplasm (•) have dissociated and penetrated the normal axo-glial junction between the axon (a) and myelin loops.

sheaths; g-ratio, (as described elsewhere (7)), of the thinly myelinated fibers was 0.8 (range 0.76–0.87) for patient 1, 0.85 (range 0.81–0.94) for Patient 2, 0.8 (range 0.78–0.85) for Patient 3, and 0.74 (range 0.69–0.79) for the normal control subject whose biopsy is shown in Figure 2B. EM examination confirmed these aspects and showed that several myelinated fibers were surrounded by proliferations of basal membrane laminae, i.e. 'onion-bulb'-like formations. Myelin debris in the cytoplasm of some Schwann cells and in a few macrophages in the endoneurial space was also seen. In longitudinal sections, we observed a frequent and marked widening of the nodes of Ranvier, typical of a demyelinating neuropathy.

All the examined paranodal regions were characterized by the absence of any transverse band of the myelin loops, with absence of close contact between myelin and axolemma (Figs. 3, 4). The space between paranodal loops and axolemma had a mean width of 17 ± 3 nm (range 11–21, 38 loops from 3 patients counted) in normal controls, and a mean width of 21 ± 4 nm (range 13–28, 17 loops from 2 controls counted) in mutated samples. In this area, which was characterized by a loss of attachment between the axon and the paranodal loops, processes of Schwann cell cytoplasm sometimes penetrated and dissociated the virtual space between the axon and the loops (Fig. 5). We did not observe these features in 3 normal controls, 4 cases of various congenital hereditary hypomyelinating neuropathies of about the same age (induced by *PMP22*, *MFN2*, and *MTMR2* mutations), or in more than 10 acquired neuropathies.

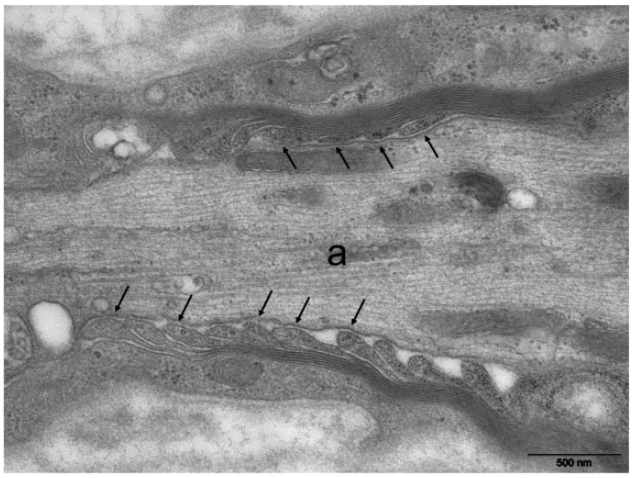

FIGURE 4. Sural nerve biopsy (electron microscopy–longitudinal section). Patient 3. Paranodal transverse bands are entirely absent resulting in a widening of the paranodal junctional gap (arrows) between the loops and the axolemma.

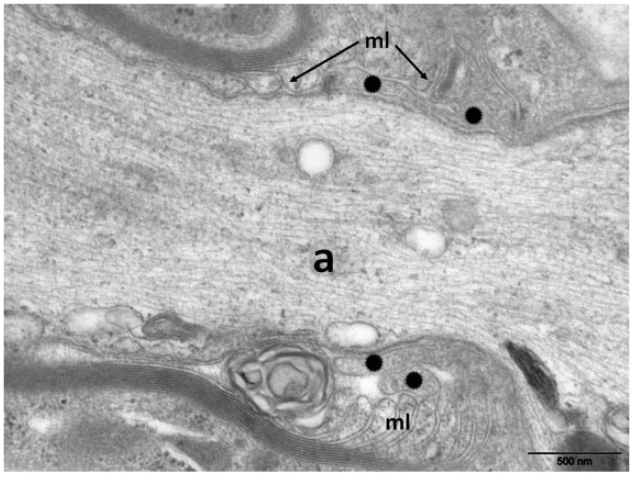

FIGURE 5. Sural nerve biopsy (electron microscopy–longitudinal section). On both sides of this paranodal region (patient 2) there are cytoplasmic processes (•) that penetrate between the axon (a) and myelin loops (ml).

DISCUSSION

We describe novel mutations in *CNTNAP1* associated with CHN. Caspr-1 is a transmembrane protein present on the axonal surface that is an essential component of the paranodal junctions of the PNS and CNS. It is involved in the maintenance of septate-like paranodal axoglial junctions that display transverse bands between the terminal Schwann cell loops and the axon, and in paranodal function. Caspr-1 interacts with contactin-1, a glycosyl phosphatidyl inositol-anchored neural cell adhesion molecule (8). The contactin-1/caspr-1 complex associates with neurofascin-155 (NF155) located on the paranodal Schwann cell membranes and hence contribute to junctional attachment. The extracellular regions of caspr-1 contain several domains (Fig. 1B), whose functions are not completely elucidated but appear important for the interaction with contactin-1 and NF155. The p.Arg764Cys and p.Cys323Arg missense mutations are not located within the putative domains and their impact on caspr-1 function is unclear. However, the nonsense mutations p.Gln671* and p.Trp623* are suspected to generate a truncated protein lacking half the extracellular domains, the transmembrane domain, and the cytosolic regions. Such truncated proteins are unlikely to bind contactin-1 and caspr-1.

The lesions in nerve specimens from these 3 patients indicate severe hypomyelinating process; they are in keeping with the finding of Laquerrière et al in other patients with *CNTNAP1* mutations (1). Moreover, we observed identical ultrastructural lesions in the nerve biopsies of our 3 patients. Our EM study detected no transverse band in these cases; such microscopic abnormalities have never previously been described in humans. In addition, the spaces between the myelin loops and the axolemma were wider than in controls with a loss of attachment of the myelin loops on the axon. Schwann cell cytoplasmic processes were detected in these widened spaces between the axons and the loops. Such anomalies could be the results of *CNTNAP1* mutations because identical dissociations of the loops from the axon have been described in peripheral nerves (8–10) and in optic nerves of caspr-1 and contactin-1 null mice (11). These genetically induced perturbations of the specific organization of paranodal regions likely reduce nerve conduction velocities in mouse models (12), as in our Patient 2. Bhat *et al* consider that cellular processes that are interposed between the terminal loops and the axon correspond to Schwann cell microvilli processes (8). These lesions (which to date, have never been described in other conditions) involved the area in which caspr-1 is located. We have not observed by EM such anomalies in genetic neuropathies induced by other gene mutations (*PMP22, MPZ, MFN2*, *MTMR2*) or in *PMP22* duplication. This indicates that these findings are characteristic of *CNTNAP1* mutations. The ultrastructural observations of these human nerve biopsies and the anomalies observed in *CNTNAP1* mice emphasize the crucial role of caspr-1 in the junctional attachment of myelin at the paranode. It is worth noting that *CNTNAP1* mutations appear to have a stronger impact than the genetic deletion of caspr-1 gene because amyelination was detected in the 3 patients but not in caspr-1 null mice. The reasons behind this are unclear. One possibility could be that *CNTNAP1* mutations may affect contactin-1 expression, which is not the case in caspr-1 null mice (9).

In conclusion, these ultrastructural observations indicate that *CNTNAP1* mutations induce characteristic ultrastructural lesions of the paranodal region, and that caspr-1 protein is essential for the formation of normal axoglial junctions. Furthermore, they show that detailed microscopic analysis of nerve lesions may orientate the search for mutations in specific genes in patients with genetic neuropathies (13).

REFERENCES

1. Laquerrière A, Maluenda J, Camus A, et al. Mutations in *CNTNAP1* and *ADCY6* are responsible for severe arthrogryposis multiplex congenita with axoglial defects. Hum Mol Genet 2014;23:2279–89

2. Warner LE, Mancias P, Butler IJ, et al. Mutations in the early growth response 2 (*EGR2*) gene are associated with hereditary myelinopathies. Nat Genet 1998;18:382–4
3. Szigeti K, Wiszniewski W, Saifi GM, et al. Functional, histopathologic and natural history study of neuropathy associated with *EGR2* mutations. Neurogenetics 2007;8:257–62
4. Funalot B, Topilko P, Arroyo MA, et al. Homozygous deletion of an *EGR2* enhancer in congenital amyelinating neuropathy. Ann Neurol 2012;71:719–23
5. Pizzino A, Murphy J, Bloom M, et al. Mutations in CNTNAP1 cause severe arthrogryposis multiplex congenita with distinct neuroradiologic features. Columbus, Ohio: 43rd Annual Meeting of the Child-Neurology-Society (October 22–25, 2014)
6. Vallat JM, Funalot B, Magy L. Nerve biopsy: requirements for diagnosis and clinical value. Acta Neuropathol 2011;121:313–26
7. Goebbels S, Oltrogge JH, Kemper R, et al. Elevated phosphatidylinositol 3,4,5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination. J Neurosci 2010;30:8953–64
8. Bhat MA, Rios JC, Lu Y, et al. Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 2001;30:369–83
9. Boyle ME, Berglund EO, Murai KK, et al. Contactin orchestrates assembly of the septate-like junctions at the paranode in myelinated peripheral nerve. Neuron 2001;30:385–97
10. Gordon A, Adamsky K, Vainshtein A, et al. Caspr and caspr2 are required for both radial and longitudinal organization of myelinated axons. J Neurosci 2014;34:14820–6
11. Colakoglu G, Bergstrom-Tyrberg U, Berglund EO, et al. Contactin-1 regulates myelination and nodal/paranodal domain organization in the central nervous system. Proc Natl Acad Sci USA 2014;111:E394–403
12. Rosenbluth J. Multiple functions of the paranodal junction of myelinated nerve fibers. J Neurosci Res 2009;87:3250–8
13. Mathis S, Goizet C, Tazir M, et al. Charcot-Marie-Tooth diseases: an update and some new proposals for the classification. J Med Genet 2015; 52:681–90