Pediatric Neurology 93 (2019) 43–49

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Clinical Observations

CNTNAP1-Related Congenital Hypomyelinating Neuropathy

Harry Lesmana, MD [a, b], Marissa Vawter Lee, MD [c], Seyed Ali Hosseini, MD, PhD [d], T. Andrew Burrow, MD [e], Barbara Hallinan, MD, PhD [c], Kevin Bove, MD [f, g], Mark Schapiro, MD [c], Robert J. Hopkin, MD [h, *]

[a] Department of Hematology, St. Jude Children's Research Hospital, University of Tennessee Health Science Center, Memphis, Tennessee
[b] Department of Oncology, St. Jude Children's Research Hospital, University of Tennessee Health Science Center, Memphis, Tennessee
[c] Division of Neurology, Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, Ohio
[d] Claritas Genomics, Boston, Massachusetts
[e] Section of Genetics and Metabolism, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, Arkansas
[f] Division of Pathology, Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, Ohio
[g] Division of Laboratory Medicine, Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, Ohio
[h] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, Ohio

ARTICLE INFO

Article history:
Received 11 July 2018
Accepted 24 December 2018
Available online 28 December 2018

Keywords:
Congenital hypomyelinating neuropathy
CNTNAP1
Congenital hypotonia
Cranial nerve palsies
Seizures

ABSTRACT

Background: Congenital hypomyelinating neuropathy is a rare form of hereditary peripheral neuropathy characterized by nonprogressive weakness, areflexia, hypotonia, severely reduced nerve conduction velocities, and hypomyelination. Mutations in contactin-associated protein 1 (*CNTNAP1*) were recently described as a cause of congenital hypomyelinating neuropathy. *CNTNAP1*-associated congenital hypomyelinating neuropathy is characterized by severe hypotonia, multiple distal joint contractures, and high mortality in the first few months of life.
Methods: Whole-exome sequencing was performed in two siblings with congenital hypotonia. Detailed phenotyping data were compared with previously reported cases.
Results: A novel, heterozygous compound mutation of CNTNAP1 was identified in both siblings. We also reviewed 17 patients harboring 10 distinct mutations from previously published studies. All patients presented with severe hypotonia, respiratory distress, and multiple cranial nerve palsies at birth. Six of 19 patients survived beyond infancy and required chronic mechanical ventilation. Seizures were common in the surviving patients.
Conclusions: These findings suggest that *CNTNAP1*-related congenital hypomyelinating neuropathy is a distinct form of hereditary neuropathy that affects both the central and peripheral nervous systems with no clear phenotype-genotype correlation. Our findings also indicate that arthrogryposis multiplex congenita and early lethality are not universal outcomes for patients with congenital hypomyelinating neuropathy.

Introduction

Congenital hypomyelinating neuropathies (CHNs, OMIM 605253) are a rare group of hereditary peripheral neuropathies presenting as hypotonia, areflexia, distal muscle weakness and atrophy, slow nerve conduction velocities, and hypomyelination of nerve fibers.[1,2] CHNs are clinically and genetically heterogeneous and can be autosomal dominant or recessively inherited.

The clinical spectrum of CHN is associated with mutations in myelination genes, including myelin protein zero (*MPZ*), myotubularin-related 2 (*MTMR2*), peripheral myelin protein 22 (*PMP22*), and early growth response 2 (*EGR2*).[3-5] Homozygous truncating mutations in contactin-associated protein 1 (*CNTNAP1*) were recently described in four unrelated consanguineous families presenting with multiple contractures at birth and reduced conduction velocities attributed to hypomyelinated axons.[6]

Here, we report two siblings from a nonconsanguineous African American family with biallelic mutations in *CNTNAP1*. We also performed a systematic review of research articles published from 1990 to 2018 in PubMed with the following search terms: "congenital hypomyelinating syndrome," "arthrogryposis multiplex congenital," "*CNTNAP1*," and "nodopathies." We reviewed five

Conflicts of interest: The authors declare no conflict of interest.

* Communications should be addressed to: Hopkin; Division of Human Genetics; Cincinnati Children's Hospital Medical Center; 3333 Burnet Avenue; MLC 4006; Cincinnati, OH 45229.
E-mail address: rob.hopkin@cchmc.org (R.J. Hopkin).

https://doi.org/10.1016/j.pediatrneurol.2018.12.014
0887-8994/

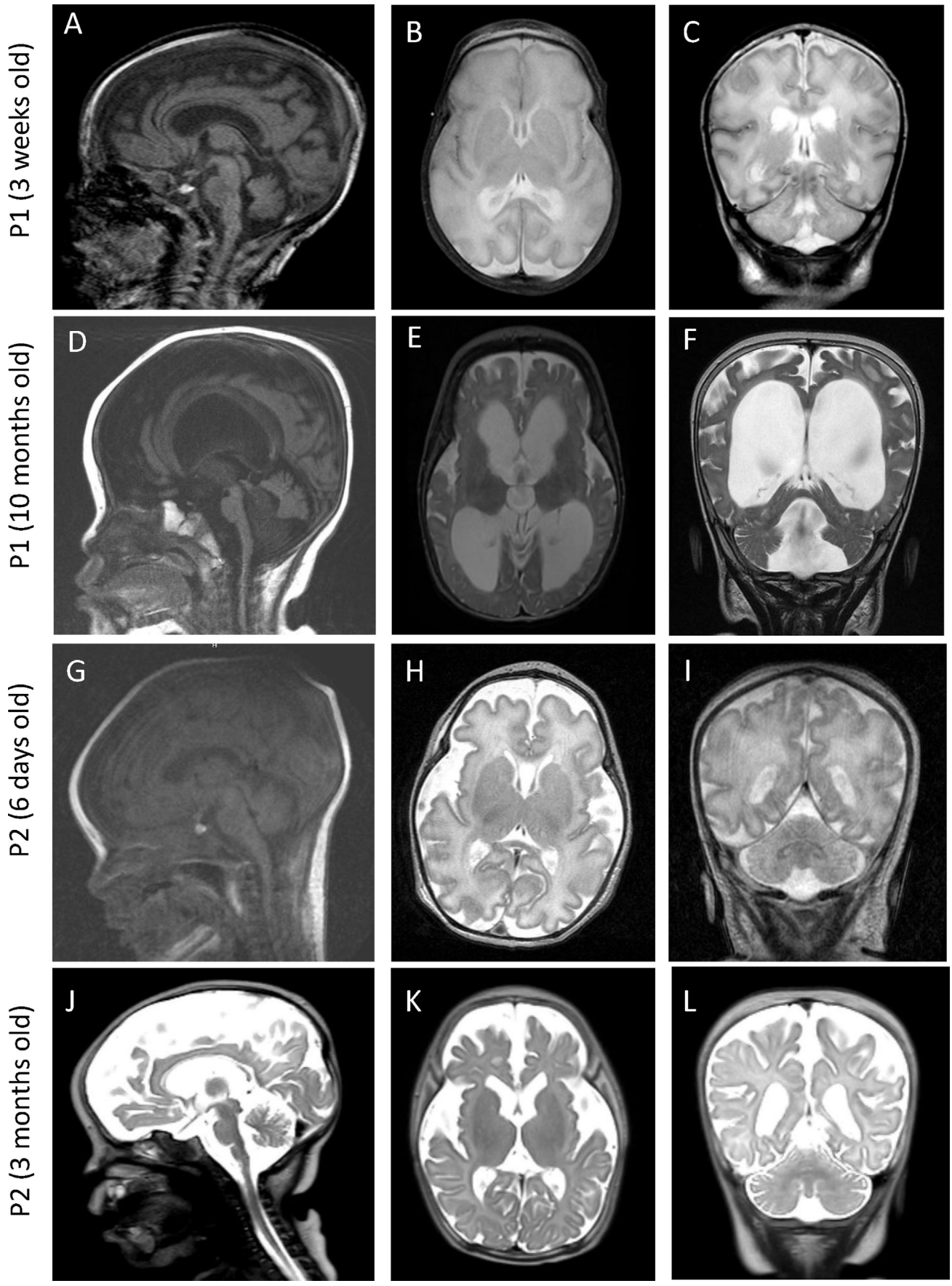
P1 (3 weeks old)
P1 (10 months old)
P2 (6 days old)
P2 (3 months old)
A
B
C
D
E
F
G
H
I
J
K
L

FIGURE 2. Dysmorphology findings. Both siblings exhibited myopathic facial expression with absence of facial and orobulbar muscle movement, narrow face, flat facial profile, and micrognathia. These dysmorphic facial features are secondary to facial nerve palsy. Pictures were obtained at six years for Patient 1 (P1) and at one year for Patient 2 (P2). The color version of this figure is available in the online edition.

previous publications and extracted phenotype and genotype data.[6-10]

Patient Descriptions

Patient 1

This girl was born at 30-weeks' gestation via spontaneous vaginal delivery. Decreased fetal movement was noted throughout pregnancy. Prenatal ultrasounds revealed polyhydramnios. She required resuscitation with positive-pressure ventilation and intubation at birth. Micrognathia, generalized hypotonia, minimal spontaneous movements, myopathic facies, multiple cranial nerve palsies (i.e., bilateral vocal cord paralysis, bilateral sensorineural hearing loss, facial diplegia, impaired gagging, and swallowing), and areflexia were observed on initial examination. Joint contractures were not evident. A tracheostomy tube was placed after multiple failed extubation attempts. She required surgical placement of a gastrostomy tube for nutrition.

Brain magnetic resonance imaging (MRI) at three weeks of age revealed delayed sulcation and myelination, consistent with prematurity. A follow-up MRI at four months (not shown) revealed prominent extra-axial spaces, delayed myelination, and moderate ventricular enlargement, which had progressed at 10 months (Fig 1). Because of progressive enlargement of head circumference (z score of +3.25 at 10 months old) and cardiopulmonary decompensation, communicating hydrocephalus was diagnosed, and a ventriculoperitoneal shunt was placed at 11 months.

She had nonepileptic abnormal movements as a neonate and experienced infantile spasms at nine months, which were treated with adrenocorticotropic hormone therapy. Background electroencephalograph activity remained abnormal, with moderate slowing and multifocal spike waves. She is now six years old and currently receives levetiracetam monotherapy, with no further seizures. Her most recent neurological examination noted macrocephaly, myopathic facies, visual fixation without tracking, facial diplegia with intact eyelid blinking, global hypotonia of extremities, focal spasticity, and limited spontaneous movements. She is nonverbal, nonambulatory, feeding tube dependent, and ventilator dependent when sleeping.

Patient 2

This boy was born at 35-weeks' gestation because of premature rupture of membranes and preterm labor. Pregnancy was complicated by prenatally diagnosed congenital pulmonary airway malformation and polyhydramnios, requiring amnioreduction at 32-weeks' gestation. Fetal akinesia was not evident. After birth, he required positive-pressure ventilation and intubation. Generalized hypotonia, multiple cranial nerve palsies (including facial diplegia, impaired gag, and vocal cord paralysis), minimal spontaneous movements, and areflexia were noted on initial examination (Fig 2). Joint contractures were not evident. He received surgical resection of the congenital pulmonary airway malformation, and a tracheotomy tube was placed after multiple failed extubation attempts.

Brain MRIs at one week and three months revealed diffuse enlargement of ventricles and extra-axial fluid spaces, suggestive of parenchymal volume loss (Fig 1). He also had progressive enlargement of head circumference but did not require a ventriculoperitoneal shunt. Although he had no clinical evidence of seizures, background electroencephalograph activity was abnormal, with excessive discontinuity. He is now eight months

FIGURE 1. Brain magnetic resonance imaging findings. (A) Sagittal, (B) axial, and (C) coronal images of Patient 1 (P1) at three weeks, demonstrating delayed sulcation and myelination pattern consistent with prematurity but otherwise no structural brain abnormalities. (D) Sagittal, (E) axial, and (F) coronal follow-up images at 10 months, demonstrating prominent extra-axial spaces consistent with brain atrophy, delayed myelination, and moderate ventricular enlargements. (G) Sagittal, (H) axial, and (I) coronal images of Patient 2 (P2) at one week, demonstrating delayed myelination interval developmental of diffuse enlargement of ventricles and extra-axial fluid spaces, suggestive of parenchymal volume loss. (J) Sagittal, (K) axial, and (L) coronal images of Patient 2 at three months.

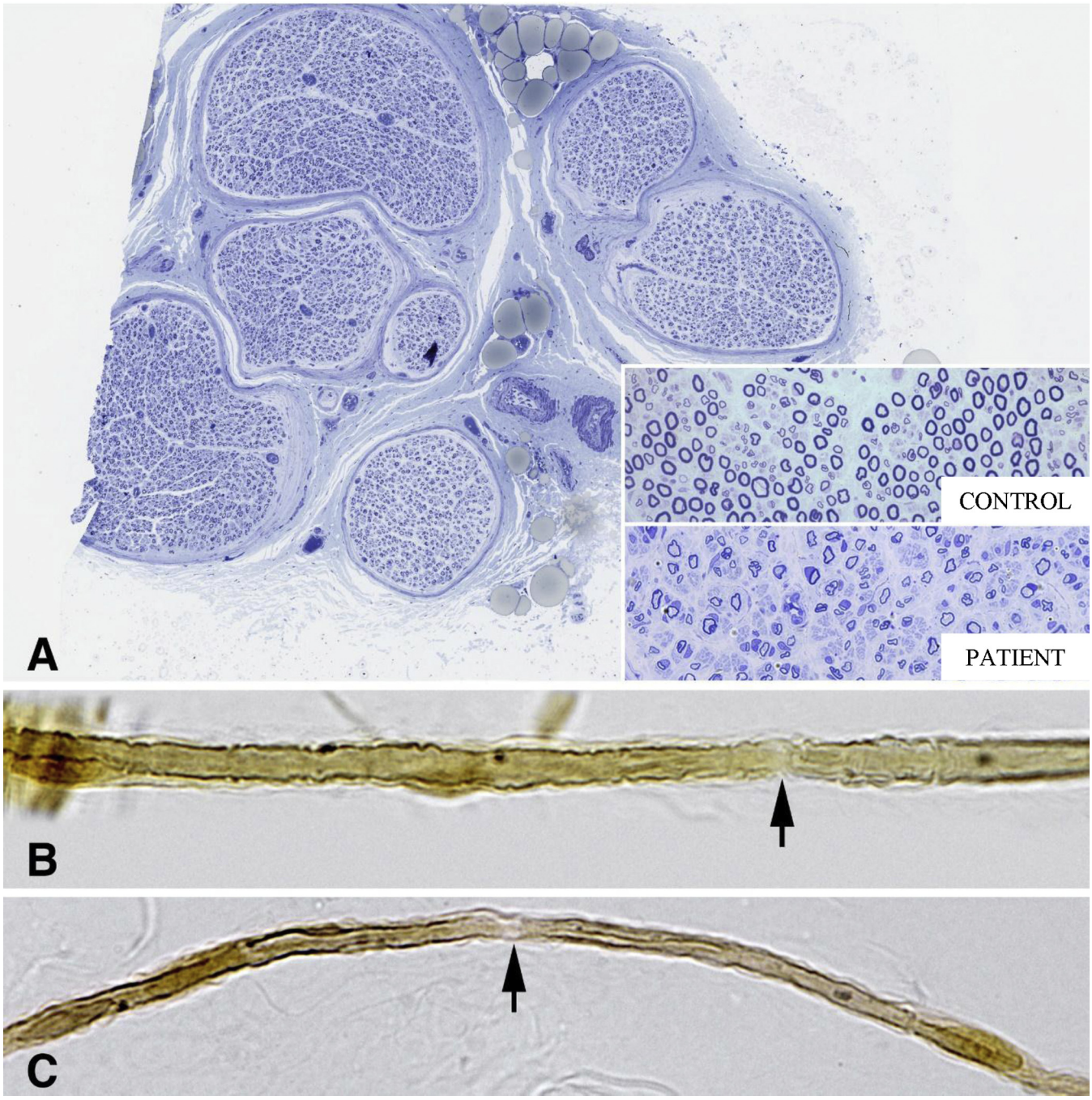

FIGURE 3. Electron microcopy findings of sural nerve biopsy. (A) All fascicles of this mixed myelinated sensory nerve exhibited uniform hypomyelination, with a striking absence of large-diameter myelinated axons. Endoneural stroma was not conspicuously increased, and no inflammatory infiltrate was apparent. Inset: Myelin thickness and axon caliber in patient sural nerve (below) and control sural nerve (above); magnification, ×400. (B, C) Individual teased healthy sensory axons of different calibers show uniform hypomyelination. Internodes of Ranvier (arrows) are wider for Patient 1 than for control tissue and are flanked by tapered zones of increasing hypomyelination. Attached to the outside of each axon are the fine filaments of excess stroma. The color version of this figure is available in the online edition.

old, feeding tube and tracheostomy tube dependent, and requires 24-hour ventilatory support. His most recent neurological examination was similar to that of his sister's, although he kicks his legs more actively and will pull away when stimulated.

Clinical findings

No prenatal genetic testing was performed for Patient 1. Routine chromosome analysis revealed a 46, XX karyotype, and single-nucleotide polymorphism microarray findings were non-anomalous. Serum creatine kinase, lactate, pyruvate, ammonia, amino acid profiles, urine organic acids, cerebrospinal fluid neurotransmitters, and lysosomal enzymes were all within normal range. Electromyography and nerve conduction velocity performed at four months revealed mixed demyelinating and axonal motor peripheral neuropathy. At 10 months, she received skeletal muscle and sural nerve biopsies. The muscle biopsy revealed type 1 myofiber size disproportion. The nerve biopsy revealed reduced

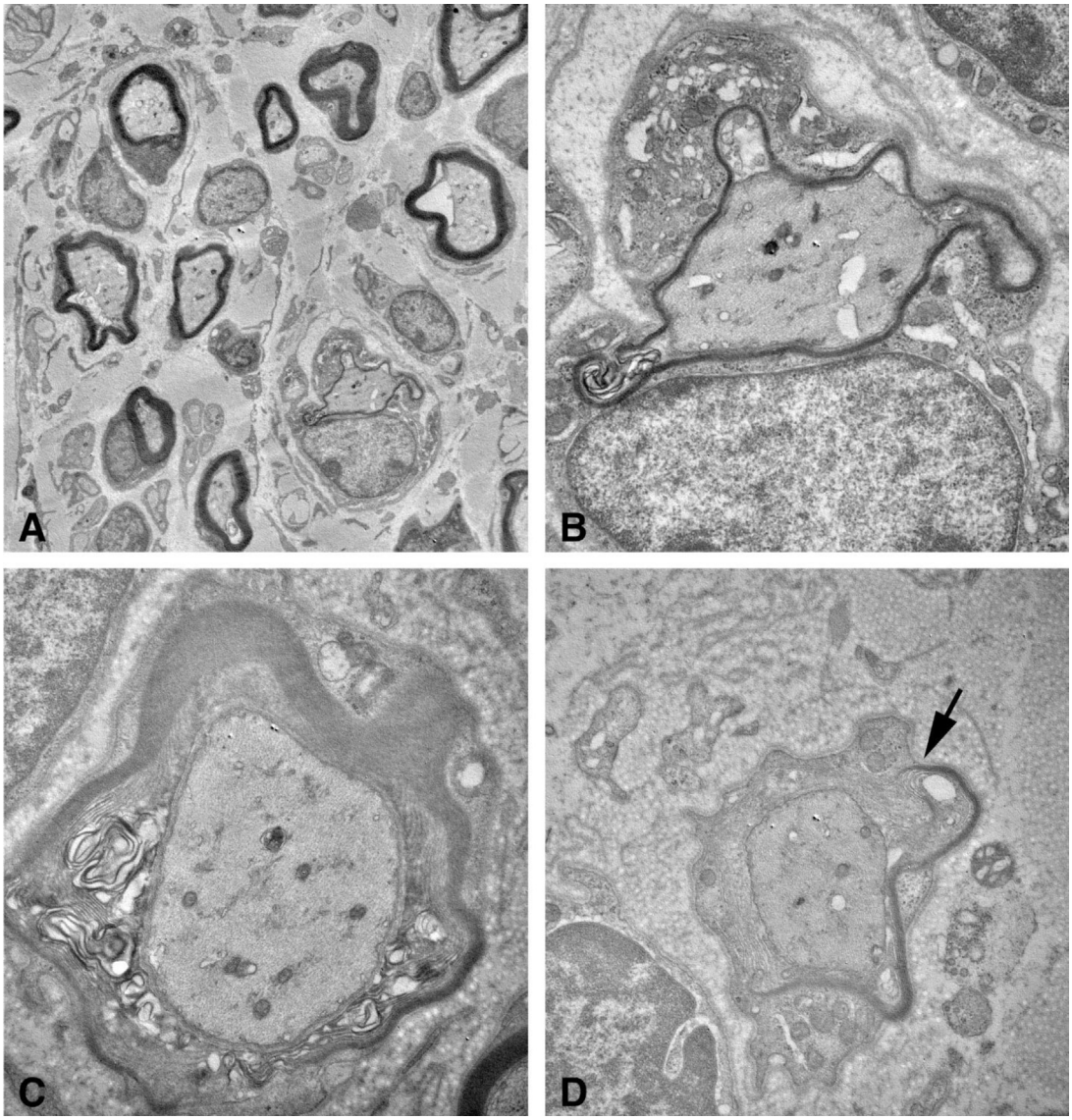

FIGURE 4. Ultrastructure of hypomyelinating peripheral neuropathy. (A) Cluster of variably hypomyelinated axons with differing diameters. Periaxonal stroma is variably increased. Scattered small autonomic fibers are interspersed. (B) Severely hypomyelinated axon is enclosed within ample cytoplasm of a Schwann cell with duplicated basement membrane (upper right). (C) Extensive degeneration of inner myelin layers of a hypomyelinated but otherwise healthy axon. (D) Axon profile at the node of Ranvier is partially myelinated. The tapered myelin laminae at the node (arrow) appear healthy for this site. Fragmented excessive basement membrane material (upper left) is abnormal.

numbers of large myelinated axons and abnormally thin myelin in smaller axons, consistent with mild hypomyelination (Figs 3 and 4). Because active demyelination or remyelination was not evident on nerve biopsy, the possibility of a CHN was considered, and a CHN genetic panel (including *MPZ* and *EGR2*) was performed by Athena Diagnostics (Marlborough, MA). The CHN panel was negative for pathogenic variants.

Because of generalized hypotonia, Patient 2 received a clinical evaluation for neonatal hypotonia. Electromyography and nerve conduction velocity revealed very similar findings to those of his sister. Given similar clinical findings and previously unresolved genetic evaluations, we performed whole-exome sequencing of both children and their parents. We identified compound heterozygous c.413_417delACTTC (p.His138Profs*121) and c.1754G>A (p.Cys585Tyr) variants in *CNTNAP1*. The first variant is a five-nucleotide deletion (c.413-417) that creates a premature of stop codon 120 nucleotides downstream of the variant. This deletion occurs in a position much earlier than other previously reported frameshift variants in *CNTNAP1* (Table) and is expected to exert a loss-of-function effect because of nonsense-mediated decay or by translation of a nonfunctioning or abnormal protein. The missense variant (c.1754G>A) affects a highly conserved nucleotide and amino acid and is predicted to be deleterious by sorting intolerant from tolerant, PolyPhen-2, and MutationTaster. This variant has not been reported in any disease-specific or gene-specific databases, such as ClinVar or human gene mutation database, nor has it been reported in the 1000 Genomes or National Heart, Lung, and Blood Institute exome sequencing project control data sets. Although further functional studies are needed to confirm pathogenicity of this missense variant, the clinical presentation of the patients supports the pathogenicity of this variant.

Systematic review

We reviewed 17 previously reported patients from nine unrelated families with confirmed mutations in *CNTNAP1* (Table). All

TABLE.
Clinical Features of Molecularly Confirmed Cases of *CNTNAP1*-Related Congenital Hypomyelinating Neuropathy

Case No.	Reference	Patient Code	Mutation	PH	FA	RD	Hypotonia	Areflexia	CNP	MGN	CP	AMC	ICV	Hypomyelination (Nerve Biopsy)	Seizures	BA	Age at Death
1	*	P1	p. [H138PfsX121]; p. [C585Y]	+	+	+	+	+	+	+	−	−	+	+	+†	+	Alive at 6 years‡
2	*	P2	p. [H138PfsX121]; p. [C585Y]	+	−	+	+	+	+	+	−	−	+	+	−	+	Alive at 8 months‡
3	7	NA	p. [R388P]; p.[R388P]	−	−	+	+	+	+	−	−	+	+	+	NA	−	1 month
4	8	IV.1	p. [L521PfsX12]; p. [L521PfsX12]	+	NA	+	+	NA	+	NA	NA	NA	+	NA	−	+	Alive at 13 years‡
5	8	IV.5	p. [L521PfsX12]; p. [L521PfsX12]	+	NA	+	+	NA	NA	NA	NA	+	NA	NA	NA	NA	1 hour
6	9	P1	p. [Q671*]; p. [R764C]	+	NA	+	+	−	+	+	+	+	NA	NA	+§	+	Alive at 12 years‡
7	9	P2	p. [Q671*]; p. [R764C]	+	NA	+	+	−	+	+	+	+	NA	NA	+§	+	Alive at 9 years†
8	9	P3	p. [Q671*]; p. [R764C]	+	NA	+	+	NA	+	+	−	−	NA	NA	−	NA	Alive at 6 months‡
9	9	P4	p. [Q671*]; p. [R764C]	+	+	+	+	+	+	+	−	−	+	NA	+‖	+	4 months
10	9	P5	p. [Q671*]; p. [R764C]	+	+	+	+	+	+	+	−	−	NA	NA	−	NA	4 hours
11	10	P1	p. [C323R]; p. [W623*]	+	+	+	+	+	+	−	−	+	+	+	NA	NA	2 months
12	10	P2	p. [C323R]; p. [W623*]	+	+	+	+	+	+	+	−	+	+	+	NA	NA	1 hour
13	6	A641	p. [P967PfsX12]; p. [P967PfsX12]	+	+	+	+	+	+	NA	NA	+	+	+	NA	NA	10 days
14	6	A641	p. [P967PfsX12]; p. [P967PfsX12]	+	+	+	+	+	+	NA	NA	+	+	+	NA	NA	10 days
15	6	A641	p. [P967PfsX12]; p. [P967PfsX12]	+	+	+	+	+	+	NA	NA	+	+	+	NA	NA	10 days
16	6	K182	p. [F1003fs]; p. [F1003fs]	+	+	+	+	+	+	NA	NA	+	+	+	NA	NA	33 days
17	6	K182	p. [F1003fs]; p. [F1003fs]	+	+	+	+	+	+	NA	NA	+	+	+	NA	NA	33 days
18	6	K199	p. [F1003fs]; p. [F1003fs]	+	NA	+	+	+	+	NA	NA	+	+	+	NA	NA	40 days
19	6	B207	p. [I999WfsX5]; p. [I999WfsX5]	+	NA	+	+	+	+	NA	NA	+	+	+	NA	NA	10 days
Total (%)				18/19 (95)	10/12 (83)	19/19 (100)	19/19 (100)	14/16 (87)	18/18 (100)	8/9 (88)	2/9 (22)	13/18 (72)	14/14 (100)	12/12 (100)	4/8 (50)	6/7 (85)	

Abbreviations:
AMC = arthrogryposis multiplex congenita
BA = brain atrophy
CNP = cranial nerve palsy
CP = cleft palate
FA = fetal akinesia
ICV = impaired conduction velocity
MGN = micrognathia
NA = not available
PH = polyhydramnios
RD = respiratory distress
* Patients described in the present study.
† Infantile spasms.
‡ Tracheotomy and ventilator dependent with global developmental delays.
§ Generalized tonic-clonic seizures.
‖ Generalized clonic, brief tonic, and myoclonic seizures.

cases shared similar clinical presentations, including respiratory distress, hypotonia, and multiple cranial nerve palsies. Including the two patients described previously, prenatal polyhydramnios was present in all cases except one, which most likely resulted from absent swallowing. We identified fetal hypokinesia or akinesia in 10 of 12 (83%) cases and arthrogryposis multiplex congenita in 13 of 18 (72%). Six of 19 (32%) patients survived beyond infancy, with the oldest reported patient being 13 years old. Seizures were reported in half of the surviving patients. All survivors required tracheotomy and mechanical ventilation.

Discussion

CHNs are subcategorized into neonatal and infantile forms based on the onset of symptom presentation. Neonatal CHNs are associated with high mortality in the first few years of life, whereas infantile CHNs are associated with better prognosis, although survivors experience considerable disabilities requiring complex medical needs.[11] *CNTNAP1* was recently reported to cause neonatal CHN. The survival of patients and inconsistent presence of arthrogryposis multiplex congenita suggest that categorizing CHNs as a subset of lethal congenital contracture syndromes is not appropriate. A clear genotype-phenotype correlation has not emerged based on previous reports or our current findings. Prolonged survival is most likely driven by the extent of medical care received rather than by the severity of phenotype.

Both patients in our study were born normocephalic but demonstrated progressive postnatal enlargement of occipitofrontal circumference, with occipitofrontal circumference values greater than two standard deviations above the mean starting at three to four months. Serial brain MRIs revealed diffuse prominence of cerebral sulci and extra-axial spaces and interval enlargement of the lateral and third ventricles. Although interval changes may represent a sequela of communicating hydrocephalus, they may also be caused by parenchymal brain volume loss. Profound cerebral and cerebellar atrophy are associated with this syndrome.[8,9] Both patients experienced considerable impairment in cognitive functions, multiple cranial nerve palsies, and lack of voluntary muscle control. Together, these findings support the expansion of the clinical presentation of CHN to include central nervous system involvement. Communicating hydrocephalus has not been previously reported.

Contrary to other forms of CHN, *CNTNAP1*-related CHN affects myelination of both the central and peripheral nervous systems. In addition to typical hypomyelination of peripheral axons, delayed myelination of white matter was evident in the brain MRIs of our patients, with delayed myelination in the corpus callosum and internal capsule, consistent with previously reported roles of *CNTNAP1*. Its protein product (contactin-associated protein [CASPR]) is a crucial element of the paranodal (axoglial) junction in myelinated axons of the central and peripheral nervous system. The structure of the paranodal junction is complex and requires cooperation of many proteins to ensure saltatory propagation of nerve impulses.[12-16] CASPR is highly expressed in neural progenitor cells and is essential for the timing of neuron and astrocyte generation in the developing mouse cerebral cortex.[17] Loss of *Cntnap1* in mice results in delayed generation of neurons and precocious astrocyte formation. Similar findings have been observed in humans with neurodevelopmental disorders such as RASopathies, further highlighting the importance of CASPR in the pathogenesis of developmentally based neurological diseases.

In summary, we describe two patients with CHN with novel *CNTNAP1* mutations. Our observations suggest that biallelic mutations in *CNTNAP1* cause a distinct recognizable syndrome characterized by neuropathy of both central and peripheral nervous systems. Survival beyond infancy is possible with aggressive medical intervention. Further studies are needed to characterize the pathogenesis of brain atrophy in this form of CHN.

Acknowledgment

The authors thank the family for their participation in this study. Drs. Paulomi Mehta, MD, and Mathilde Nizon, MD, personally provided additional clinical information beyond those reported in their respective manuscripts. Author contributions: H.L. and R.J.H. were responsible for the concept and design of the study. H.L. drafted the main manuscript. H.L., K.B., M.V.L., and S.A.H. analyzed and interpreted the data. B.H., H.L., M.S., M.V.L., R.J.H., and T.A.B. contributed to the clinical data. All authors reviewed the manuscript and made comments regarding its structure, details, and grammar.

References

1. Warner LE, Garcia CA, Lupski JR. Hereditary peripheral neuropathies: clinical forms, genetics, and molecular mechanisms. Annu Rev Med. 1999;50:263–275.
2. Harati Y, Butler IJ. Congenital hypomyelinating neuropathy. J Neurol Neurosurg Psychiatry. 1985;48:1269–1276.
3. Kochanski A, Drac H, Kabzińska D, et al. A novel MPZ gene mutation in congenital neuropathy with hypomyelination. Neurology. 2004;62:2122–2123.
4. Bolino A, Lonie LJ, Zimmer M, et al. Denaturing high-performance liquid chromatography of the myotubularin-related 2 gene (MTMR2) in unrelated patients with Charcot-Marie-Tooth disease suggests a low frequency of mutation in inherited neuropathy. Neurogenetics. 2001;3:107–109.
5. 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;18:382–384.
6. Laquerriere 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;23:2279–2289.
7. Mehta P, Küspert M, Bale T, et al. Novel mutation in CNTNAP1 results in congenital hypomyelinating neuropathy. Muscle Nerve. 2017;55:761–765.
8. Lakhani S, Doan R, Almureikhi M, et al. Identification of a novel CNTNAP1 mutation causing arthrogryposis multiplex congenita with cerebral and cerebellar atrophy. Eur J Med Genet. 2017;60:245–249.
9. Hengel H, Magee A, Mahanjah M, et al. CNTNAP1 mutations cause CNS hypomyelination and neuropathy with or without arthrogryposis. Neurol Genet. 2017;3:e144.
10. Nizon M, Cogne B, Vallat JM, et al. Two novel variants in CNTNAP1 in two siblings presenting with congenital hypotonia and hypomyelinating neuropathy. Eur J Hum Genet. 2017;25:150–152.
11. Phillips JP, Warner LE, Lupski JR, Garg BP. Congenital hypomyelinating neuropathy: two patients with long-term follow-up. Pediatr Neurol. 1999;20:226–232.
12. 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;34:14820–14826.
13. Sherman DL, Tait S, Melrose S, et al. Neurofascins are required to establish axonal domains for saltatory conduction. Neuron. 2005;48:737–742.
14. Gollan L, Salomon D, Salzer JL, Peles E. Caspr regulates the processing of contactin and inhibits its binding to neurofascin. J Cell Biol. 2003;163:1213–1218.
15. 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;30:385–397.
16. Rios JC, Melendez-Vasquez CV, Einheber S, et al. Contactin-associated protein (Caspr) and contactin form a complex that is targeted to the paranodal junctions during myelination. J Neurosci. 2000;20:8354–8364.
17. Wu ZQ, Li D, Huang Y, et al. Caspr controls the temporal specification of neural progenitor cells through notch signaling in the developing mouse cerebral cortex. Cereb Cortex. 2017;27:1369–1385.