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Novel Mutation in *CNTNAP1* results in Congenital Hypomyelinating Neuropathy

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Abstract

Introduction—Congenital hypomyelinating neuropathy is a rare congenital neuropathy that presents in the neonatal period and has been linked previously to mutations in a number of genes associated with myelination. A recent study has linked 4 homozygous frameshift mutations in the *CNTNAP1* gene with this condition. We report a neonate with congenital hypomyelinating neuropathy who was found to have absent sensory nerve and compound muscle action potentials and hypomyelination on nerve biopsy.

Methods/Results—On whole exome sequencing we identified a novel *CNTNAP1* homozygous missense mutation (p.Arg388Pro) in the proband, and both parents were carriers. Molecular modeling suggests that this variant disrupts a β -strand to cause an unstable structure and likely significant changes in protein function.

Discussion—This report links a missense *CNTNAP1* variant to the disease phenotype previously associated only with frameshift mutations.

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Keywords

CNTNAP1; Hypomyelination; Nerve conduction; Congenital neuropathy; Missense mutation; Exome sequencing

Introduction

Congenital hypomyelinating neuropathy (CHN) is a rare congenital neuropathy, often accompanied by arthrogryposis, that is characterized by prenatal onset, areflexia, hypotonia, hypomyelination, and slowed nerve conduction velocities¹. The clinical presentation varies, but most patients come to medical attention in infancy, with respiratory difficulties and profound hypotonia². Previous reports of genetic analyses of patients with this spectrum of signs have described specific mutations in genes known to be important in myelination, including *de novo* mutations in the myelin protein zero (*MPZ*), peripheral myelin protein 22 (*PMP22*), and early growth response 2 (*EGR2*) genes³⁻⁶. Recessive mutations in other genes may result in a less severe phenotype, but since many of these patients are not evaluated with molecular studies, these lists are incomplete².

Contactin-associated protein 1 (CNTNAP1) is a cell-adhesion molecule, commonly referred to as CASPR in the literature, that was the first identified constituent of paranodal junctions in the peripheral nervous system (PNS)⁷. CNTNAP1 complexes with contactin, another neural cell adhesion molecule, to form a high molecular weight complex in paranodal junctions⁸. This complex is thought to be targeted to the paranodal junctions via extracellular interactions with myelinating glia⁸. A homozygous null mouse model initially does not display an abnormal phenotype through the first week of life, but later demonstrates progressive neurologic defects that reach maximal severity by the third week of life⁹. These defects include hypomotility, tremors, wide-based gait, and generalized motor paresis, and death occurs generally within the first month of post-natal life⁹. Of note, heterozygotes were unaffected throughout all ages. This mouse model also demonstrates disorganization of the paranodal junctions, including dysregulation of ion channel interactions (Na⁺ and K⁺) and slowing of nerve conduction velocity⁹. Important functional activity occurs at the paranodal junction at the axon-Schwann cell interface in the PNS, including anchoring of myelin loops to the axon, preventing diffusion of membrane protein complexes, and creating an ionic diffusion barrier into the periaxonal space. These all suggest that this interface serves as a site for bidirectional signaling between axons and Schwann cells¹⁰.

In humans, *CNTNAP1* mutations have been implicated in nonsyndromic arthrogryposis multiplex congenita, in which all individuals had frameshift mutations leading to premature termination of transcription and resulting in death within the first several weeks of life¹¹. Here we report on a male infant born with a congenital hypomyelinating neuropathy who carried a novel homozygous missense mutation in the *CNTNAP1* gene likely responsible for the disease phenotype.

Material and Methods

Clinical presentation

The proband is a male infant born vaginally at 38 3/7 weeks gestation to a 29-year old G2P2 mother with an uncomplicated pregnancy. At delivery, the infant emerged limp without respiratory effort and required extensive resuscitation effort, including positive pressure ventilation and chest compressions. His Apgar scores were 1, 4, and 4 at 1, 5, and 10 minutes, respectively. His birth weight was 2.9 kg, length 43.5 cm, and head circumference 34 cm (all within the twenty-fifth to fiftieth percentile for gestational age). His initial physical examination was remarkable for the following: bilateral club feet, flexion posture with ulnar deviation of the wrist, deep palmar creases, fingers curled back with hypermobility of finger joints, and undescended testes. On neurological examination there were minimal spontaneous movements, hypotonia, a minimal gag reflex, absent primitive reflexes including doll's-head eye movements, pupillary reflexes, and tendons stretch reflexes, and poor spontaneous respiratory efforts with an inability to be extubated.

He underwent therapeutic hypothermia for 72 hours after birth in the setting of low Apgar scores and an abnormal physical examination concerning for hypoxic ischemic encephalopathy. Neurological investigations included continuous video electroencephalography (EEG) monitoring that showed a discontinuous immature pattern without electrographic seizures. Brain magnetic resonance imaging (MRI) revealed patchy signal abnormalities within the cerebral white matter, and a small lactate peak with unremarkable MR-arterial (MRA) and MR-venous (MRV) sequences. After discontinuation of therapeutic hypothermia, repeat physical examinations reflected proximal >distal strength, with antigravity movements noted in deltoids, biceps, iliopsoas, hamstrings, and quadriceps as well as some spontaneous shoulder girdle and hip movements, but minimal spontaneous activity of elbows/wrists/fingers and feet/toes. He continued to have absent reflexes throughout as well as reactivity to exam (primarily withdrawal from cold touch).

Further routine laboratory investigations were normal, including blood tests for electrolytes, glucose, liver function, complete blood count, and coagulation profiles. Serum ammonia, lactate, and pyruvate were also within normal ranges. Urine organic acids, serum amino acids, and acylcarnitine and acylglycine panels were reportedly normal.

Based on these findings, a preliminary diagnosis of a neuromuscular disorder was made. Serum creatinine kinase (CK) was normal at 151 units/liter, and survival of motor neuron 1 (*SMN1*) gene testing did not reveal a homozygous deletion of exon 7, which ruled out spinal muscular atrophy. A chromosomal microarray was reported to be absent for clinically significant copy number variants. Electromyography and nerve conduction studies were performed and were consistent with a neurogenic rather than a myopathic pattern. A muscle and sural nerve biopsy were performed, and a diagnosis of congenital hypomyelinating neuropathy was made, as described below. After pathological confirmation of the diagnosis of congenital hereditary neuropathy, the decision was made to withdraw care, and the patient passed away at age 1 month.

Histopathological Findings

Biopsies from the left quadriceps muscle and the left sural nerve were obtained and studied by both light and electron microscopy. Muscle histochemistry showed unremarkable caliber muscle fibers, with normal lipid and glycogen content. The distribution of Type 1 and type 2 fibers was random, as normally expected. There was no evidence of an underlying myopathic or neurogenic process. Electron microscopy confirmed normal myofibrillar structure, as well as normal mitochondrial number and distribution. Nerve biopsy showed hypomyelination without evidence of onion bulbs or myelin breakdown products. Toluidine blue stained semi-thin sections and electron microscopy demonstrated reduction in the number of large myelinated fibers (Fig. 1A). Additionally, scattered large axons demonstrated abnormal myelination including thin, abnormal, or incomplete myelin sheaths (Fig. 1B,C).

EMG

Nerve conduction studies were conducted, and there were absent right median, medial plantar, and ulnar sensory nerve action potentials (Fig. 1D-F). Compound muscle action potentials were noted to be reduced in amplitude in the right median nerve (1.1mV at the wrist; 1.0mV at the elbow) and absent in tibial and fibular motor nerve studies (Fig. 1G-J). The median motor nerve conduction velocity was 25.0m/s. Abundant fibrillation potentials were noted in the left tibialis anterior, gastrocnemius, and hamstring muscles with large polyphasic motor unit potentials of prolonged duration and decreased recruitment.

Family Enrollment and Whole Exome Sequencing

The proband and both parents were enrolled in an institutional review board (IRB) approved study at Boston Children's Hospital (BCH), and a family pedigree was obtained. Of note, there was no consanguinity noted. Deoxyribonucleic acid (DNA) was extracted from maternal, paternal, and proband blood samples for whole exome sequencing. Samples were prepared as a sequencing library (Illumina) and enriched for exonic sequences using the Nimblegen Exome Enrichment protocol (SeqCap EZ VCRome 2.0). The captured libraries were sequenced using an Illumina HiSeq 2000 Sequencer, and paired-end 100 base pair reads were obtained. The sequenced files were filtered and aligned, and variants were filtered and annotated by Codified Genomics (proprietary algorithm, Houston, TX).

Computational modelling

A suitable structural model of CNTNAP1 was identified in Protein Data Bank (PDB) through sequence search. The protein was the ectodomain of Neurexin-1 α (PDB code: 3R05). It shared 43% homology and 27% identity with the first 559 residues of CNTNAP1, which contained the mutation R388P. After a protein sequence alignment, the residue 388 of CNTNAP1 was mapped to the residue 449 of Neurexin-1 α . Based on the structure of Neurexin-1 α , structural models of CNTNAP1 and its mutant were generated by PYMOL (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

Results

Identification of CNTNAP1 mutation

Whole exome sequencing was performed on the DNA extracted from the proband and both parents. Sequencing did not reveal any variants in genes previously implicated in CHN (*MPZ*, *PMP22*, *ERG2*, *MTMR2*, *SOX10*). A homozygous missense mutation in the *CNTNAP1* gene (ch17:40839856G>C; c.1163G>C, p.Arg388Pro) was identified that segregated appropriately in the parents and was confirmed by Sanger sequencing (Fig. 2A). The variant frequency in healthy controls is 1/121368 (<0.0001%) according to ExAC database¹², and the gene is highly depleted for functional variation relative to all genes (Residual Variation Intolerance Score (RVIS) score -2.3 and percentile score 1.2%)¹³. The variant affects a residue that is highly conserved evolutionarily among vertebrates and is deemed pathogenic by various software programs including SIFT, Polyphen-2 and MutationTaster (Fig. 2B).

Computer modeling revealed that the mutation replaced an arginine residue with a proline on a beta-strand within multiple beta-sheets (Fig. 2C,D). Proline often breaks alpha-helices and beta-strands; therefore, this mutation likely breaks the local beta strand and destabilizes the structure, altering the inherent function of CNTNAP1.

Discussion

Early hereditary neuropathies were first described by Dejerine and Sottas in the late 1800s when they described a hereditary neuropathy with progressive muscle wasting. Since that time, a larger spectrum of these conditions has been identified through a variety of diagnostic criteria. Congenital hypomyelinating neuropathy is a heterogeneous disorder that is comprised of 2 clinically distinct groups of patients. In the first group, the disease manifests in the neonatal period and patients often die at an early age from associated complications, including pneumonia. In the second group, patients come to medical attention later in infancy and, despite severe disability, have a better prognosis, often becoming capable of ambulation without significant support within the first decade of life^{1,5}.

Detailed electrophysiological testing is required to confirm the clinical diagnosis, but given the variable clinical course during the first few years of life, counseling about the prognosis should be guarded. Nerve biopsy is now less often performed, as genetic testing in patients with hereditary neuropathies is becoming increasingly utilized². However, as in our case of a family with a novel mutation, nerve biopsy may be required. In patients with novel mutations and those with known hereditary neuropathies, nerve biopsy examination has shown either complete absence of peripheral nerve myelin or severe hypomyelination without evidence of myelin destruction. Congenital hypomyelinating neuropathy is distinguished in part by the absence of onion bulbs on nerve histology from clinically similar diseases such as Dejerine-Sottas or Charcot-Marie-Tooth (CMT), which are also disorders of myelin³. These diseases, which fall under the family of hereditary motor and sensory neuropathies (HMSN), also share causative genes with CHN¹⁴. These causative genes include *PMP22*, *ERG2*, and *MPZ*, the first of which is often the responsible mutation in CMT1 patients^{14,15}.

The role of the paranodal junction in the peripheral nervous system is complex and requires coordination of several protein complexes, including that of CNTNAP1 and its cofactors. On its own, CNTNAP1 has been shown to play an important role in the stability and organization of the many members of the paranodal junction, including organization of paranodal loops and localization of ion channels essential to normal saltatory conduction^{9,16}. Animal studies have shown that movement of these paranodal ion channels may contribute to loss of nerve conduction and action potential propagation in demyelination pathologies¹⁷. CNTNAP1, together with CNTNAP2, has also been shown to play an important role in the radial and longitudinal organization of myelinated axons¹⁶.

Frameshift mutations in *CNTNAP1* have been implicated recently in the pathogenesis of non-syndromic arthrogryposis multiplex congenita¹¹. Four families (3 consanguineous) were found to carry frameshift mutations (1bp insertion or deletion in either exon 18 or 19) that led to the ensuing similar fetal phenotypes in all 7 offspring. Polyhydramnios first appeared late in pregnancy as did distal joint contractures (e.g. club feet, proximal and distal contractures of the hands) with sparing of proximal joints. Postnatal clinical deficits included severe hypotonia, facial diplegia, lack of swallowing, absent tendon stretch reflexes, and spontaneous respiratory effort. Death typically occurs within the first 2 months of life in all patients¹¹. In each patient, motor nerve conduction studies demonstrated markedly reduced conduction velocities (<10m/s). Sciatic nerve samples were analyzed by transmission electron microscopy in 4 patients, and they revealed thinner than normal myelin sheaths and widened nodes of Ranvier.

Our patient's mutation would certainly explain the decreased nerve conduction velocities and hypotonia, given the known functions of CNTNAP1 as well as the previously documented findings in patients with *CNTNAP1* mutations. While this patient does not have classic arthrogryposis, there are a number of features that suggest a congenital contracture syndrome, including bilateral club feet and flexion posture with ulnar deviation of the wrists. The hypomyelination noted on our patient's biopsies is similar to those seen in patients who carry *CNTNAP1* frameshift mutations¹¹. However, this is a curious finding, given that animal studies have not shown a difference in myelin formation in the *Cntnap1*-null mouse model⁹. There is much to be understood regarding axo-glial junction relationships and the many factors that play into the proper development of these junctions. This report links a missense variant in the *CNTNAP1* gene to the hypotonia/hypomyelination phenotype in a patient, and it further emphasizes the role of CNTNAP1 in the production and maintenance of myelin in the peripheral nervous system.

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Abbreviations

CHN	Congenital Hypomyelinating Neuropathy
CK	Creatinine Kinase
CMT	Charcot-Marie-Tooth
CNTNAP1	Contactin-Associated Protein 1
DNA	Deoxyribonucleic Acid
EEG	Electroencephalogram
EGR2	Early Growth Response 2
IRB	Institutional Review Board
HMSN	Hereditary Motor and Sensory Neuropathies
MRA	MR-arterial
MRI	magnetic resonance imaging
MRV	MR-venous
MPZ	Myelin Protein Zero
PDB	Protein Data Bank
PMP22	Peripheral Myelin Protein 22
PNS	Peripheral Nervous System
PYMOL	PyMOL Molecular Graphics System
RVIS	Residual Variation Intolerance Score
SMN1	Survival of Motor Neuron 1

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Figure 1.

(A) Plastic embedded toluidine blue stained cross-sections (semi-thin) demonstrate numerous axons with thinly/incompletely myelinated sheaths (arrows). (B) Electron microscopy of sural nerve shows a significantly enlarged axon with a thin to absent myelin sheath (arrowhead). For comparison note a normally sized and myelinated axon (arrow). (C) EM of sural nerve with a cluster of thinly myelinated axons (arrowhead). A normally myelinated axon is noted in the upper left hand corner (arrow). (D-F) Absent sensory nerve action potentials are noted in several nerves, including right median (D), medial plantar (E), and ulnar (F). (G-J) Motor studies show a low amplitude right median compound muscle action potential (CMAP) and absent CMAPs in the left fibular and bilateral tibial nerves.

Figure 2.

CNTNAP1 mutation in the proband and computer modeling of the variant. (A) Sanger sequencing confirms that the proband is homozygous for the c.1163G>C variant (shaded) in the *CNTNAP1* gene, while both parents are heterozygous. (B) The altered arginine residue (in red) in the p.Arg388Pro mutation is highly conserved in vertebrates, including mouse and zebrafish. (C-D) Computer modeling reveals R388 is located on a beta-strand within multiple beta-sheet (C). The proline residue in place of arginine (D) likely breaks the alpha helix and beta sheet, thereby destabilizing the protein.