**CHCHD10** variant p.(Gly66Val) causes axonal Charcot-Marie-Tooth disease

**ABSTRACT**

**Objective:** We describe the phenotype consistent with axonal Charcot-Marie-Tooth disease type 2 (CMT2) in 4 families with a c.197G>T (p.Gly66Val) variant in CHCHD10.

**Methods:** We sequenced the **CHCHD10** gene in a cohort of 107 families with CMT2 of unknown etiology. The patients were characterized by clinical examination and electroneuromyography. Muscle MRI and biopsy of the muscle or nerve were performed in selected cases. Neuropathologic autopsy was performed in 1 case.

**Results:** The c.197G>T variant in **CHCHD10** was found in 6 families, 4 of which included multiple individuals available for detailed clinical study. Variants in this gene have recently been associated with amyotrophic lateral sclerosis-frontotemporal dementia, mitochondrial myopathy, or spinal muscular atrophy Jokela type (SMAJ), but not with CMT2. Our patients had a late-onset distal axonal neuropathy with motor predominance, progressing to involve sensory nerves. Neuropathologic and neuropathologic studies confirmed the diagnosis of sensorimotor axonal neuropathy with no loss of anterior horn neurons. Muscle biopsies showed occasional cytochrome c oxidase-negative fibers, combined with small amounts of mitochondrial DNA deletions.

**Conclusions:** **CHCHD10** c.197G>T (p.Gly66Val) is a cause of sensorimotor axonal neuropathy. This gene should be considered in patients presenting with a pure CMT2 phenotype, particularly when motor symptoms predominate.  

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**GLOSSARY**

ALS = amyotrophic lateral sclerosis; CMT2 = Charcot-Marie-Tooth disease type 2; COX = cytochrome c oxidase; ENMG = electroneuromyography; FTD = frontotemporal dementia; IHC = immunohistochemistry; MF = myelinated fibers; mtDNA = mitochondrial DNA; SMAJ = spinal muscular atrophy Jokela type; WES = whole-exome sequencing.

Axonal Charcot-Marie-Tooth disease type 2 (CMT2) is a genetically heterogeneous group of hereditary sensorimotor neuropathies. The ~20 known disease genes for CMT2 take part in diverse processes of axon maintenance, including cytoskeletal organization, axoplasmic transport, and mitochondrial dynamics. The most common disease gene is **MFN2**, which is required for mitochondrial fusion. However, the genetics of CMT2 is only partially understood, as disease mutations are found in only 25% of cases.

Abnormal mitochondrial function was recently also suggested as a pathogenic mechanism in amyotrophic lateral sclerosis (ALS). Dominant variants in the 22q11.23-mapped gene **CHCHD10**, encoding a protein localized to the mitochondrial intermembrane space and regulating mitochondrial crista architecture, were discovered in patients with familial ALS or frontotemporal dementia (FTD). Muscle biopsies showed aberrant mitochondrial organization and abundant mitochondrial DNA (mtDNA) deletions. This opened the possibility of mechanistic similarities between ALS-FTD and CMT. The spectrum of **CHCHD10** disease has also expanded to include mitochondrial...
myopathy\(^4\) and late-onset spinal motor neurophy,\(^5\) also known as spinal muscular atrophy Jokela type (SMAJ, OMIM #615048).

In this study, we establish a **CHCHD10** variant as the cause of CMT2 in 4 families. Our results give further support to the importance of functional mitochondrial network in the maintenance of peripheral nerves.

**METHODS Patients.** Our current study cohort of CMT2 patients with unknown genetic etiology consists of 107 unrelated families. Here, we examined in detail 4 families in whom the CHCHD10 variant c.197G>T (NM_001301339.1) was identified. Diagnosis was based on clinical examination and electromyography (EMG). Selected patients underwent muscle MRI or muscle and/or nerve biopsy. Detailed autopsy was performed in 1 patient. Blood samples were taken from patients and healthy family members, in accordance with the Declaration of Helsinki.

**Standard protocol approvals, registrations, and patient consents.** All participants gave informed consent, and the study was approved by the ethics review board of Helsinki University Hospital (dnro 399/E9/07).

**Sequencing.** Whole-exome sequencing (WES) and targeted gene panel sequencing to exclude variants in known CMT2 disease genes had been previously performed for individuals from families 1 and 2 (WES for F1:IV-3, F1:III-9, F2:II-2, and F2:III-7, figure 1); targeted sequencing for F1:IV-3 and F2:II-2,\(^7\) table e-1 at Neurology.org/ng). In this study, we sequenced the coding exons and flanking intronic regions of the CHCHD10 gene in the index patient samples of our unsolved CMT2 cohort. The following oligonucleotide primers were used (forward followed by reverse): 5’-AGCTGCTGAAAGGGGATG-3’, 5’-CCGGAGAGATGGACGACC-3’ (exon 1); 5’-TTAACCCTGTCTCCTCCAC-3’, 5’-GGAGCTGCTGCCTCTAAGTGA-3’ (exon 2); 5’-CAACTCAAAGCTGATCCTGC-3’, 5’-GAGTCTGCACCGACCTCTT-3’ (exon 3); 5’-ACATGTTGAGTGA GTGGAC-3’, 5’-GGTCCCCACAGTGGAGAT-3’ (exon 4).

**Neurophysiologic findings.** ENMG showed chronic motor neuropathy with regeneration in all studied patients. In families 1, 2, and 14, sensory findings were evident in the first ENMG, consistent with typical CMT2 neuropathy (table e-2). In family 1, 2, and 14, sensory abnormalities such as loss of tendon reflexes, muscle weakness, and atrophy. Sensory abnormalities such as loss of sensation for vibration or cold were strong enough to be detected on clinical examination in 7 of 12 patients. Creative kinase was normal in 4 of 12 patients. Progression tended to be slow, and so far all patients are alive or died from a cause unrelated to neuropathy. No signs of upper motor neuron disease, bulbar symptoms, or progressive cognitive problems were observed in the patients. Lower limb muscle MRI showed edema or fatty degeneration that was pronounced distally, particularly in calves, and milder in thighs.

**Neuropathologic findings.** ENMG showed chronic motor neuropathy with regeneration in all studied patients. In families 1, 2, and 14, sensory findings (decreased sural nerve action potential amplitude) were evident in the first ENMG, consistent with typical CMT2 neuropathy (table e-2). In family 42, motor findings predominated and sensory abnormalities became evident only at a later age.

**RESULTS Genetic findings.** Our previous WES or targeted gene panel sequencing for families 1 and 2 did not identify potential disease-causing mutations (table e-1).\(^6,7\) The newly reported ALS gene, CHCHD10,\(^3\) was poorly covered by our WES and was not included in the targeted panel. By Sanger sequencing, we found a c.197G>T variant in CHCHD10 predicting a p.(Gly66Val) amino acid change to segregate with CMT2 in families 1 and 2. Within our CMT2 cohort, the entire CHCHD10 coding region was sequenced, and we found the same variant in 4 additional index patients. Of these, 2 were from families with multiple members available for detailed study, families 14 and 42 (figure 1). The remaining 2 patients had been diagnosed with CMT2 elsewhere, with samples sent to us for DNA analysis, but detailed clinical information was not available. The p.(Gly66Val) variant was not found in 104 Finnish control individuals\(^5\) or in the 1000 Genomes database (http://www.1000genomes.org/, accessed January 2015). The variant was predicted to be “probably damaging” by PolyPhen-2, “tolerated” by SIFT, and “disease causing” by MutationTaster.

**Neuropathologic findings.** Muscle biopsies of selected patients demonstrated denervation atrophy: small and large group atrophy, some single atrophic fibers, variable fiber type grouping, some secondary myopathic changes, and end-stage atrophy in 1 case (F1:III-9) (figure 2A). Fibers deficient of cytochrome c oxidase (COX) staining (figure 2A) were detected in 2 patients (F1:III-8 and F2:II-2).

Small amounts of mtDNA deletions were present in 4 of 6 tested patients (figure 2B). Patient F1:IV-3 also had marginal inflammatory features on biopsy, but this was not observed in the other patients (data not shown).
Nerve biopsy samples from the sural nerve of 4 patients showed unspecific axonal neuropathy, with thick (≥7 mm) myelinated fibers (MF) most affected (figure 2C). In the available sural nerve biopsies from 3 patients, the perineurial sheath was slightly fibrotic at places, but no significant changes were observed in epineurium, perineurium, or vessels, and no amyloid could be demonstrated. In the endoneurial compartment, the MF density varied between 2,500 and 3,600 MF/mm² and large MF represented between 5% and 25% of the total MF (normal values in our adult patients: approximately 7,000–9,000 MF/mm² total count, large MF fraction 35%–45%). No marked hypertrophic changes, including marked sprouting, regenerative clusters, or demyelination features, were observed.
Neuropathologic autopsy was available for patient F1:III-9. He died of metastasized microcellular neuroendocrine prostate cancer and also presented with epidural metastatic changes compressing the spinal cord at thoracic levels T5 to T6. Otherwise, the spinal cord was macroscopically normal; no anterior root atrophy was observed. Histologically, there was a marginal unspecific reduction of anterior horn motor neuron density, but changes typical for motor neuron disease/ALS were not observed. p62 and phosphorylated TDP-43 immunohistochemistry (IHC) was also negative in the spinal cord and medullary hypoglossal nuclei. In the brain, no specific focal or otherwise diagnostic findings could be observed; IHC for β-amyloid, phosphorylated tau, and p62 was negative in hippocampus and frontal cortex.

**DISCUSSION** Defects in CHCHD10 have emerged as an important cause of variable neurodegenerative phenotypes, underlying ALS-FTD,3 SMAJ,5 and mitochondrial myopathy.4 The c.197G>T (p.(Gly66Val)) variant has been previously described in patients with SMAJ5 or ALS.11 Some of our patients showed clinical overlap with SMAJ. For instance, cramps or fasciculations were common presenting symptoms in SMAJ5 and were among the main symptoms in 3 of our 12 patients. However, several lines of evidence suggested length-dependent degeneration of sensory and motor axons, i.e., CMT2, rather than SMAJ or ALS, in the patients described here. First, the typical initial symptom was distal lower limb muscle weakness, and predominantly distal muscle involvement was shown by MRI. In addition, clinical examination showed sensory impairment in 7 of 12 patients. Sensory impairment in patients with SMAJ was mild and had a later onset.5 Second, ENMG revealed axonal neuropathy involving both sensory and motor fibers in 10 of 12 patients, including decreased sensory nerve action potential at first presentation in 9 of 12 patients. Third, axonal neuropathy was found in all 3 patients who underwent sural nerve biopsy. Fourth, autopsy excluded degeneration of anterior horn neurons. Therefore, our results demonstrate that the CHCHD10 p.(Gly66Val) variant can cause variable phenotypes ranging from ALS or lower motor neuron diseases to CMT2.
neuronopathy to sensorimotor axonopathy. CHCHD10 should thus be considered a candidate gene in patients with CMT2.

The CHCHD10 c.197G>T variant was found in 4.8% (6/126) of our cohort, when including solved and unsolved unrelated families.6,7 Our patients are likely to have the same disease haplotype as the previously described patients with SMAJ or ALS, because of shared Finnish ethnicity. Such founder haplotypes are typical in Finland, which is a genetic isolate. Other factors than the haplotype are likely to modify the clinical phenotype in the different patients who carry the same variant. The CHCHD10 protein localizes to the mitochondrial intermembrane space, where it enriches at cristae junctions and appears to be important for maintenance of cristae morphology and oxidative phosphorylation.3 In our patients, the mitochondrial pathology in muscle was mild, with only occasional COX-negative fibers and small amounts of mtDNA deletions. In contrast, p.(Gly58Arg) caused a pure myopathy,4 while p.(Ser59Leu) led to a more aggressive ALS-FTD phenotype that included clear involvement of both the central and peripheral nervous system in addition to pathologic evidence of mitochondrial myopathy.3 The disease spectrum of CHCHD10 resembles that of MFN2, where CMT2 is the typical presentation but some patients have mitochondrial myopathy with mtDNA deletions12 or CNS involvement13 as additional features. Peripheral neurons may be exquisitely sensitive to mitochondrial network defects owing to the length of their axons. Further functional studies are needed to fully understand the function of CHCHD10, the disease mechanisms of its variants, and the factors that modify the phenotypic outcome in the patients.

AUTHOR CONTRIBUTIONS
M.A. collected and analyzed patient data. E.Y. analyzed patient and sequencing data. M.S. performed sequencing and mtDNA analysis. A.P. performed neuropathology. S.K.-E. contributed to clinical analysis. J.P.T. performed and analyzed neurophysiology. M.A., E.Y., and H.T. designed the study and drafted the manuscript. All authors revised the manuscript.

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