Complex hereditary peripheral neuropathies caused by novel variants in mitochondrial-related nuclear genes

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Abstract
Mitochondrial disorders are a group of clinically and genetically heterogeneous multisystem disorders and peripheral neuropathy is frequently described in the context of mutations in mitochondrial-related nuclear genes. This study aimed to identify the causative mutations in mitochondrial-related nuclear genes in suspected hereditary peripheral neuropathy patients. We enrolled a large Japanese cohort of clinically suspected hereditary peripheral neuropathy patients who were mutation negative in the prescreening of the known Charcot–Marie–Tooth disease-causing genes. We performed whole-exome sequencing on 247 patients with autosomal recessive or sporadic inheritance for further analysis of 167 mitochondrial-related nuclear genes. We detected novel bi-allelic likely pathogenic/pathogenic variants in four patients, from four mitochondrial-related nuclear genes: pyruvate dehydrogenase beta-polypeptide (PDHB), mitochondrial poly(A) polymerase (MTPAP), hydroxyacetyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, beta subunit (HADHB), and succinate-CoA ligase ADP-forming beta subunit (SUCLA2). All these patients showed sensory and motor axonal polyneuropathy, combined with central nervous system or multisystem involvements. The pathological analysis of skeletal muscles revealed mild neurogenic changes without significant mitochondrial abnormalities. Targeted screening of mitochondria-related nuclear genes should be considered for patients with complex hereditary axonal polyneuropathy, accompanied by central nervous system dysfunctions, or with unexplainable multisystem disorders.

Keywords Peripheral neuropathy · Whole-exome sequencing · Nuclear genes · Mitochondrial disease
Introduction

Peripheral neuropathy has various causes, one of which is mitochondrial abnormalities. Mitochondrial-related nuclear genes, such as MFN2 and GDAP1 that are involved in mitochondrial dynamics, are major causes of Charcot–Marie–Tooth disease (CMT) [1, 2], the most common subtype of hereditary peripheral neuropathy. In MFN2, known as CMT2A and HMSN6A, phenotypes may cause optic atrophy, and in GDAP1, they may cause vocal cord paresis [2] so the spectrum of CMT has considerably broadened and multisystem involvement is frequently observed similar to other disorders caused by mutations in mitochondrial DNA (mtDNA) or mitochondria-related nuclear genes. Moreover, mitochondrial disorders associated with defects in mitochondrial DNA (mtDNA) maintenance and replication or defects in the respiratory chain complex are often associated with peripheral neuropathy [2]. Although the severity of these disorders is usually mild or subclinical, peripheral neuropathy can be severe and might be the main feature of a mitochondrial disorder [3]. Given that > 1100 mitochondrial-related nuclear genes have been identified and > 240 nuclear genes cause mitochondrial disorders [4, 5]; we speculate that more mutations in mitochondrial-related nuclear genes cause patients to manifest the CMT-like phenotype. In this study, through whole-exome sequencing (WES) data, we examined a large cohort of Japanese patients with clinically suspected hereditary peripheral neuropathy patients to determine the presence of variants in a panel of mitochondrial-related nuclear genes.

Materials and methods

Patient selection and extraction of genomic DNA

After preliminary exclusion of the PMP22 duplication or deletion mutation, using fluorescence in situ hybridization or multiplex ligation probe amplification, we enrolled a nationwide cohort of 854 Japanese patients clinically suspected with pure or complex hereditary peripheral neuropathy, between April 2007 and July 2014. All of their clinical information, electrophysiological and radiological records, and pathological findings, were provided by local neurologists or pediatricians. The protocol was reviewed and approved by the Institutional Review Board of Kagoshima University (Kagoshima, Japan). All patients and family members provided written informed consent to participate in this study. The study conforms with the World Medical Association Declaration of Helsinki published on the website of the Journal of American Medical Association.

Genomic DNA was isolated from peripheral blood leukocytes, using the Qiagen Puregene Core Kit C (Qiagen, Valencia, CA, USA), or from the saliva, using the Oragene DNA self-collection kit (DNA Genotek, Ottawa, ON, Canada), according to the manufacturer’s protocol.

Gene panel screening and WES

All 854 DNA samples were processed on 1 of the 2 types of CMT-related gene panel screening platforms. Between April 2007 and April 2012, 417 cases were screened using a customized MyGeneChip® CustomSeq® Custom Resequencing Array (Affymetrix, Inc., Santa Clara, CA, USA), targeting 28 genes known to cause CMT or related diseases following a protocol described previously [6]. Between May 2012 and July 2014, we used the Illumina MiSeq next-generation sequencing platform to screen 437 patients for 40 known CMT disease-causing and 20 candidate genes [7]. After target resequencing, we used WES to further analyze 399 mutation-negative patients, including 247 patients with autosomal recessive (AR) or sporadic inheritance.

We used a SureSelect v4 + UTRs or v5 + UTRs kit, then sequenced on Illumina HiSeq 2000® (Illumina, San Diego, CA, USA). WES data were aligned to the human reference genome (NCBI37/hg19) with Burrows–Wheeler Aligner [8], and variant call was performed using SAM tool [9]. The CLC Genomic Workbench software program (Qiagen, Hilden, Germany) and an in-house R script were applied for variant annotation and filtering.

Variant identification and segregation analysis

We concentrated on the WES variants in a list of 167 known mitochondrial-related nuclear genes, which was modified from the Baylor Genetics (https://www.bcm.edu/research/medical-genetics-labs/) BCM-MitomeNGS panel (Supplementary Table 1). Sanger sequencing was applied to validate the suspected pathogenic variants. We carried out segregation studies for other family members whenever available. All variants were checked against the single nucleotide polymorphism database (dbSNP: http://www.ncbi.nlm.nih.gov/SNP/), the gnomAD browser (https://gnomad.broadinstitute.org) as a global control database, the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp) and Japanese Multi Omics Reference Panel (jMorp 8.5 K: https://jmorlp.megabank.tohoku.ac.jp/jjgv/) as Japanese databases, and the in-house database to assess whether they were normal variants. Moreover, to perform in silico analysis, we used four prediction algorithms: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2, cut-off > 0.9), SIFT (http://sift.jcvi.org, cut-off < 0.05), PROVEAN (http://provean.jcvi.org/index.php, cut-off < −2.5), Mutation Taster (http://mutatiorntaster.org, scores ranging between 0 and 215,
variants suspected of pathogenicity are classified as “dis-
dease-causing” and variants suspected of less pathogenicity
are classified as “polymorphisms”). We interpreted variants
according to the American College of Medical Genetics
and Genomics and the Association for Molecular Pathol-
ogy (ACMG/AMP) standards and guidelines [10].

RNA extraction and reverse-transcription
polymerase chain reaction (RT-PCR)

Total RNA was extracted from whole blood of Patient 4
using the PAXgene Blood RNA Kit (Qiagen). Subsequently,
complementary DNA (cDNA) was produced with a high-
capacity cDNA reverse-transcription kit (Applied Biosys-
tems, Carlsbad, CA, USA) according to the manufacturer’s
instructions. To analyze the effect of the splice site vari-
ant in the succinate-CoA ligase ADP-forming beta subunit
(SUCLA2) gene, we amplified SUCLA2 cDNA using the
following primer pairs:

- Forward primer located in exon 4: 5'-GGAAGTTCACAT
  GGTGGTGC-3'
- Reverse primer located in exon 7: 5'-TGAGATTTGCCT
  TAGCAGCA-3'

Clinical studies

Clinical findings and laboratory data, nerve conduction stud-
ies (NCS), and image examinations were based on the cur-
rently available information for all patients. The primary
physician performed histological investigations of the sural
nerve biopsy in Patient 1 as well as skeletal muscle biopsies
in Patients 3 and 4, after obtaining informed consent. In
Patient 4, respiratory chain enzyme activities in the skeletal
muscle homogenate were also assayed as described earlier
[11].

Results

From WES data of 247 CMT patients with AR or sporadic
inheritance, we concentrated all uncommon variants (allele
frequency <0.05) in the mitochondria-related 167 nuclear
inheritance, we concentrated all uncommon variants (allele
frequency <0.05) in the mitochondria-related 167 nuclear
gene panel. Therein, bi-allelic variants were identified in
four patients, from four distinct genes. These genes were
pyruvate dehydrogenase, beta-polypeptide (PDHB), mito-
ochondrial poly(A) polymerase (MTPAP), hydroxyacyl-
CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA
hydratase, beta subunit (HADHB), and SUCLA2. These vari-
ants comprise c.880G > A (p.G294R) homozygous variants
in PDHB (Fig. 1a), c.833G > T (p.R278I) and c.1531C > T
(p.Q511*) compound heterozygous variants in MTPAP
(Fig. 1b), c.1192 T > C (p.F398L) homozygous variants
in HADHB (Fig. 1c), and c.664-1G > A and c.1,300delG
(p.D434fs) compound heterozygous variants in SUCLA2
(Fig. 1d). All the missense variants were novel and located
at a highly conserved amino acid residues (Fig. 1e–h), and
most variants were located in each protein domain region
(Fig. 1i–l). All variants were absent in the control database
and predicted to be damaging or deleterious during in silico
analysis; the in silico analysis results as well as the classi-
fication by ACMG standards and guidelines are shown in
Supplementary Table 2. The genetic and clinical presenta-
tions of the patients are summarized in Table 1.

Segregation analyses were performed for the parents
of all four patients, except Patient 3, whose parents were
deceased. The same genotype was detected in an affected
elder brother of Patient 2 (Fig. 2).

Patient 1 [PDHB c.880G > A (p.G294R)]

This patient was a woman in her 30 s who was born weigh-
ing 2400 g. By 2 years of age, she had a ventricular septal
defect and seizures four times. She began walking at 1 year
and 6 months but had gait disturbance. The surgery of the
strabismus was performed at age 4 and of the ileus at age
15. Gait disturbance progressed, and sural nerve biopsy
was performed at age 25. A decrease in the density of large
myelinated fibers with thin small myelinated clusters was
revealed by nerve analysis (Fig. 3a). Simultaneously, adjust-
dment disorder associated with mild-to-moderate intellectual
disability was observed. Cerebral atrophy with no white mat-
ter lesions was shown by brain magnetic resonance imaging
(MRI) (Fig. 3b). Clinically, the patient had weakness and
atrophy of the distal limb muscles, pes cavus, and hallux
valgus. Disturbance of the leg vibration sense and decreased
tendon reflexes were also noted. Axonal polyneuropathy was
revealed by NCS (Table 1).

Patient 2 [MTPAP c.833G > T(p.R278I) c.1531C > T
(p.Q511*)]

This male patient in his 30 s who had gait disturbance at the
age of 1 year and 6 months. At 28 years, scoliosis, equinus
foot, and paresthesia were present, and he could not walk.
Sensory-dominant axonal polyneuropathy was revealed by
NCS (Table 1), and he had skin acne through the lumbar
region from the head. Low levels of vitamin B12 (70 ng/
ml; normal: 180–914 ng/mL) and folate (1.3 ng/mL; nor-
mal: > 3 ng/mL) were revealed in laboratory investigations,
but the intrinsic factor antibody and gastric parietal cell anti-
body were negative. An upper gastrointestinal endoscopy
showed only atrophic gastritis. Patchy T2 hyperintensities in
the white matter of the temporal and occipital lobes (Fig. 3c)
were revealed by brain MRI.
During elementary school, his elder brother, who had the same MTPAP variants, also had gait disturbance, amblyopia, and scoliosis, and he began using a wheelchair at 38 years of age. We observed hypoesthesia and optic atrophy, and axonal polyneuropathy was revealed by NCS. His vitamin B12 levels were low, and despite supplementation, the symptoms worsened.

**Patient 3 [HADHB c.1192 T>C (p.F398L)]**

Details about this case were previously established [12]. A man in his 50s had languor of the lower extremity and brown urine after exercise at the age of 10. For a while, symptoms improved but recurred at age 45, and gait disturbance progressed. At age 55, deep tendon reflexes were...
absent, and muscle weakness and atrophy in his distal lower limbs were present. His senses of vibration and position were deeply disturbed, and sensory ataxia was present. Axonal sensorimotor polyneuropathy was shown by NCS (Table 1) and normal creatine kinase levels by laboratory investigations. After gene analysis, he suffered from recurrent rhabdomyolysis. Neurogenic changes, such as marked fiber-type grouping were revealed by open muscle biopsy (Fig. 3d), but there was no mitochondrial abnormality. His 3-hydroxy-tetradecanoyl carnitine (C14-OH) level was slightly increased (0.06 nmol/mL; normal: < 0.05 nmol/mL).

Patient 4 [SUCLA2 c.1300delG (p.D434Mfs*8) c.664-1G > A (p.L222_Q224del)]

Details about this case were previously established [13]. This infant girl was born weighing 2,450 g. At 3 months, ptosis, deafness, scaphocephaly, and axial hypotonia were observed. At 7 months, profound intellectual disability was noted. At 1 year, she developed dysphagia, and tube feeding was started. At 2 years, gastroesophageal reflux and scoliosis were observed accompanied by cyclic vomiting and hypercapnia during sleep. NCS showed that axonal polyneuropathy predominantly affected the motor nerves (Table 1). Laboratory tests showed increased levels of mild methylmalonic aciduria (34.1 μg/mg creatinine; normal: < 10 μg/mg creatinine), blood lactate (58.5 mg/dL; normal: 4–17 mg/dL), and pyruvate (2.17 mg/dL; normal: 0.3–0.9 mg/dL), and cerebrospinal fluid lactate and pyruvate levels were 27.9 mg/dL and 1.81 mg/dL, respectively. Brain MRI revealed cerebral atrophy and T2 hyperintensities in the bilateral putamen and caudate nuclei (Fig. 3e) and a lactate peak in magnetic resonance spectroscopy (Fig. 3f). Therefore, Leigh syndrome was suspected; however, there was no known mitochondrial DNA mutation in peripheral blood lymphocytes. Open muscle biopsy showed a neurogenic change in the fiber-type grouping (Fig. 3g), but no insufficient cytochrome c oxidase activity, ragged red fibers, or increased staining for succinate dehydrogenase were observed. Hence, she was tentatively diagnosed with CMT2 or other complex hereditary peripheral neuropathy.

### Table 1 Genetic and clinical features of four patients with mitochondrial-related nuclear gene variants

| Patient No | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|------------|-----------|-----------|-----------|-----------|
| Gene symbol | PDHB      | MTPAP     | HADHB     | SUCLA2    |
| Mutation   | c.880G > A (p.G294R) | c.833G > T (p.R278I) | c.1192 T > C (p.F398L) | c.1300delG (p.D434Mfs*8) |
| Genotype   | Homo      | C Hetero  | Homo      | C Hetero  |
| Age at the most recent exam (years)/Sex | 37/F | 37/M | 56/M | 4/F |
| Onset      | 0 years   | 1 years   | 10 years  | 3 months |
| Consanguinity | No        | No        | No        | No        |
| Segregation | Yes       | Yes       | No        | Yes       |
| Initial symptom | Seizure   | Gait disturbance | Gait disturbance | Hypotonia, Ptosis, Deafness, Scaphocephaly |
| MMT<sup>a</sup> | 2         | 1         | 3         | 3         |
| Sensory disturbance | Yes      | Yes       | Yes       | No        |
| DTRs       | All absent bilaterally | All absent bilaterally | All absent bilaterally | All absent bilaterally |
| NCS        | dCMAP (mV) | Median 4.2 Tibial 2.53 | Median 18.9 Tibial 1.98 | Median 0.41 Tibial 1.49 |
|            | MCV (m/s) | Median 46.4 Tibial 43.9 | Median 59.7 Tibial 53.7 | Median 54.5 Tibial 39.1 |
|            | SNAP (μV) | Median 4.2 Sural 1.66 | Median ND Sural ND | Median 3.62 Sural ND |
|            | SCV (m/s) | Median 53.8 Sural 45.6 | Median ND Sural ND | Median 48.4 Sural ND |
| Other findings | Pes cavus | Equinus foot | Pes cavus | Encephalopathy |
|            | Hallux valgus small-for-date infant | Scoliosis | Hammer toes | Intellectual disability |
|            | Intellectual disability | Skin acne | Brown urine | Dystonia |
|            | Strabismus | Vitamin B<sub>12</sub> and folate deficiency | Rhabdomyolysis | Ophthalmoplegia |
|            | VSD | | | Scoliosis |
|            | Ileus | | | Periodic vomiting |
|            | | | | Lactic acidosis |
|            | | | | Vitamin B<sub>12</sub> and folate deficiency |

<sup>C Hetero</sup> compound heterozygous, <sub>dCMAP</sub> distal compound muscle action potential, <sub>DTRs</sub> deep tendon reflexes, <sup>Homo</sup> homozygous, <sub>MCV</sub> motor conduction velocity, <sub>MMT</sub> manual muscle testing, NA not available, <sub>NCS</sub> nerve conduction study, ND not detected (evoked), <sub>SCV</sub> sensory conduction velocity, <sub>SNAP</sub> sensory nerve action potential, VSD ventricular septal defect

<sup>a</sup>Scores indicating manual muscle testing (MMT) grade in the distal lower limbs
After the gene analysis, the muscle respiratory chain enzyme activities showed deficiencies in complexes I (45%) and IV (43%), whereas complex II (the only complex that does not contain mtDNA-encoded proteins) was normal (105%; each activity was expressed as the citrate synthase ratio).

Regarding heterozygous splice acceptor site variant (c.664-1G > A) in intron 5, we detected two RT-PCR products comprising a wild-type band and a smaller band at 370 bp (Fig. 4a). Sanger sequencing of the RT-PCR product revealed 9 bp heterozygous deletion (Fig. 4b), leading to the in-frame deletion of three amino acids in exon 6 of SUCLA2 (p.L222_Q224del; Fig. 4c).

Discussion

Using WES, we performed further genetic analysis targeting 167 mitochondrial-related nuclear genes among a group of patients with clinically suspected with pure or complex hereditary peripheral neuropathy. In four patients, we identified four disease-causing genes, of which variants in PDHB, MTPAP, and SUCLA2 have been reported not as a cause of CMT but of other diseases associated with symptomatic or subclinical peripheral neuropathy [14–16]. Although recessive mutations in HADHB have been assumed to be associated with axonal CMT, no pathophysiological mechanism was elucidated [17, 18]. We indicated the pathological significance of the PDHB and HADHB genes using Drosophila neuron-specific knockdown models in a previous study [19, 20]. Although all four patients presented with various phenotypes, motor and sensory axonal neuropathy was the major clinical feature.

The pyruvate dehydrogenase alpha (PDHA) and PDHB encode pyruvate dehydrogenase, a component enzyme of the pyruvate dehydrogenase multienzyme complex in mitochondria [21]. Recessive PDHB mutation causes pyruvate dehydrogenase complex (PDC) deficiencies with severe clinical consequences that primarily affect the nervous system, such as developmental delay, seizures, central hypotonia, ataxia, peripheral neuropathy, microcephaly, congenital brain malformations, and degenerative changes, such as Leigh syndrome [14, 21, 22]. Given that cases of thiamine-sensitive PDC deficiency have been reported and a ketogenic diet may alleviate the symptoms [21], it is important to diagnose this disease. In 3–21% of patients, peripheral neuropathy has been found to be linked to PDC deficiencies [21], whereas progressive peripheral neuropathy in adult patients has been linked only to mutations in PDHA1 [22]. In Patient 1, in addition to peripheral neuropathy, central nervous system...
involvement was suggested by multiple manifestations, comprising seizures, intellectual disability, and cerebral atrophy in brain MRI. The G294R variant is located at the transketolase coding region of the C-terminal domain of the PDHB protein (Fig. 1i) and is close to the previously reported mutations of PDC deficiencies [14].

*MTPAP* gene encodes the mitochondrial polyadenylation polymerase, involved in DNA maintenance and repair [23]. Recessive mutation in *MTPAP* would result in delayed DNA repair, elevation of the level of DNA double-strand breaks, reactive oxygen species, and cell death after irradiation, leading to spastic ataxia and optic atrophy or encephalopathy. A previous case report described progressive lower motor neuron involvement due to *MTPAP* mutation [15] but progressive peripheral neuropathy, especially sensory nerves, without pyramidal signs, as observed in family 2, has not previously been described.

Importantly, low levels of vitamin B$_{12}$ and folate, as found in Patient 2 and his brother, might also cause peripheral neuropathy. Folate and vitamin B$_{12}$, especially methylcobalamin, are coenzymes for cytosolic methionine synthase involved in vital cellular processes, such as methylation and DNA synthesis [24, 25]. Bi-allelic mutations of *MTPAP* would elevate both DNA double-strand breaks and cell death, whereas over-consumption of vitamin B$_{12}$ and folate for DNA synthesis could cause deficiencies of...
these substances. Despite the normalization of vitamin B₁₂ and folate levels by supplementary treatment, their symptoms progressed, suggesting other contributive reasons. In family 2, segregation analysis confirmed that p.R278I and p.Q511* of MTPAP are compound heterozygotes, which would affect both DNA strands, thereby influencing the MTPAP protein function. The p.R279I variant is located in the nucleotidyltransferase (NT) domain of poly(A) polymerases and terminal uridylyl transferases (Fig. 1j); no mutation is reported in this domain. Because the p.Q511* variant is located in the last exon, and loss-of-function has not been a recognized mechanism in MTPAP-related disease, this variant was classified as “strong,” as per the ACMG standards and guidelines. Further investigations are needed to clarify the role of vitamin B₁₂ and folate in the pathogenesis of MTPAP-related peripheral neuropathy.

The alpha and beta subunits of the mitochondrial trifunctional protein (MTP) are encoded by the HADHA and HADHB genes [26]. These subunits catalyze three steps in the beta-oxidation of fatty acids, including the long-chain 3-hydroxyacyl-CoA dehydrogenase step. Recessive HADHB mutation results in the dysfunction of the beta-oxidation of fatty acids, leading to MTP deficiency, characterized in a severe heterogeneous syndrome, such as cardiomyopathy, recurrent Leigh-like encephalopathy, hepatopathy, and neonatal or unexpected infant death [27]. In contrast, the milder form is characterized by later-onset progressive axonal peripheral neuropathy (approximately 50–80%) and myopathy with or without episodic myoglobinuria [17, 26]. Phenylalanine 398 is located in the thiolase, C-terminal domain of HADHB protein (Fig. 1k), and is close to known mutations linked to the milder form [26]. After the genetic diagnosis, Patient 3 experienced recurrent episodes of brown urine after exercise and rhabdomyolysis, and his C_{13}-OH level increased slightly; all these characteristic phenotypes were considered supportive evidence to his diagnosis. As described for Patient 3, peripheral neuropathy may be the main symptom in patients with HADHB mutations, or at least in certain stages of the disease course.

The SUCLA2 gene is involved in the citric acid cycle and mtDNA synthesis [28]. In the citric acid cycle, SUCLA2 catalyzes the reversible formation of succinate and ATP from succinyl-CoA and ADP. This protein also interacts with nucleoside diphosphate kinase, which is involved in mtDNA synthesis [28]. Recessive SUCLA2 mutation causes mtDNA depletion syndrome, which could cause mild methylmalonic aciduria, recurrent vomiting, hypotonia, dehydration, respiratory distress, neonatal encephalopathy, and progressive lethargy [16]. Peripheral neuropathy could also be observed, as described for Patient 4. We identified two heterozygous variants in SUCLA2 from Patient 4: frame shift (c.1300delG) and splice site (c.664-1G > A); the latter was found to produce a protein that is three amino acids smaller than normal (p.L222_Q224del). The c.1300delG variant, located close to the 3′-terminal of SUCLA2, is likely to produce a truncated protein (Fig. 1l). Taken together with the segregation analysis, we classified these bi-allelic variants as pathogenic/probably pathogenic.

Peripheral nerves have long axons wrapped in myelin lamellae provided by Schwann cells and are highly dependent on energy metabolism. ATP synthesis might eventually be influenced by any abnormalities in energy production and

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**Fig. 4** RNA analysis of SUCLA2. a Agarose gel electrophoresis of cDNA fragments obtained from the RT-PCR of Patient 4. Patient 4 had the expected 379 bp band and an additional 370 bp band, which is the result of the deletion of exon 6 in one allele. b, c Sequence chromatogram of the RT-PCR product from Patient 4 showing heterozygote 9 bp deletion in exon 6. bp base pairs, NC normal control.
Table 2 Genetic pathophysiology, phenotype, inheritance pattern, and neuropathy type of the mitochondrial-related nuclear genes described in this report (upper table) and previously reported genes (lower table)

| Gene      | Pathophysiology                                                                 | Phenotype                                                                 | Inheritance | Neuropathy type                  |
|-----------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------|-------------|----------------------------------|
| PDHB      | Pyruvate dehydrogenase complex                                                  | Pyruvate dehydrogenase E1-beta deficiency                                 | AR          | Sensory-motor axonal             |
| MTPAP     | mtDNA maintenance and repair                                                     | SPAX4                                                                    | AR          | Sensory-motor axonal             |
| HADHB     | Mitochondrial energy production (beta-oxidation)                                | Trifunctional protein deficiency                                           | AR          | Sensory-motor axonal             |
| SUCLA2    | Mitochondrial energy production (tricarboxylic acid cycle), mtDNA synthesis     | Mitochondrial DNA depletion syndrome 5                                     | AR          | Sensory-motor axonal             |
| MFN2      | Mitochondrial dynamics (fusion)                                                 | CMT2A2, HMSN6 (CMT6A)                                                    | AR and AD   | Sensory-motor axonal             |
| OPA1      | Mitochondrial dynamics (fusion)                                                 | Optic Atrophy I, Mitochondrial DNA depletion syndrome                     | AD          | Sensory-motor axonal             |
| GDAP1     | Mitochondrial dynamics (fission)                                                | CMT4A, CMT2K, CMTRIA, CMT with vocal cord paresis                         | AR and AD   | Sensory-motor axonal (with or without secondary demyelinating changes) |
| SLC25A46  | Mitochondrial dynamics (fission)                                                | HMSN6B (CMT6B)                                                           | AR          | Motor or sensory-motor axonal    |
| MYH14     | Mitochondrial dynamics (fission)                                                | Peripheral neuropathy, myopathy, hoarseness, and hearing loss              | AD          | Motor axonal (with or without sensory demyelinating changes) |
| MFF       | Mitochondrial dynamics (fission)                                                | Encephalopathy due to defective mitochondrial and peroxisomal fission 2   | AR          | Motor demyelinating or mixed     |
| DHTKD1    | Mitochondrial energy production (tricarboxylic acid cycle)                      | CMT2Q                                                                    | AD          | Sensory-motor axonal             |
| HK1       | Mitochondrial energy production (glycolytic system)                             | CMT4G                                                                    | AD          | Sensory-motor demyelinating      |
| COX6A1    | Mitochondrial respiratory chain (complex IV)                                   | CMTRID                                                                   | AR          | Sensory-motor axonal or mixed    |
| SURF1     | Mitochondrial respiratory chain (complex IV)                                   | CMT4K, Leigh syndrome                                                    | AR          | Sensory-motor demyelinating      |
| AIFM1     | Oxidative phosphorylation and redox control in healthy cells                   | CMTX4 (Cowchock syndrome)                                               | XLR         | Sensory-motor axonal             |
| PDK3      | Pyruvate dehydrogenase complex                                                  | CMTX6                                                                    | XLD         | Sensory-motor axonal (with or without secondary demyelinating changes) |
| C12orf65  | Mitochondrial energy production (oxidative phosphorylation), Mitochondrial translation | Combined oxidative phosphorylation deficiency 7 SPG55, CMT6                | AR          | Sensory-motor axonal             |
| POLG1     | mtDNA replication and maintenance                                               | Childhood MCHS, Alpers syndrome, ANS disorders, MEMSA, MNGIE-like, SANDO autosomal recessive and dominant PEO | AR and AD   | Sensory axonal; hypomyelinating when early onset |
| C10orf2   | mtDNA replication and maintenance                                               | Ans disorders, Mitochondrial DNA Depletion Syndrome, PEO                 | AR and AD   | Usually sensory axonal           |
| TYMP      | mtDNA replication and maintenance                                               | Mitochondrial DNA Depletion Syndrome, MNGIE                               | AR          | Sensory-motor demyelinating      |
| RRM2B     | mtDNA replication and maintenance                                               | Mitochondrial DNA Depletion Syndrome, MNGIE-like, PEO                    | AR and AD   | Sensory-motor demyelinating      |
| MPV17     | mtDNA maintenance                                                               | Mitochondrial DNA Depletion Syndrome Navajo neurohypopatophathy          | AR          | Sensory-motor axonal or demyelinating |
| SLC25A19  | mtDNA replication and maintenance                                               | Bilateral striatal degeneration and progressive polynuropathy            | AR          | Motor or sensory-motor axonal    |
| COA7      | Assembling mitochondrial respiratory chain complexes                             | Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy       | AR          | Sensory-motor axonal             |

AD autosomal dominant, ANS ataxia neuropathy spectrum, AR autosomal recessive, CMT Charcot–Marie–Tooth disease, CMTRIA Charcot–Marie–Tooth disease, recessive intermediate A, CMTRID Charcot–Marie–Tooth disease, recessive intermediate D, HMSN hereditary motor and sensory neuropathy, MCHS myocerebrohepatopathy spectrum disorders, MEMSA myoclonus epilepsy myopathy sensory ataxia, MNGIE mitochondrial neurogastrointestinal encephalomyopathy, mtDNA mitochondrial DNA, PEO progressive external ophthalmoplegia, SANDO sensory ataxic neuropathy with dysarthria and ophthalmoparesis, SPAX4 spastic ataxia autosomal recessive Type 4, SPG55 spastic paraplegia 55, XLD X-linked dominant, XLR X-linked recessive
depletion of mitochondria, leading to peripheral neuropathy. Moreover, various mitochondrial dysfunctions, such as dysfunctions in mitochondrial energy production or PDC, or assembling mitochondrial respiratory chain complex have been reported to cause CMT (Table 2) [2, 29–35]. Pathological mechanisms of each gene remain unclear, because peripheral nerves have long axons wrapped in myelin lamellae provided by Schwann cells and are highly dependent on energy metabolism; however, ATP synthesis might eventually be influenced by any abnormalities in energy production and depletion of mitochondria, leading to peripheral neuropathy (Fig. 5). As observed in patients with bi-allelic variants in PDHB and SUCLA2, in mitochondrial disorders, multisystem manifestation representative of central nervous system involvement is a common feature suggestive of clinical diagnosis. Conversely, like MTPAP and HADHB, these genes could hardly result in central nervous system abnormalities; thus, more attention should be paid to clinical assessments, particularly for certain symptoms that transiently emerged in their disease course and naturally disappeared with age. Moreover, because of the clinical diversity of these patients, peripheral neuropathy has always been recognized as part of the mitochondrial disorder rather than of CMT. Heterogeneous phenotypes of both mitochondrial disorders and CMT make the clinical diagnosis of either difficult. However, the development of diagnostic genetics has facilitated diagnoses made on a genetic basis.

In this study of a large Japanese cohort of patients with clinically suspected pure or complex hereditary peripheral neuropathy, we identified novel likely pathogenic/pathogenic
variants in four mitochondrial-related nuclear genes. Mitochondrial abnormalities should be considered as a differential diagnosis in cases of axonopathy with suggestive symptoms or other unexplainable multisystem manifestations. Considering the limited number of gene panels targeted in our study, the discovery of more mitochondrial-related nuclear genes leading to mitochondrial-related neuropathy is highly likely. Regarding treatment, early diagnosis would provide more effective and prompt therapy strategies and medicines for the improvement of mitochondrial function might one day be a common target for mitochondrial neuropathy.

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Data availability statement Data are available on request from the authors.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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References

1. Pipis M, Rossor AM, Laura M, Reilly MM (2019) Next-generation sequencing in Charcot–Marie–Tooth disease: opportunities and challenges. Nat Rev Neurol 15:644–650. https://doi.org/10.1038/s41582-019-0254-5
2. Pareyson D, Piscosquito G, Moroni I, Salsano E, Zeviani M (2013) Peripheral neuropathy in mitochondrial disorders. Lancet Neurol 12:1011–1024. https://doi.org/10.1016/S1477-4422(13)70158-3
3. Luigetti M, Sauchelli D, Primiano G, Cuccagna C, Bernardo D, Lo Monaco M, Servidei S (2016) Peripheral neuropathy is a common manifestation of mitochondrial diseases: a single-centre experience. Eur J Neurol 23:1020–1027. https://doi.org/10.1111/ene.12954
4. Rath S, Sharma R, Gupta R, et al. (2021) MitoCarta3.0: An updated inventory of the mitochondrial proteome, now with sub-organelle localization and pathway annotations. Nucleic Acids Res 49:D1541–D1547. https://doi.org/10.1093/nar/gkaa1011
5. Koopman WI, Willems PH, Smeitink JA (2012) Monogenic mitochondrial disorders. N Engl J Med 366:1132–1141
6. Hashiguchi A, Higuchi Y, Nomura M et al (2014) Neurofilament light mutation causes hereditary motor and sensory neuropathy with pyramidal signs. J Peripher Nerv Syst 19:311–316. https://doi.org/10.1111/jns.12102
7. Maeda K, Idehara R, Hashiguchi A, Takashima H (2014) A family with distal hereditary motor neuropathy and a K141Q mutation of small heat shock protein HSPB1. Intern Med 53:1655–1658. https://doi.org/10.2169/internalmedicine.53.2843
8. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324
9. Li H, Handsaker B, Wysoker A et al (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352
10. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30
11. Murayama K, Nagasaka H, Tsuruoka T et al (2009) Intractable secretory diarrhea in a Japanese boy with mitochondrial respiratory chain complex I deficiency. Eur J Pediatr 168:297–302. https://doi.org/10.1007/s00431-008-0753-7
12. Yamamoto Y, Matsui N, Hiramatsu Y et al (2017) Mitochondrial trifunctional protein deficiency: an adult patient with similar progress to Charcot–Marie–Tooth disease. Rinsho Shinkeigaku 57:82–87. https://doi.org/10.5692/clinicalneurol.cn-000976
13. Fumihito N, Mioko M, Tomohiro K et al (2020) A rare case of SUCLA2-related mitochondrial DNA depletion syndrome. No To Hattatsu 52:318–322. https://doi.org/10.11251/ojcn.52.318
14. Okajima K, Korotchkina LG, Prasad C et al (2008) Mutations of the E1beta subunit gene (PDHB) in four families with pyruvate dehydrogenase deficiency. Mol Genet Metab 93:371–380. https://doi.org/10.1016/j.ymgme.2007.10.135
15. Crosby AH, Patel H, Chioza BA et al (2010) Defective mitochondrial mRNA maturation is associated with spastic ataxia. Am J Hum Genet 87:655–660. https://doi.org/10.1016/j.ajhg.2010.09.013
16. Carrozzo R, Dionisi-Vici C, Steuerwald U et al (2007) SUCLA2 mutations are associated with mild methylmalonic aciduria, Leigh-like encephalomyopathy, dystonia and deafness. Brain 130:862–874. https://doi.org/10.1093/brain/awl389
17. Hong YB, Lee JH, Park JM et al (2013) A compound heterozygous mutation in HADHB gene causes an axonal Charcot–Marie–Tooth
18. Lu Y, Wu R, Meng L et al (2018) HADHB mutations cause infantile-onset axonal Charcot–Marie–Tooth disease: a report of two cases. Clin Neuropathol 37:232–238. https://doi.org/10.5414/np301097

19. Dung VM, Suong DNA, Okamaoto Y et al (2018) Neuron-specific knockdown of Drosophila PDHB induces reduction of lifespan, deficient locomotive ability, abnormal morphology of motor neuron terminals and photoreceptor axon targeting. Exp Cell Res 366:92–102. https://doi.org/10.1016/j.yexcr.2018.02.035

20. Li J, Suda K, Ueoka I et al (2019) Neuron-specific knockdown of Drosophila HADHB induces a shortened lifespan, deficient locomotive ability, abnormal motor neuron terminal morphology and learning disability. Exp Cell Res 379:150–158. https://doi.org/10.1016/j.yexcr.2019.03.040

21. DeBrosse SD, Okajima K, Zhang S et al (2012) Spectrum of neurological and survival outcomes in pyruvate dehydrogenase complex (PDC) deficiency: lack of correlation with genotype. Mol Genet Metab 107:394–402. https://doi.org/10.1016/j.ymgme.2012.09.001

22. Sedel F, Challe G, Mayer JM, Boutron A, Fontaine B, Saudubray JM, Brivet M (2008) Thiamine responsive pyruvate dehydrogenase deficiency in an adult with peripheral neuropathy and optic neuropathy. J Neurol Neurosurg Psychiatry 79:846–847. https://doi.org/10.1136/jnnp.2007.136630

23. Martin NT, Nakamura K, Paila U et al (2014) Homozygous mutation of MTPAP causes cellular radiosensitivity and persistent DNA double-strand breaks. Cell Death Dis 5:e1130. https://doi.org/10.1038/cddis.2014.99

24. Coelho D, Suormala T, Stucki M et al (2008) Gene identification for the cblD defect of vitamin B12 metabolism. N Engl J Med 358:1454–1464. https://doi.org/10.1056/NEJMoa072200

25. Bailey LB, Gregory JF III (1999) Folate metabolism and requirements. J Nutr 129:779–782

26. Naiki M, Ochi N, Kato YS et al (2014) Mutations in HADHB, which Encodes the β-Subunit of mitochondrial trifunctional Protein, Cause Infantile Onset Hypoparathyroidism and peripheral Polyneuropathy. Am J Med Genet A 164A:1180–1187. https://doi.org/10.1002/ajmg.a.36434

27. Spiekerkotter U, Bennett MJ, Ben-Zeev B, Strauss AW, Tein I (2004) Peripheral neuropathy, episodic myoglobinuria, and respiratory failure in deficiency of the mitochondrial trifunctional protein. Muscle Nerve 29:66–72. https://doi.org/10.1002/mus.10505

28. Elpeleg O, Miller C, Hershkovicz E et al (2005) Deficiency of the ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. Am J Hum Genet 76:1081–1086. https://doi.org/10.1086/430843

29. Copeland WC (2008) Inherited mitochondrial diseases of DNA replication. Annu Rev Med 59:131–146. https://doi.org/10.1146/annurev.med.59.053006.104646

30. Almutawwa W, Smith C, Sabouny R et al (2019) The R941L mutation in MYH14 disrupts mitochondrial fission and associates with peripheral neuropathy. EBioMedicine 45:379–392. https://doi.org/10.1016/j.ebiom.2019.06.018

31. Koch J, Feichtinger RG, Freisinger P et al (2016) Disturbed mitochondrial and peroxisomal dynamics due to loss of MFF causes Leigh-like encephalopathy, optic atrophy and peripheral neuropathy. J Med Genet 53:270–278. https://doi.org/10.1136/jmedgenet-2015-103500

32. Abrams AJ, Hufnagel RB, Rebelo A et al (2015) Mutations in SLC25A46, encoding a UGO1-like protein, cause an optic atrophy spectrum disorder. Nat Genet 47:926–932. https://doi.org/10.1038/ng.3354

33. Tucci A, Liu YT, Preza E et al (2014) Novel C12orf65 mutations in patients with axonal neuropathy and optic atrophy. J Neurol Neurosurg Psychiatry 85:486–492. https://doi.org/10.1136/jnnp-2013-306387

34. Tamiya G, Makino S, Hayashi M et al (2014) A mutation of COX6A1 causes a recessive axonal or mixed form of Charcot–Marie–Tooth disease. Am J Hum Genet 95:294–300. https://doi.org/10.1016/j.ajhg.2014.07.013

35. Higuchi Y, Okunushi R, Hara T et al (2018) Mutations in COA7 cause spinocerebellar ataxia with axonal neuropathy. Brain 141:1622–1636. https://doi.org/10.1093/brain/awy104