

Neurophysiological profile of peripheral neuropathy associated with childhood mitochondrial disease

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1 Abstract

2 **Introduction:** Peripheral nerve involvement is common in mitochondrial disease but often
3 unrecognised due to the prominent central nervous system features. Identification of the
4 underlying neuropathy may assist syndrome classification, targeted genetic testing and
5 rehabilitative interventions.

6 **Methods:** Clinical data and the results of nerve conduction studies were obtained
7 retrospectively from the records of four tertiary children's hospital metabolic disease,
8 neuromuscular or neurophysiology services. Nerve conduction studies were also performed
9 prospectively on children attending a tertiary metabolic disease service. Results were
10 classified and analysed according to the underlying genetic cause.

11 **Results:** Nerve conduction studies from 27 children with mitochondrial disease were
12 included in the study (mitochondrial DNA (mtDNA) – 7, *POLG* – 7, *SURF1* – 10, PDHc
13 deficiency – 3). Four children with mtDNA mutations had a normal study while three had
14 mild abnormalities in the form of an axonal sensorimotor neuropathy when not acutely
15 unwell. One child with MELAS had a severe acute axonal motor neuropathy during an acute
16 stroke-like episode that resolved over 12 months. Five children with *POLG* mutations and
17 disease onset beyond infancy had a sensory ataxic neuropathy with an onset in the second
18 decade of life, while the two infants with *POLG* mutations had a demyelinating neuropathy.
19 Seven of the 10 children with *SURF1* mutations had a demyelinating neuropathy. All three
20 children with PDHc deficiency had an axonal sensorimotor neuropathy. Unlike CMT, the
21 neuropathy associated with mitochondrial disease was not length-dependent.

22 **Conclusions:** This is the largest study to date of peripheral neuropathy in genetically-
23 classified childhood mitochondrial disease. Characterising the underlying neuropathy may
24 assist with the diagnosis of the mitochondrial syndrome and should be an integral part of the
25 assessment of children with suspected mitochondrial disease.

26 **Key words:** mitochondrial disease, childhood, neuropathy, demyelinating, mitochondrial

27 DNA

1 **1. Introduction**

2 Childhood mitochondrial diseases have a heterogeneous phenotype with many
3 different systems being affected including the peripheral nervous system. Around 30% of
4 children with a mitochondrial disease have an associated peripheral neuropathy (*Colomer, et*
5 *al., 2000*), but the neuropathy is often unrecognised due to the overwhelming central nervous
6 system manifestations. Mutations in nuclear genes responsible for mitochondrial dynamics
7 and axonal transport, including *MFN2* and *GDAP1*, are recognised causes of Charcot-Marie-
8 Tooth disease (CMT) (*Niemann, et al., 2005; Züchner, et al., 2004*). Recently, mutations in
9 *MT-ATP6* and *SURF1*, genes known to cause Leigh syndrome and other multisystemic
10 mitochondrial diseases, have also been shown to cause phenotypes characterised
11 predominantly by a peripheral neuropathy (*Echaniz-Laguna, et al., 2013; Pitceathly, et al.,*
12 *2012*). Identifying the presence of a peripheral neuropathy and defining its characteristics
13 may help with classifying the mitochondrial syndrome and targeted genetic testing (*Menezes*
14 *and Ouvrier, 2012*). The associated peripheral neuropathy may be symptomatic and disabling
15 and specific treatment and rehabilitative intervention may be needed.

16

17 **2. Methods**

18 Children with mitochondrial disease and identified mutations who had previously
19 undergone nerve conduction studies were identified from the mitochondrial diseases database
20 at the Murdoch Childrens Research Institute, Melbourne, Australia, the records of the Genetic
21 Metabolic Disorders Clinic at The Children's Hospital at Westmead, Sydney, Australia, the
22 Sydney Children's Hospital Randwick Nerve and Muscle Clinic and the Neurophysiology
23 Department at Great Ormond Street Children's Hospital, London, UK. Nerve conduction
24 studies were also performed prospectively according to a defined protocol (see
25 Supplementary Methods) on children from the Genetic Metabolic Disorders Clinic at The

1 Children's Hospital at Westmead who had identified mitochondrial mutations and consented
2 to inclusion in the study. Children with pyruvate dehydrogenase complex (PDHc) deficiency
3 were included if there was biochemical confirmation of the PDHc deficiency, even if a
4 genetic mutation had not been identified. The data from both retrospective and prospective
5 groups were combined and classified according to the underlying genetic cause. The results
6 were compared with age-matched normative values (*Cai and Zhang, 1997*). Because of a lack
7 of published paediatric electrodiagnostic criteria for demyelination in inherited neuropathies,
8 the EFNS/PNS electrodiagnostic criteria for demyelination in chronic inflammatory
9 demyelinating neuropathy were used (*Hughes, et al., 2006*). These criteria require a 30%
10 reduction of motor conduction velocity below the lower limit of normal in at least one nerve.
11 Both retrospective and prospective studies were approved by the Sydney Children's Hospital
12 Network Ethics Committee (10/56), and the retrospective study at Great Ormond Street
13 Hospital by the National Research Ethics Committee London Bloomsbury, UK.

14

15 **3. Results**

16 Nerve conduction data were available from 27 children from 25 families with a
17 genetically classified mitochondrial disease or biochemically-defined PDHc deficiency. The
18 data were collected over a six-year period (2010-2015). Retrospective nerve conduction
19 studies were available for 20 children and prospective nerve conduction studies were
20 performed on seven children. All nerve conduction studies in the retrospective series were
21 performed because of the clinical suspicion of a neuropathy, except in individual 3, who was
22 investigated because of the known association of a neuropathy with retinitis pigmentosa. One
23 child in the prospective study also had data included from another nerve conduction study
24 performed four years previously. Seven children had mitochondrial genome mutations and 20
25 had nuclear DNA mutations (*POLG* – 7, *SURF1* – 10, PDHc deficiency – 3). All genetic

1 diagnoses were established after Sanger sequencing of individual nuclear or mitochondrial
2 genes or after testing of a panel of common mitochondrial genome mutations. None of the
3 diagnoses were established by next generation sequencing technologies. All identified
4 mutations have been previously reported as pathogenic except the c.897G>A (p.Met299Ile)
5 variant in *POLG* (individual 9 in table 2). The neurophysiological results were categorised by
6 the causative gene (Tables 1-4).

7

8 *3.1. Mitochondrial DNA mutations*

9 Neurophysiologic findings in seven children (from six families) with mutations in the
10 mitochondrial genome were evaluated (Table 1). Three had Leigh/NARP (neurogenic muscle
11 weakness, ataxia, and retinitis pigmentosa) syndrome due to the m.8993T>C mutation in *MT-*
12 *ATP6*, one had MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and
13 stroke-like episodes) due to the m.3243A>G mutation in *MT-TL1* and two individuals had
14 single large mtDNA deletions. This group of children had normal studies or mild
15 abnormalities, usually in the form of an axonal sensorimotor neuropathy.

16

17 Of the children with ***MT-ATP6* mutations**, two (individuals 1 and 3) had presented in
18 the second year of life with developmental delay. Individual 1 had learning difficulties,
19 choreoathetosis, cerebellar ataxia, hypotonia, areflexia and episodes of acute weakness with
20 illness. His nerve conduction studies, done when not acutely ill, showed an axonal motor
21 neuropathy affecting the lower limbs. Individual 3 had a NARP-like phenotype with retinitis
22 pigmentosa but no clinical or neurophysiological evidence of a neuropathy. Individual 2
23 presented at 11 years of age with severe brainstem involvement (central hypertension,
24 hypoventilation, eye movement abnormality) following a respiratory infection with

1 widespread cortical, basal ganglia and brainstem changes on MRI. Her nerve conduction
2 study showed an absent sural SNAP but was otherwise normal.

3

4 One of the siblings with the m.3243A>G mutation in *MT-TL1* had **MELAS** (patient
5 5) while the other was asymptomatic (patient 4). The symptomatic sibling had presented at 10
6 years of age with frequent headache and had a classical MELAS clinical profile with
7 recurrent stroke-like episodes, multiple cortical infarcts, growth failure and seizures. Nerve
8 conduction tests done when he was admitted with an acute stroke-like episode with acute
9 sensorineural hearing loss, ataxia, bilateral intention tremor and reduced reflexes in the lower
10 limbs showed an axonal predominantly motor neuropathy. The studies were repeated a year
11 later, when he was well, and showed almost complete resolution of neuropathy with only
12 borderline reduction of lower limb motor amplitudes.

13

14 Prospective neurophysiological studies were undertaken in two individuals with
15 **single large mtDNA deletions**. One child had Pearson syndrome with sideroblastic anaemia,
16 renal Fanconi syndrome, growth failure, developmental delay and primary adrenal
17 insufficiency while the other had Kearns-Sayre syndrome with sensorineural hearing loss,
18 complete heart block, renal tubular acidosis, cerebellar ataxia, ptosis and ophthalmoplegia.
19 Neither had neurophysiological evidence of a peripheral neuropathy.

20

21 *3.2. Nuclear DNA mutations*

22 *3.2.1. POLG (Polymerase (DNA Directed), Gamma)*

23 Seven children with compound heterozygous or homozygous mutations in *POLG*, a
24 nuclear gene responsible for mtDNA maintenance, were included in this study (Table 2). All
25 the identified mutations have been previously reported as pathogenic except the c.897G>A

1 (p.Met299Ile) variant in *POLG* (individual 9). This mutation was seen in conjunction with a
2 recurrent mutation, was in a mutational hotspot and was not found on the Exome Aggregation
3 Consortium (ExAC) database. Two children presented within the first two years of life, one
4 with a Myocerebrohepatopathy Spectrum phenotype and the other with gross motor delay.
5 Both had failure to thrive, lactic acidemia, generalised hypotonia and areflexia. The other five
6 patients presented between four and 15 years of age, four with focal seizures and stroke-like
7 episodes while patient 12 presented with intestinal pseudo-obstruction. Two patients had liver
8 dysfunction when treated with sodium valproate. Among the later presentations, features of a
9 neuropathy (sensory ataxia, distal weakness and areflexia) were identified between 10 and 17
10 years of age. Two patients also had sural nerve biopsies that showed an axonal neuropathy
11 with loss of large myelinated fibres. Four of the seven patients had died between the ages of 2
12 weeks and 31 years.

13

14 Nerve conduction studies in the two youngest patients showed a demyelinating motor
15 neuropathy. Nerve conduction studies in the older children invariably showed a severe axonal
16 sensory neuropathy, irrespective of the age at which the studies were performed, with three of
17 the older children having additional motor nerve involvement.

18

19 3.2.2. *SURF1* (*Surfeit 1*)

20 Eleven nerve conduction studies from 10 children (from nine families; individuals 16
21 and 23 were siblings) with homozygous or compound heterozygous *SURF1* mutations were
22 included in this study (Table 3). Seven of the children described here (patients 16, 17, 18, 19,
23 20, 22, 23) were also included in a description of 44 individuals with *SURF1* mutations by
24 Wedatilake et al (*Wedatilake, et al., 2013*). Presentation was between three days and 18
25 months of age with poor feeding, vomiting and poor weight gain. The associated neuropathy

1 was usually evident by 18 months of age with gait ataxia and tremor in the upper limbs. Other
2 common features included growth failure, developmental regression, nystagmus and lactic
3 acidemia. Brain MRI showed changes consistent with Leigh syndrome in all except one child
4 who had a normal MRI at one year of age. Reduced or absent cytochrome c oxidase (COX)
5 activity in muscle or cultured fibroblasts was seen in all nine children who had this tested.
6 Nine of the children died between 20 months and seven years of age and one child was alive
7 at 12 years.

8

9 Seven children had a demyelinating neuropathy, with four having sensorimotor
10 involvement and three isolated motor involvement. Three studies showed predominantly
11 axonal changes with a mild reduction in motor conduction velocity. Unlike typical forms of
12 CMT, the nerve conduction abnormalities were not length-dependent. Three children had
13 only motor involvement on nerve conduction studies.

14

15 *3.2.3. PDHc Deficiency*

16 Three children with PDHc deficiency were included in this study. They had presented
17 in the first two years of life with hypotonia and global or isolated motor delay. Episodes of
18 lactic acidosis associated with generalised weakness and hypotonia were frequent initially but
19 decreased in frequency with advancing age and dietary therapy. Two children also had
20 intellectual delay while one had choreoathetosis and nystagmus. Brain MRI was
21 characteristic of PDHc deficiency with abnormalities in the basal ganglia, substantia nigra
22 and cerebellar white matter. The study in patient 27 was performed 8 weeks into an episode
23 of acute weakness. The nerve conduction studies showed a patchy axonal, non-length
24 dependent sensorimotor neuropathy (Table 4). Sensory responses were universally absent,
25 even in studies done at a young age.

1 3. Discussion

2 Normal mitochondrial function is essential for neuronal growth, function and survival
3 (*Sheng, 2014*). The genes that affect mitochondrial function may cause peripheral neuropathy
4 by alteration in the mitochondrial dynamics of fusion, fission and axonal transport, or due to
5 abnormalities in energy production. (*Cassereau, et al., 2009; Chen, et al., 2003; Hollenbeck*
6 *and Saxton, 2005; Misko, et al., 2010; Sheng and Cai, 2012; Song, et al., 2009; Vallat, et al.,*
7 *2008*). The neuropathy associated with CMT is usually length-dependent, with weakness
8 starting and being more pronounced distally, and the lower limbs being affected earlier and
9 more severely than the upper limbs. Neurophysiology in CMT often parallels this clinical
10 picture with axonal degeneration (primary in CMT2 and secondary to a demyelinating
11 process in CMT1), as measured by the CMAP and SNAP amplitudes, of greater severity in
12 the lower limbs (*Kamholz, et al., 2000; Krajewski, et al., 2000; Scherer, 1999*). In contrast,
13 this study shows that the nerve conduction abnormalities in children with mitochondrial
14 disease are generally not length-dependent.

15

16 While our study was not designed to measure the absolute frequency of neuropathy in
17 patients with mtDNA and nuclear DNA mutations, peripheral nerve involvement was more
18 common in children with nuclear DNA mutations (7/7 children with *POLG*, 10/10 children
19 with *SURF1* and 3/3 children with PDHc deficiency) when compared with those with
20 mtDNA mutations (3/7 children). Horga et al. found that in individuals with progressive
21 external ophthalmoplegia, the presence of a peripheral neuropathy had the highest specificity
22 (91%), negative predictive value (83%) and positive likelihood ratio (5.87) for the diagnosis
23 of a nuclear DNA defect, as opposed to a mitochondrial gene defect (*Horga, et al., 2014*).

24

25

1 4.1. Mitochondrial DNA mutations

2 Peripheral neuropathy was uncommon in those with mtDNA mutations, with affected
3 children having either normal nerve conduction studies or a mild neuropathy when not
4 acutely ill. Out of the nine children in the cohort of adults and children with MELAS
5 described by Kaufmann et al., five had normal nerve conduction studies while another three
6 had only borderline reductions in peroneal CMAP amplitudes (*Kaufmann, et al., 2006b*). Of
7 the 67 individuals with Leigh and Leigh-like syndrome described by Rahman et al, none of
8 12 individuals with a mutation involving the mitochondrial genome had an identified
9 peripheral neuropathy, although not all were investigated with nerve conduction studies
10 (*Rahman, et al., 1996*). In individuals with the NARP phenotype, the presence of a peripheral
11 neuropathy in the first two decades of life is specific to the m.9185T>C mutation (*Childs, et*
12 *al., 2007*). Peripheral neuropathy is uncommon with single large mitochondrial deletions and
13 has been described in only a single case report (*McDonald, et al., 2002*).

14

15 4.2. Nuclear DNA mutations

16 4.2.1. POLG

17 In our study, in those who presented beyond infancy, *POLG* mutations were
18 associated with an axonal sensory neuropathy with variable motor involvement, with clinical
19 onset in the second decade of life. While a ‘sensory ataxic neuropathy’ almost universally
20 develops in individuals with late-onset *POLG* syndromes, its onset is usually late in the
21 second decade or beyond (*Hakonen, et al., 2005; Neeve, et al., 2012; Schulte, et al., 2009*),
22 although an earlier onset has occasionally been described (*Tzoulis, et al., 2006; Wong, et al.,*
23 *2008*). While hypotonia and areflexia are listed as features of *POLG* mutations in the first
24 year of life, there are very few reports of the nerve conduction findings of affected children.
25 Our study included two young children with *POLG* mutations and demyelinating motor

1 neuropathies. In a cohort of eight children with Alpers syndrome and *POLG* mutations,
2 Ferrari et al. reported two infants with neurophysiological or biopsy evidence of a
3 demyelinating neuropathy (Ferrari, et al., 2005). Recessive mutations in *PEO1*, the gene
4 encoding mitochondrial Twinkle helicase, present with a similar phenotypic spectrum to
5 *POLG* and are also associated with an early-onset sensory neuropathy (Lönnqvist, et al.,
6 2009).

7

8 4.2.2. *SURF1*

9 *SURF1* mutations were predominantly associated with a demyelinating neuropathy.
10 In the report by Wedatilake et al, 13 of 16 individuals who had undergone nerve conduction
11 studies had a neuropathy, which was demyelinating in seven cases. Echaniz-Laguna and
12 colleagues have reported two families presenting with *SURF1* mutations with a childhood-
13 onset demyelinating sensorimotor neuropathy, initially diagnosed as CMT (Echaniz-Laguna,
14 et al., 2013). MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) due to
15 mutations in the *TYMP* gene is also reported to be associated with a childhood-onset
16 demyelinating neuropathy (Garone, et al., 2011; Hirano, et al., 2004).

17

18 4.2.3. *PDHc* deficiency

19 *PDHc* deficiency was characterised by axonal sensorimotor neuropathy. Previously
20 published case reports and a single case series of children with *PDHc* deficiency and
21 neuropathy describe a predominantly axonal neuropathy with significantly reduced CMAP
22 amplitudes and mildly reduced nerve conduction velocities (Bonne, et al., 1993; Chabrol, et
23 al., 1994; Di Rocco, et al., 2000; Federico, et al., 1990; Koga, et al., 2012; Marsac, et al.,
24 1997). In these reports, the axonal neuropathy was recognised in both those investigated prior
25 to institution of treatment with thiamine as well as those on treatment. Only a single

1 individual had a more significant reduction of median motor conduction velocity, which
2 improved from 19.8m/s to 37.9m/s following treatment with thiamine (*Di Rocco, et al.,*
3 *2000*). Long term treatment with dichloroacetate, used to treat lactic acidosis in children
4 with PDHc deficiency, has been shown to cause a reversible axonal neuropathy and animal
5 studies have shown that thiamine may mitigate the severity of this neuropathy (*Berendzen, et*
6 *al., 2006; Kaufmann, et al., 2006a; Stacpoole, et al., 1990*).

7

8 *4.3. Acute neuropathy with mitochondrial disease*

9 An acute neuropathy has only rarely been described in mitochondrial disease (*Coker,*
10 *1993; Hara, et al., 1994; Stickler, et al., 2003*). In our study, one child with MELAS
11 developed an acute axonal neuropathy during an acute stroke-like episode. Acute reversible
12 axonal dysfunction related to energy failure in peripheral nerves has been shown to occur
13 during stroke-like episodes in MELAS (*Farrar, et al., 2010*). It is possible that an acute
14 neuropathy accompanies stroke-like episodes in MELAS but is under-recognised due to the
15 prominent central nervous system features. In our study, two children with PDHc deficiency
16 had evidence of an axonal sensorimotor neuropathy on studies performed when they were not
17 acutely ill. Debray et al. described 13 individuals with PDHc deficiency and acute weakness.
18 Of the seven who had undergone nerve conduction studies, five had evidence of a peripheral
19 neuropathy (*Debray, et al., 2006*). The lack of nerve conduction studies before the onset of
20 weakness or after recovery makes it difficult to determine if the neuropathy was chronic or if
21 an acute metabolic neuropathy or worsening of a pre-existing neuropathy was responsible for
22 the acute weakness.

23

24 The combination of retrospective and prospective data is a limitation of this study,
25 with the retrospectively collected studies having been performed with different protocols. As

1 the retrospective group included only those known to have had nerve conduction studies on
2 the clinical suspicion of a neuropathy, this study was not designed to characterise the
3 frequency of peripheral neuropathy in different mitochondrial diseases. It is, however, the
4 largest study to date of peripheral neuropathy in genetically- classified childhood
5 mitochondrial disease and provides valuable data on the characteristics of the peripheral
6 neuropathy associated with different mitochondrial diseases.

7

8 **3. Conclusion**

9 We have characterised the neuropathy associated with the major genetic classes of
10 childhood mitochondrial disease. Our findings may help to classify the mitochondrial
11 syndrome and direct genetic testing. Detailed phenotyping including the characteristics of the
12 associated neuropathy may also be useful in confirming the pathogenicity of variants found in
13 whole exome/genome sequencing. In contrast to CMT, the neuropathy of paediatric
14 mitochondrial disease is not length-dependent. Nerve conduction studies should be an
15 integral component of the diagnostic evaluation of suspected childhood mitochondrial
16 disease.

17

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20 Australia (NH&MRC) postgraduate scholarship.

Table 1: Neurophysiological profile of children with mitochondrial genome mutations (n=7)

Pt./ Sex	Syndrome	Mutation	Age at presentation	Signs suggestive of neuropathy	Age at NCS, Retrospective /Prospective	Upper limb motor [CMAP(mV)/ CV(m/s)]	Lower limb motor [CMAP(mV)/ CV(m/s)]	Upper limb sensory [μ V]	Lower limb sensory (μ V)
1/M	Leigh	<i>MT-ATP6</i> m.8993T>C	2y	hypotonia, ataxia, generalised weakness (acute with illness)	5y/R	(M) 14.2/54	(P) 1.7/46 (T) 1.4/38	(M) 25	(S) 38
2/F	Leigh	<i>MT-ATP6</i> m.8993T>C	12y	-	12y/P	(M) 4.9/52	(P) 3.5/43	(M) 16	(S) NR
3/F	NARP	<i>MT-ATP6</i> m.8993T>C	16y	-	16y/R	(M) NA/46	(P) NA/46	(M) 15	(S) 10
4/M	MELAS	<i>MT-TL1</i> m.3243A>G	9y	-	11y/P	(M) 9/54 (U) 9.6/60	(P) 5.3/46 (T) 17.3/49	(M) 26 (U) 20	(S) 5
5/M	MELAS	<i>MT-TL1</i> m.3243A>G	10y	ataxia, areflexia (during acute episode)	12y/P	(M) 2.7/56 (U) NR/NR	(P) NR/NR (T) NR/NR	(M) 23 (U) 15	(S) NR
					13y/P	(M) 10.3/59 (U) 9.5/59	(P) 2.0/47 (T) 8.7/51	(M) 26 (U) 12	(S) NR
6/M	Pearson	single mtDNA deletion	7m	-	4y/P	(M) 7.6/45 (U) 6.7/62	(T) 9.9/48	(M) 35	(S) 9
7/M	Kearns-Sayre	single mtDNA deletion	11y	-	15y/P	(M) 14.8/68 (U) 13.9/65	(P) 5.1/58 (T) 23.2/58	(M) 10 (U) 11	(S) 16

Abnormal results (< 2SD) in bold. Reference values from Cai et al. (Cai and Zhang, 1997). Hz – homozygous, w-weeks, m– months, y-years, R – retrospective, P – prospective, CMAP – compound muscle action potential, CV – conduction velocity, SNAP – sensory nerve action potential,

M – median, U – ulnar, P – peroneal, T – tibial, S – sural, Sp – superficial peroneal, Mp – medial plantar, NR – not recordable, NA – not available, sup – superficial, empty box indicates this nerve was not studied

Table 2: Neurophysiological profile of children with *POLG* mutations (n=7)

Pt./ Sex	Mutation	Age at presentation	Signs suggestive of neuropathy	Age at NCS, Retrospective/ Prospective	Upper limb motor [CMAP(mV)/ CV(m/s)]	Lower limb motor [CMAP(mV)/ CV(m/s)]	Upper limb sensory [μ V]	Lower limb sensory (μ V)
8/M	c.1399G>A/ c.695G>A	2w	hypotonia, areflexia	2w/R	(U) NA/ 8	(T) NA/ 10		
9/M	c.2551A>G/ c.897G>A	7 m	hypotonia, areflexia	1y/R	(M) 4.5/ 16.7	(P) 1.1/25.5 (T) 1.7/25.7	(M) NR	(S) NR
10/F	Hz c.911T>G	4y	foot drop, ataxia, distal lower limb weakness, distal lower limb sensory loss, areflexia	10y/R	(M) 8.8/56	(P) 4.0/49 (T) 9.0/47	(M) NR (U) NR	(S) NR
11/F	Hz c.1399G>A	7y	ataxia, tremor, areflexia	13y/R	(M) 10.4/48	(P) 2.7/45	(M) 20	(S) NR (Sp) NR
				16y/R	(U) 6.4/46	(P) 1.8/42 (T) 4.6/NA	(M) 19	(S) NR (Sp) NR
12/M	c.1943C>G/ c.926G>A	12y	pes cavus, foot drop, ataxia, distal lower limb weakness, distal lower limb sensory loss, areflexia	16y/R	(U) NA/23	(T) NR/NR	(M) NR	
13/M	c.2551A>G/ c.3140G>A	17y	pes cavus, ataxia, distal lower limb weakness, areflexia	17y/R	(U) NA/47.8	(P) NR/NR	(M) 6.7 (U) 6.1	(S) NR
14/F	Hz c.1399G>A	15y	ataxia, areflexia	19y/R	(M) 14.1/ 46.8 (U) 6.1/49.4	(P) 3.0/45 (T) 2.7/39.3	(M) NR (U) NR	(S) NR (Sp) NR

Abnormal results (< 2SD) in bold. Reference values from Cai et al.(Cai and Zhang, 1997). Hz – homozygous, w-weeks, m– months, y-years, R – retrospective, P – prospective, CMAP – compound muscle action potential, CV – conduction velocity, SNAP – sensory nerve action potential,

M – median, U – ulnar, P – peroneal, T – tibial, S – sural, Sp – superficial peroneal, Mp – medial plantar, NR – not recordable, NA – not available, sup – superficial, empty box indicates this nerve was not studied

Table 3: Neurophysiological profile of children *SURF1* mutations (n=10)

Pt./ Sex	Mutation	Age at presentation	Signs suggestive of neuropathy	Age at NCS, Retrospective /Prospective	Upper limb motor [CMAP(mV)/ CV(m/s)]	Lower limb motor [CMAP(mV)/ CV(m/s)]	Upper limb sensory [μ V]	Lower limb sensory (μ V)
15/M	c.312_320del10insAT/ c.532_535delAATA	10m	Areflexia	12m/R	(M) NA/ 28.4	(T) NA/ 22.2	(M) 17.1	(S) NR
16/M	H _z c.516-2A>G	3d	Ataxia, Tremor	14m/R		(T) 4.1/21		(S) 14
17/M	H _z c.324-11T>G	9m	Ataxia	18m/R		(T) 1.5/14		(S) NR
18/M	H _z c.312_320del10insAT	10m	Ataxia, Tremor	18m/R		(T) 4.5/43		(S) 3
19/M	H _z c.516-2A>G	4m	Ataxia	21m/R		(T) 5.4/35.8		(S) 9.2
20/F	H _z c.751C>T	9m	Ataxia, Tremor	2y/R		(T) 7.5/22	(M) NR	
				7y/R	(M) 0.6/NA (U) 0.6/19.5	(T) 4/NA	(M) NR	(S) NR
21/M	H _z c.312_320del10insAT	18m	Ataxia, Tremor, Areflexia	2y/R	(M) 9.4/ 36.9		(M) 6.8	(S) NR
22/F	c.240+1G >T, c.575G>A	18m	Ataxia, Tremor	2y/R	(U) 4.7/ 43	(T) 5.3/26	(R) 23	(Pm) 21
23/M	H _z c.516-2A>G	10	NA	4y/R	(U) 4.9/ 28	(P) 3.5/28 (T) 3.9/18	(R) 24	(S) NR
24/F	H _z c.792_793delAG	2y	Ataxia, Tremor, Areflexia	5y/R	(M) 5.6/ 31.9	(P) 1.4/31.3	(M) 6 (U) 7.6	

Abnormal results ($< 2SD$) in bold. Reference values from Cai et al. (*Cai and Zhang, 1997*). Hz – homozygous, w-weeks, m– months, y-years, R – retrospective, P – prospective, CMAP – compound muscle action potential, CV – conduction velocity, SNAP – sensory nerve action potential, M – median, U – ulnar, P – peroneal, T – tibial, S – sural, Sp – superficial peroneal, Mp – medial plantar, NR – not recordable, NA – not available, sup – superficial, empty box indicates this nerve was not studied

Table 4: Neurophysiological profile of children with PDHc deficiency (n=3)

Pt./Sex	Mutation	Age at presentation	Signs suggestive of neuropathy	Age at NCS, Retrospective /Prospective	Upper limb motor [CMAP(mV)/ CV(m/s)]	Lower limb motor [CMAP(mV)/ CV(m/s)]	Upper limb sensory [μ V]	Lower limb sensory (μ V)
25/M	PDHA1 c.787C>G	11m	episodic acute weakness, areflexia	1yr/R	(M) 3.7/39 (U) 4.0/52	(P) 2.1/52 (T) 3.9/42	(M) NR (U) NR	(S) NR
				5y/P	(M) 4.8/52 (U) 2.7/58	(P) 1.6/52 (T) 4.9/47	(U) NR	(S) NR
				6y/P	(M) 6.9/50 (U) 3.8/55	(P) 1.9/47 (T) 2.5/37	(M) NR	(S) NR
26/M	NT	5m	-	7y/P	(M) 4.9/43 (U) 5.4/47	(P) 1.9/35 (T) 4.4/35	(M) NR (U) NR	(S) NR
27/M	NT	3y	distal weakness, areflexia	11y/R (8 weeks into acute episode)	(M) 0.7/57 (U) 5.5/51	(P) NR/NR (T) 0.2/36	(M) NR	(S) NR

Abnormal results (< 2SD) in bold. Reference values from Cai et al.(Cai and Zhang, 1997). Hz – homozygous, w-weeks, m– months, y-years, R – retrospective, P – prospective, CMAP – compound muscle action potential, CV – conduction velocity, SNAP – sensory nerve action potential, M – median, U – ulnar, P – peroneal, T – tibial, S – sural, Sp – superficial peroneal, Mp – medial plantar, NR – not recordable, NA – not available, sup – superficial, empty box indicates this nerve was not studied

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SUPPLEMENTARY METHODS

Nerve conduction tests

Nerve conduction tests were performed using a Viking™ On Nicolet™ EDX

Electrodiagnostic System from CareFusion Nicolet with surface electrodes. Sedation with

nitrous oxide was used in some children above the age of 12 months. For the motor studies, a supramaximal rectangular pulse direct current stimulus was delivered using a two-prong stimulator, to elicit a compound muscle action potential (CMAP). The median nerve was stimulated at the wrist and elbow and the CMAP was recorded over the bulk of the abductor pollicis brevis (APB) muscle. The ulnar nerve was stimulated over the wrist and behind the elbow and the CMAP was recorded over the bulk of the abductor digiti minimi (ADM) muscle. The common peroneal nerve was stimulated over the ankle and knee and the CMAP was recorded over the bulk of the extensor digitorum brevis (EDB) muscle. The tibial nerve was stimulated over the ankle and behind the knee and the CMAP was recorded over the bulk of the abductor hallucis (AH) muscle. The sweep speed and sensitivities were 5 ms/division and 5 mV/division respectively. The duration of the stimulus was 0.1ms.

The sensory nerve action potential (SNAP) was recorded by an orthodromic technique for the upper limb (median and ulnar) nerves and by an antidromic technique for the lower limb (sural) nerve. For the median nerve, the stimulus was delivered by ring electrodes over the index finger (digital branch of the median nerve) and SNAPs were recorded over the wrist. For the ulnar nerve, the stimulus was delivered by ring electrodes over the little finger (digital branch of the ulnar nerve) and SNAPs were recorded over the wrist. For the sural nerve, the stimulus was delivered above the lateral malleolus, approximately 14 cm from the proximal recording electrode which was placed on the lateral border of the foot. All sensory potentials were recorded using a recurrent stimulus and with averaging using a supramaximal stimulus. The stimulus duration was 0.1ms and the sweep speed and sensitivities were 1 ms/division and 10 μ V/division respectively.

The distal motor latencies, CMAP amplitudes and conduction velocities were recorded using conventional methods. CMAP and SNAP amplitudes were measured from baseline to the negative peak of the action potential. The distal motor latency was calculated from the stimulus artefact to the initial negative deflection from the baseline. Normative data was sourced from Cai et al. (*Cai and Zhang, 1997*). The neuropathy was designated as demyelinating when the nerve conduction velocity was reduced to < 70% of the lower limit for that age range (lower limit = mean-2SD). The neuropathy was designated as axonal when there was a reduction in the CMAP amplitude and there was no reduction in the conduction velocity, or the reduction did not satisfy criteria for demyelinating neuropathy. Care was taken when the CMAP amplitude was <1mV as it is known that measures of conduction velocity in this setting can be erroneous and appear pseudo-demyelinating due to preferential loss of faster conduction fibres.