

# Charcot-Marie-Tooth disease

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## ABSTRACT

**Objective:** To investigate whether mutations in the *SURF1* gene are a cause of Charcot-Marie-Tooth (CMT) disease.

**Methods:** We describe 2 patients from a consanguineous family with demyelinating autosomal recessive CMT disease (CMT4) associated with the homozygous splice site mutation c.107-2A>G in the *SURF1* gene, encoding an assembly factor of the mitochondrial respiratory chain complex IV. This observation led us to hypothesize that mutations in *SURF1* might be an unrecognized cause of CMT4, and we investigated *SURF1* in a total of 40 unrelated patients with CMT4 after exclusion of mutations in known CMT4 genes. The functional impact of c.107-2A>G on splicing, amount of SURF1 protein, and on complex IV activity and assembly was analyzed.

**Results:** Another patient with CMT4 was found to harbor 2 additional *SURF1* mutations. All 3 patients with *SURF1*-associated CMT4 presented with severe childhood-onset neuropathy, motor nerve conduction velocities <25 m/s, and lactic acidosis. Two patients had brain MRI abnormalities, including putaminal and periaqueductal lesions, and developed cerebellar ataxia years after polyneuropathy. The c.107-2A>G mutation produced no normally spliced transcript, leading to SURF1 absence. However, complex IV remained partially functional in muscle and fibroblasts.

**Conclusions:** We found *SURF1* mutations in 5% of families (2/41) presenting with CMT4. *SURF1* should be systematically screened in patients with childhood-onset severe demyelinating neuropathy and additional features such as lactic acidosis, brain MRI abnormalities, and cerebellar ataxia developing years after polyneuropathy. **Neurology® 2013;81:1523-1530**

## GLOSSARY

**BNGE** = blue native gel electrophoresis; **bp** = base pair; **cdNA** = complementary DNA; **CK** = creatine kinase; **CMT** = Charcot-Marie-Tooth; **COX** = cytochrome c oxidase; **LS** = Leigh syndrome; **MNCV** = motor nerve conduction velocities; **MRC** = mitochondrial respiratory chain; **RT** = reverse transcription.

Peripheral neuropathies are a well-known complication of mitochondrial DNA and nuclear-encoded mitochondrial gene mutations. For instance, patients with mutations in the nuclear-encoded mitochondrial genes *MFN2* and *GDAP1*, which encode outer mitochondrial membrane proteins, usually present with axonal and demyelinating forms of Charcot-Marie-Tooth (CMT) disease, respectively.<sup>1</sup> Moreover, patients with mutations in the mitochondrial DNA gene *MTATP6*, which encodes the ATP6 subunit of the mitochondrial respiratory chain (MRC) complex V, may present with axonal CMT (CMT2).<sup>2</sup>

The determination of the genetic cause is a major challenge in rare neuromuscular diseases such as autosomal recessive demyelinating CMT (CMT4). We investigated a consanguineous family in which 2 patients with CMT4 harbored a homozygous splice site mutation in *SURF1*, encoding an assembly factor of the MRC complex IV (cytochrome *c* oxidase [COX]). Despite this defect, we detected some residual assembly and function of COX in fibroblasts and muscle of both patients. We then screened for *SURF1* mutations in a cohort of 40 unrelated patients

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with genetically undefined CMT4, and found compound heterozygous *SURF1* mutations in an additional patient.

**METHODS Standard protocol approvals, registrations, and patient consents.** The study was approved and performed under the ethical guidelines issued by our institutions for clinical studies. The diagnostic procedures were conducted according to the Strasbourg University Hospital Ethical Committee, and informed written consent was obtained from all patients.

**CMT4 index family.** We investigated a consanguineous Algerian family in whom 2 patients presented with CMT4. Detailed clinical assessments were performed on the 2 patients. Data obtained included age of symptom onset, clinical history and examination findings, electrodiagnostic studies, serum creatine kinase (CK) and lactate, and brain MRI studies.

**CMT4 patient cohort.** We studied 40 French families with CMT4. Patients were considered as having CMT4 when the family history clearly suggested an autosomal recessive pattern (multiple affected siblings with no parent, child, or other family members affected) or when the dominant forms of demyelinating neuropathies had been excluded. Fifteen patients originated from consanguineous families, and 25 patients originated from nonconsanguineous families including 14 patients with sporadic demyelinating polyneuropathy and 11 patients with recessive demyelinating polyneuropathy. *PMP22*, *MPZ*, and *GJB1* had been previously analyzed in all 40 patients. In addition, *GDAP1*, *MTMR2*, *PRX*, and *SH3TC2* genes had been screened in 20 patients.

**Morphologic and biochemical analyses.** Muscle and skin biopsies were performed in the proband from the index family. Muscle biopsy was processed with standard methods for histology and histochemistry. Enzymatic activities of the MRC complexes were measured in muscle and cultured fibroblasts as reported previously.<sup>3</sup>

**Molecular investigations.** *SURF1* (gene ID 6834, OMIM \*185620) exons 1–9 and intron/exon boundaries were amplified and sequenced from genomic DNA from both patients of the index family and 40 additional patients with CMT4. For reverse transcription (RT)-PCR analysis, total RNA was isolated from cultured skin fibroblasts of the proband from the index family using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). RNA samples were free of any contaminating DNA by treatment with the DNA-free Kit (Ambion Inc., Austin, TX). RT of 0.8–1 µg total RNA was performed as described.<sup>4</sup> Amplification of *SURF1* complementary DNA (cDNA) was performed with several specific primer pairs. Amplification products were cloned into the pCR2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA). Sequences of the amplification products were compared with the GeneBank reference *SURF1* sequence, NM\_003172.3.

**Western blot analysis.** Approximately 10<sup>6</sup> cells from the proband of the index family were prepared as described.<sup>5</sup> Sodium dodecyl sulfate–polyacrylamide gel of 50 µg protein/lane and Western blot analysis were performed using monoclonal antibodies against *SURF1* (MitoSciences LLC, Eugene, OR) and complex II subunit SDHB (Invitrogen), and a polyclonal antibody against *SURF1*.<sup>5</sup>

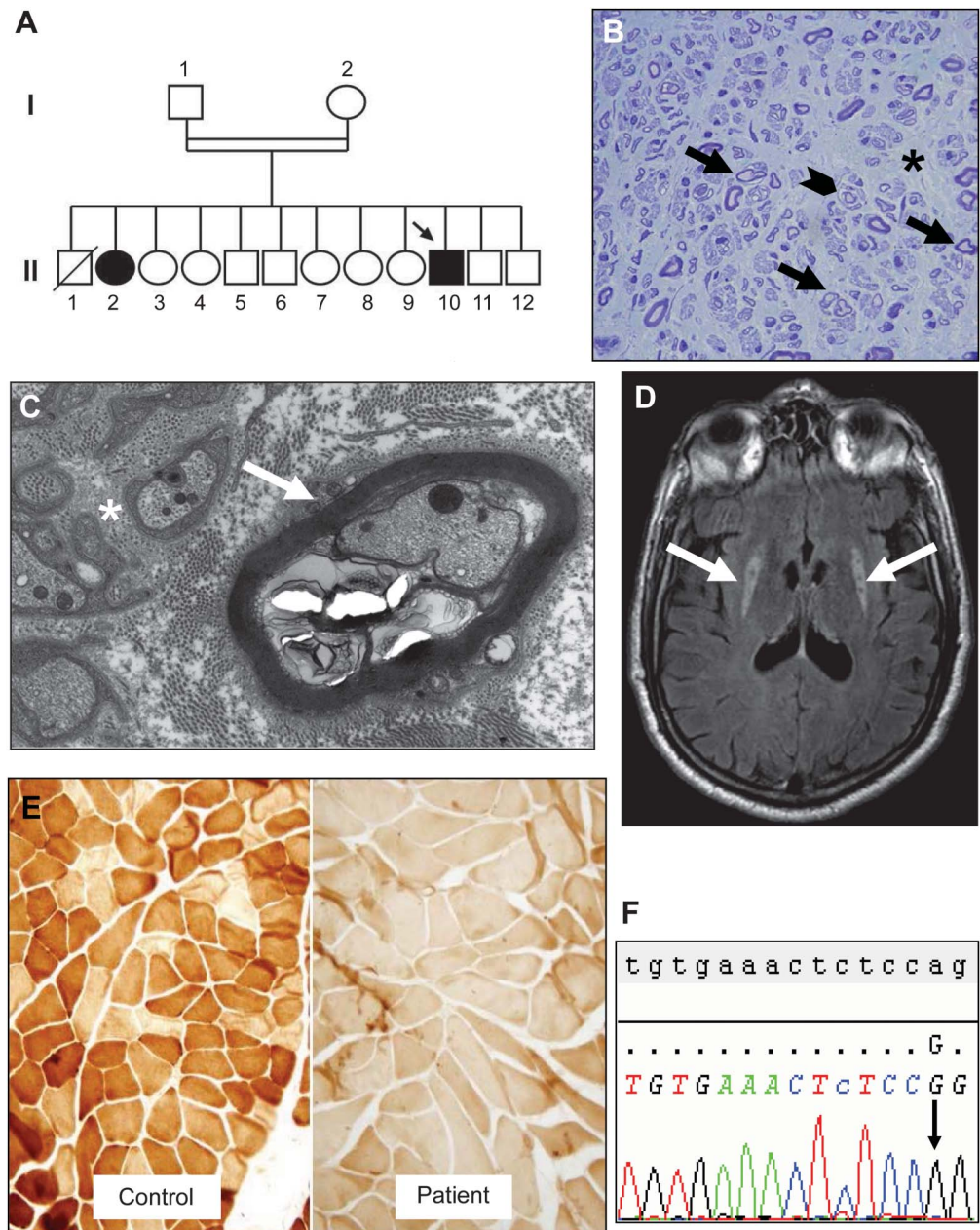
**Blue native gel electrophoresis.** The detection of the assembled respiratory chain complexes in control and proband skin fibroblasts was performed by using blue native gel electrophoresis (BNGE). Samples were obtained from 2 × 10<sup>6</sup> cultured fibroblasts as described.<sup>6</sup> Twenty microliters of the sample was loaded and run into a 5% to 13% gradient nondenaturing 1-dimension BNGE.

After electrophoresis, gels were transferred to a nitrocellulose membrane (Amersham Hybond-ECL; GE Healthcare, UK). Immunoblotting was performed with monoclonal antibodies raised against MTCO1 and COX4 complex IV subunits and complex II subunit SDHA (Invitrogen).

## RESULTS Characteristics of the CMT4 index family.

The 42-year-old proband was born at term after a normal pregnancy to consanguineous Algerian parents (figure 1A). His psychomotor development proceeded normally, with no delay in the main milestones, including independent ambulation at the age of 12 months. He was first examined at age 8 years because of easy fatigability. At physical examination, he presented with moderate kyphoscoliosis, moderate muscle wasting of hands and feet, abolished tendon reflexes in the 4 limbs, and reduced vibration and pinprick sensation in the lower limbs. Electrodiagnostic studies revealed a combination of extremely reduced conduction velocities, mildly prolonged distal latencies, and prolonged F-wave latencies that were consistent with severe demyelinating CMT disease (table 1). CSF examination was normal. Superficial peroneal nerve biopsy revealed axonal loss and hypomyelinated fibers, consistent with severe chronic demyelinating neuropathy (figure 1, B and C). Eventually, the patient was diagnosed with demyelinating CMT disease. At physical examination at age 42, the patient was still able to walk without assistance but for no more than 30 m because of easy fatigability. The physical examination was similar to the previous one, indicating very slow progression. However, he also presented with spontaneous unidirectional horizontal nystagmus and mild hearing loss. Pathologic reflexes, cerebellar ataxia, dystonia, and swallowing and respiratory disturbances were not observed. Serum CK levels at rest were normal. Under fasting conditions, lactic acidosis was observed at rest (3.3 mmol/L; normal <1.8). Karyotype was normal, and the patient refused neuropsychological testing. In this patient with severe demyelinating CMT disease, no mutations were found in candidate genes including *PMP22*, *MPZ*, *GJB1*, *GDAP1*, and *SH3TC2*. Because the patient presented with nystagmus and hearing loss, brain MRI was performed and demonstrated hyperintense lesions in both putamina (figure 1D). Mitochondrial disease was then suspected, and muscle and skin biopsies were performed. Histologic examination of muscle showed marked reduction of COX-specific reaction in all fibers (figure 1E). Spectrophotometric analysis of the MRC complexes demonstrated an isolated defect of COX activity, with 18% of the mean control values (table 2). The activity of the citrate synthase was normal. In cultured fibroblasts, an isolated COX deficiency was also detected, with 26% of the mean control values. In both muscle and fibroblasts, complex activity ratios were increased (I/IV, II/IV, and III/IV) or decreased

**Figure 1** Pedigree, brain imaging, peripheral nerve, skeletal muscle, and genetic analyses in the proband from the index family



(A) Pedigree of a consanguineous family with demyelinating Charcot-Marie-Tooth disease. Black boxes indicate the patients affected. The arrow indicates the proband. (B) Superficial peroneal nerve biopsy of proband at age 8 years. Transverse section of the nerve showing axonal loss (\*), hypomyelinated fibers (arrows), and an onion bulb formation (arrowhead) (original magnification  $\times 1,000$ ). (C) Superficial peroneal nerve biopsy. Electron micrograph showing axonal degeneration (\*) and a hypomyelinated degenerating fiber (arrow). Section contrasted with lead citrate and uranyl acetate (original magnification  $\times 11,925$ ). (D) Axial T1-weighted brain MRI of the proband at age 42 years demonstrating hyperintense lesions in both putamina (arrows). (E) Cytochrome c oxidase (COX) stain showing deficiency of COX activity in all muscle fibers of the proband (right panel) compared with a control muscle (left panel) (original magnification  $\times 200$ ). (F) Electropherogram showing the 3' end of intron 2 from the *SURF1* gene of the patient. The c.107-2A>G variation is indicated with an arrow. The patient was homozygous for this variation.

(IV/II + III), supporting the isolated defect of complex IV. No pathologic variations were identified in genes causing complex IV deficiency, including mitochondrial *MTCO1*, *MTCO2*, and *MTCO3*, and nuclear *TACO1*.<sup>7</sup>

An elder sister of the patient, aged 57, presented since before age 10 years with the same symptoms, i.e., hands and feet wasting, abolished tendon reflexes in the 4 limbs, and reduced vibration and pinprick sensation in the lower limbs. Spontaneous unidirectional

**Table 1** Results of nerve conduction studies in the proband from the index family

	Normal values	Patient
<b>Ulnar motor (W-BE)</b>		
Distal amplitude (mV)	>4.5	3.7
Proximal amplitude (mV)		3.1
Latency (ms)	<3.4	5.2
Conduction velocity (m/s)	>50	21
<b>Ulnar sensory</b>		
Amplitude ( $\mu$ V)	>6.0	NR
Latency (ms)	<3.2	NR
Conduction velocity (m/s)	>47	NR
<b>Ulnar F waves</b>		
Latency (ms)	<28	38
<b>Median motor</b>		
Distal amplitude (mV)	>5.6	5.1
Proximal amplitude (mV)		4.2
Latency (ms)	<4.0	5.6
Conduction velocity (m/s)	>49	22
<b>Median sensory</b>		
Amplitude ( $\mu$ V)	>7.5	NR
Latency (ms)	<3	NR
Conduction velocity (m/s)	>48	NR
<b>Median F waves</b>		
Latency (ms)	<29	40
<b>Tibial motor</b>		
Distal amplitude (mV)	>5.9	4.2
Proximal amplitude (mV)		3.2
Latency (ms)	<6.3	7.0
Conduction velocity (m/s)	>44	18
<b>Tibial F waves</b>		
Latency (ms)	<55	62
<b>Peroneal motor</b>		
Distal amplitude (mV)	>2.3	2.2
Proximal amplitude (mV)		1.9
Latency (ms)	<5.9	6.5
Conduction velocity (m/s)	>43	15
<b>Sural sensory</b>		
Amplitude ( $\mu$ V)	>3.5	NR
Latency (ms)	<3.8	NR
Conduction velocity (m/s)	>45	NR

Abbreviations: BE = below elbow; ms = millisecond; NR = not recordable; W = wrist.

All values presented were from the left side. Motor response amplitude was measured from baseline to peak of the negative component.

horizontal nystagmus, hearing loss, and kyphoscoliosis were also observed. Electrodiagnostic studies revealed a severe demyelinating polyneuropathy, with upper limb motor nerve conduction velocities (MNCV) at 25 m/s. Under fasting conditions, lactic acidosis was observed at rest (2.9 mmol/L; normal <1.8). Brain MRI was normal. *PMP22*, *MPZ*, *GJB1*, *GDAP1*, and *SH3TC2* genes were normal. After age 40, she developed marked cerebellar ataxia with altered finger-nose and heel-knee tests in both sides. She refused muscle and skin biopsies.

The proband and his sister harbored a homozygous change in *SURF1* gene, the recently reported A to G substitution (c.107-2A>G), abolishing the invariable consensus AG splice acceptor site of intron 2 (figure 1F).<sup>8</sup>

**Characteristics of another CMT4 patient harboring *SURF1* mutations.** This patient was born to nonconsanguineous French parents, and presented at age 3 years with hands and feet wasting, abolished tendon reflexes in the 4 limbs, and reduced vibration and pinprick sensation in the lower limbs. She had no family history of neuromuscular disorders, and electrodiagnostic studies demonstrated severe demyelinating polyneuropathy with upper limb MNCV at 22 m/s. Serum CK levels at rest were normal. Under fasting conditions, elevated plasma lactate was present at rest (2.5 mmol/L; normal <1.8). Brain MRI showed nonspecific abnormalities in the brain stem periaqueductal area. *PMP22*, *MPZ*, *GJB1*, *GDAP1*, *PRX*, *SH3TC2*, and *MTMR2* genes were normal. Muscle and skin biopsies were refused. After age 10, she developed mild cerebellar ataxia with altered finger-nose and heel-knee tests in both sides. The patient was compound heterozygous for 2 *SURF1* variants: i) a previously reported missense change c.574C>T, resulting in the substitution of the arginine residue at codon 192 with a tryptophan (p.Arg192Trp)<sup>9</sup>; and ii) a novel deletion (c.799\_800del). The patient inherited the c.574C>T from her mother and the c.799\_800del from her father. The p.Arg192Trp change affects a highly conserved residue, and is predicted to be pathogenic by different bioinformatics tools (*Polyphen-2*, *PMUT*, and *SIFT*<sup>10-12</sup>). The c.799\_800del is predicted to produce a truncated protein (p.Leu267GlufsX24) with an abnormal C-terminus.

**Remaining patients with CMT4.** *SURF1* gene analysis was normal in the remaining 39 patients with CMT4.

**RT-PCR analysis.** To identify the impact of the c.107-2A>G variation on splicing, *SURF1* cDNA from the proband and a control fibroblast cell line were amplified. Amplification of control cDNA with different primer pairs yielded fragments of the expected sizes (895, 541, 519, and 206 base pairs [bp]) (figure 2). Sequencing showed that the 895, 541, and 206 bp fragments corresponded to *SURF1* exons 1/2-9, 1/2-6, and 1/2-3/4,

**Table 2** Respiratory chain complex activities in muscle and fibroblasts of the proband from the index family

	Muscle biopsy		Cultured fibroblasts	
	Proband	Controls (n = 110)	Proband	Controls (n = 10)
<b>Enzyme activities</b>				
Complex I	13.9	14.1 (9.3-24.5)	5.2	7.2 (5.0-11.4)
Complex II + III	8.7	7.7 (4.5-12.5)	9.2	12.2 (8.3-14.1)
Complex II	19.7	18.7 (12.7-28.8)	12.6	16.7 (13.3-20.1)
Complex III	160.5	123.6 (71.0-182.9)	111.2	113.1 (95.6-139.5)
Complex IV	17.8 <sup>a</sup>	101.1 (64.5-143.9)	26.6 <sup>a</sup>	103.7 (93.2-122.9)
Citrate synthase	126.5	152.9 (73.9-311.3)	421.7	376.7 (253.1-459.7)
<b>Ratios</b>				
I/II + III	1.58	1.83 ± 0.45	0.56	0.59 ± 0.12
I/III	0.09	0.11 ± 0.03	0.05	0.06 ± 0.01
I/IV	0.78 <sup>a</sup>	0.14 ± 0.03	0.20 <sup>a</sup>	0.07 ± 0.02
II/IV	1.11 <sup>a</sup>	0.18 ± 0.04	0.47 <sup>a</sup>	0.16 ± 0.03
III/IV	9.02 <sup>a</sup>	1.22 ± 0.29	4.18 <sup>a</sup>	1.09 ± 0.13
IV/II + III	2.04 <sup>a</sup>	13.13 ± 2.87	2.89 <sup>a</sup>	8.5 ± 1.46

Respiratory chain complex activities are expressed as nanomoles per minute per milligram protein and normalized for citrate synthase activity. Control enzyme activities are given as mean with range in parentheses. Control values of activity ratios reflecting the optimal balance between respiratory chain complex activities are given as mean ± SD. Activities are measured in an 800-g supernatant of crude muscle homogenates and in mitochondria-enriched preparations from cultured fibroblasts. Mitochondria-enriched preparations were obtained as described previously.<sup>3</sup>

<sup>a</sup> Abnormal value.

respectively, while the 519 bp one corresponded to exons 2–6 (not shown). Contrariwise, cDNA samples from the proband fibroblasts yielded several DNA fragments (figure 2). Sequencing of approximately 30 cloned fragments demonstrated different *SURF1* alternative splicing. The c.107-2A>G, affecting the invariant AG dinucleotide at the acceptor splice site in intron 2, results either in exon skipping or in the use of preexisting but weaker cryptic acceptor sites. For example, the c.107-2A>G variation causes complete skipping of exon 3 or exons 3–5, partial deletion in exon 3 (r.107\_119del and r.107\_189del), or insertion of intronic nucleotides (r.106\_107ins107-51\_107-1 and r.106\_107ins107-18\_107-1). Because each abnormal transcript contains a premature termination codon, they are either degraded by the nonsense-mediated mRNA decay pathway or lead to truncated *SURF1* proteins probably disposed by degradation. The unavailability of biological samples from the sporadic patient hampered further investigation.

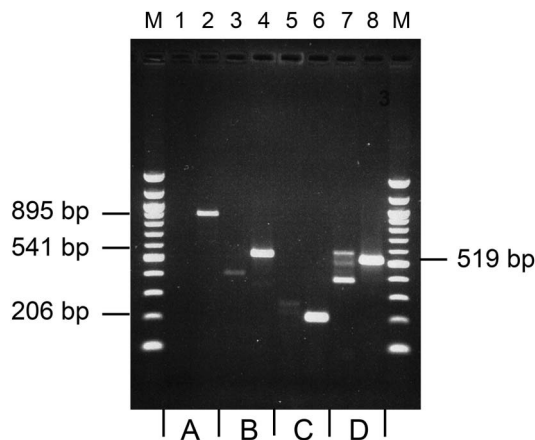
**Biochemical findings.** We analyzed the *SURF1* protein by Western blot. In mitochondria-enriched preparation from the proband fibroblasts and in a *SURF1*<sup>-/-</sup> subject carrying 2 common *SURF1* frameshift mutations (p. [Leu105X] + [Ser282CysfsX9]) (patient 5 from reference 13), no specific immunoreactive band was detected using either a monoclonal (figure 3A) or a

polyclonal antibody (not shown) against *SURF1*, confirming the virtual absence of *SURF1* protein in the proband. Next, we evaluated the amount and assembly status of COX, using 1-dimension BNGE. In the proband's fibroblasts, fully assembled COX was markedly reduced compared with the mean value from 2 controls, using both MTCO1 (18%) and COX4 (8%) antibodies, with no evidence of subassembly species. Again, the same findings were obtained in the *SURF1*<sup>-/-</sup> control cell line, which showed slightly more severe COX reduction (6% and 3% of the mean control value with MTCO1 and COX4 antibodies, respectively) (figure 3B).

**DISCUSSION** In our genetically undefined CMT4 cohort, we identified disease-causing *SURF1* variants in 2 of 41 families (5%), including one unrelated proband in addition to the index family in which we originally found a disease-segregating *SURF1* mutant allele. This finding is relevant because molecular defects are currently detected in less than 20% of CMT4 patients.<sup>14,15</sup> In both families, a mitochondrial etiology of the disease had not been initially considered because the phenotype consisted of isolated peripheral neuropathy, with very little additional multisystem involvement.

CMT is the most common inherited neuromuscular disorder affecting at least 1 in 2,500, and 13 genes

**Figure 2** *SURF1* transcripts analysis in cultured fibroblasts of the proband from the index family and a control

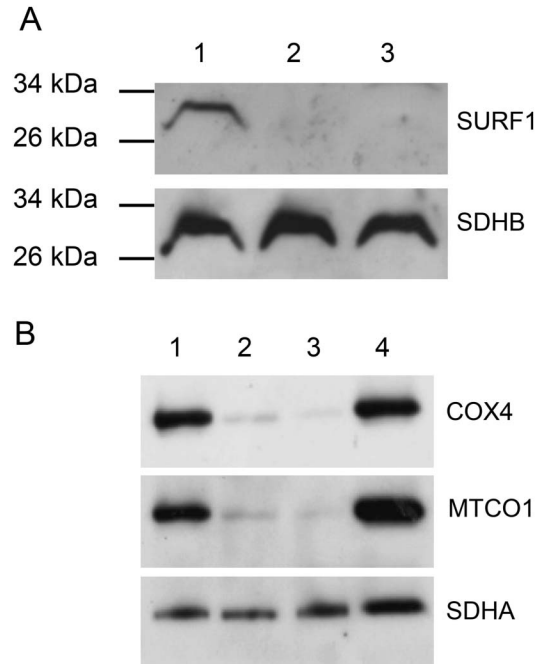


Amplification of exons from complementary DNA was performed using several primer pairs. A, Exons 1/2-9 were amplified using the exon 1/2 (forward) primer (nucleotides [nt] 47-65) and the exon 9 (reverse) primer (nt 941-920). B, Exons 1/2-6 were amplified using the exon 1/2 forward primer (nt 47-65) and the exon 6 (reverse) primer (nt 587-568). C, Exons 1/2-3/4 were amplified using the exon 1/2 forward primer (nt 47-65) and the exon 3/4 (reverse) primer (nt 252-234). D, Exons 2-6 were amplified using the exon 2 (forward) primer (nt 69-87) and the exon 6 (reverse) primer (nt 587-568). Nucleotide numbering uses the A of the first ATG translation initiation start codon as nucleotide +1. Lanes 1, 3, 5, 7: patient; lanes 2, 4, 6, 8: age-matched control. M is a 100-base pair (bp) ladder.

have been identified to cause autosomal recessive demyelinating CMT4: *GDAP1*, *MTMR2*, *MTMR13*, *SH3TC2*, *NDRG1*, *EGR2*, *PRX*, *HK1*, *FGD4*, *FIG4*, *CTDP1*, *PMP22*, and *MPZ*.<sup>15,16</sup> Interestingly, *GDAP1*, the most frequent genetic cause for CMT4, encodes a protein anchored to the mitochondrial outer membrane, thus demonstrating that mitochondrial disorders may manifest with demyelinating polyneuropathy as the predominant feature.<sup>1,15-17</sup>

The *SURF1* gene encodes one of at least 6 assembly factors of COX, the terminal component of the MRC. Studies on yeast and human mutant cells indicate for *SURF1* a role in the formation of the early subcomplexes of COX.<sup>18</sup> The mature *SURF1* protein is a 30-kDa hydrophobic polypeptide with 2 transmembrane domains at the N and C termini, which anchor the protein to the mitochondrial inner membrane.<sup>18</sup> *SURF1* mutations cause Leigh syndrome (LS), or subacute necrotizing encephalomyelopathy, a severe, usually infantile encephalopathy. The MRI of LS is characterized by symmetrical lesions in the basal ganglia, cerebellum, and brain stem, and the clinical course reflects the neuropathologic hallmarks, eventually leading to global neurologic failure; lactic acidosis is almost invariably present.<sup>8,19</sup> Patients with *SURF1*-associated LS usually exhibit a stereotypical clinical course and

**Figure 3** Immunoblot analysis of the proband from the index family and controls



(A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of mitochondria-enriched preparations from a control (lane 1), the proband (lane 2), and a patient previously published carrying 2 *SURF1* frameshift mutations (p. [Leu105X] + [Ser282CysfsX9]) used as a *SURF1*<sup>-/-</sup> control (lane 3) (patient 5 of reference 13). Monoclonal antibody against *SURF1* was used and an antibody against *SDHB* was used as loading control. (B) One-dimension blue native gel electrophoresis from 2 controls (lanes 1 and 4), the proband (lane 2), and the patient previously published carrying 2 *SURF1* frameshift mutations (p. [Leu105X] + [Ser282CysfsX9]) (lane 3). Antibodies against *COX4* and *MTCO1* were used to detect complex IV, and an antibody against *SDHA* was used for complex II and served as loading control.

mortality before 10 years of age; only a few have been reported to survive beyond age 10.<sup>8</sup> Only 2 atypical patients have been reported to date: one with isolated leukodystrophy leading to death a few months after birth; another with isolated demyelinating polyneuropathy.<sup>20,21</sup> All patients with *SURF1*-associated LS show severely reduced COX activity in muscle and fibroblasts (5%–21% of normal values).<sup>8,13,22-25</sup> In *SURF1* null human samples, residual amounts of fully assembled, functionally active complex IV were found, suggesting partial functional redundancy.<sup>5</sup> Similarly, biochemical and assembly COX defects are also present in *SURF1* knockout mice models.<sup>26,27</sup>

All 3 patients with *SURF1*-associated CMT4 had common features, including severe childhood-onset neuropathy with MNCV <25 m/s, and lactic acidosis. All patients had multisystem involvement with nystagmus, hearing loss, and kyphoscoliosis, and brain MRI abnormalities, including putaminal and periaqueductal

lesions, observed in different combinations. Two patients had an evolving clinical course characterized by cerebellar ataxia, which developed several years after the onset of the polyneuropathy.

In the c.107-2A>G samples, the absence of SURF1 protein is associated with a detectable, but markedly reduced, amount of fully assembled complex IV, which is responsible for some residual COX activity. We found a slightly more severely impaired COX assembly and reduced COX activity in a *SURF1* null LS patient (patient 5 from reference 13). It is unclear why patients harboring *SURF1* mutations, such as those presented in this study, develop a tissue-specific peripheral neuropathic phenotype, whereas several other reported patients and families with similar, or even the same, pathogenic mutations develop a multi-system neurologic syndrome such as LS. For instance, the c.107-2A>G associated in trans with the common frameshift Ser282CysfsX9 was recently reported in LS.<sup>8</sup> In yeast strains with ablated *SHY1*, the *SURF1* ortholog, adaptive changes with interacting partners (i.e., other COX assembly factors or cytochrome *c*), and/or adaptive mechanisms such as increased mitochondrial copper level can suppress, at least in part, the COX defect.<sup>28</sup> Likewise, the variable severity of the phenotype associated with the virtual absence of *SURF1* may well depend on the efficiency of compensatory genetic or epigenetic mechanisms in humans.

The patients with *SURF1*-associated CMT4 reported herein presented with a mainly demyelinating polyneuropathy, although axonal loss was also observed. The precise molecular mechanisms linking MRC dysfunction to axonal loss and demyelination have yet to be determined, although studies on CMT-causing genes encoding mitochondrial molecules such as *GDAP1*, *MFN2*, and *MRS2* suggest that perturbed axonal transport, impaired energy production, and/or defective mitochondrial Mg<sup>2+</sup> homeostasis may be involved.<sup>29,30</sup>

We suggest that *SURF1* should be considered in the molecular diagnostic evaluation of patients with CMT4. The clinical and electrophysiologic phenotype in our families was typical of CMT4; however, the following features should help to prioritize *SURF1* for mutation analysis in patients with demyelinating neuropathy: 1) disease onset in the first decade; 2) variable clinical severity; 3) associated features including nystagmus, hearing loss, and kyphoscoliosis; 4) brain MRI abnormalities, including putaminal hyperintense lesions and periaqueductal abnormalities; 5) lactic acidosis; and 6) an evolving clinical phenotype with cerebellar ataxia developing several years after polyneuropathy onset.

#### AUTHOR CONTRIBUTIONS

A.E.-L., D.G., M.C., M.M., S.P., L.M., I.R., B.L., D.B., P.L., M.Z., and B.M.deC. designed and performed research, and collected the data. A.E.-L. and B.M.deC. wrote the manuscript. D.G., M.C., M.M., S.P., L.

M., I.R., B.L., D.B., P.L., and M.Z. critically revised the manuscript for important intellectual content.

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