






CASE STUDY

Charcot–Marie–Tooth disease type 2F associated with biallelic *HSPB1* mutations

Elena Abati¹ , Stefania Magri², Megi Meneri³, Giulia Manenti¹, Daniele Velardo³ ,
Francesca Balistreri², Chiara Pisciotta⁴, Paola Saveri⁴, Nereo Bresolin^{1,3}, Giacomo Pietro Comi^{1,3} ,
Dario Ronchi¹ , Davide Pareyson⁴ , Franco Taroni² & Stefania Corti^{1,3}

¹Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, Neuroscience Section, University of Milan, Milan, Italy

²Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

³Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

⁴Rare Neurodegenerative and Neurometabolic Diseases Unit, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Correspondence

Stefania Corti, Department of Pathophysiology and Transplantation (DEPT), Dino Ferrari Centre, Neuroscience Section, Neurology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy. Tel: +39 02 55033807; Fax: +39 02 50320430; E-mail: stefania.corti@unimi.it

Funding Information

Fondazione Regionale per la Ricerca Biomedica (FRRB); Centro Dino Ferrari; Italian Ministry of Health - Foundation IRCCS Ca' Granda Ospedale; Maggiore Policlinico Ricerca Corrente 2020; European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD); Inherited Neuropathy Consortium RDCRN.

Received: 31 October 2020; Revised: 19 March 2021; Accepted: 24 March 2021

Annals of Clinical and Translational Neurology 2021; 8(5): 1158–1164

doi: 10.1002/acn3.51364

Elena Abati, Stefania Magri, and Megi Meneri equally contributed to the work; Davide Pareyson, Franco Taroni, and Stefania Corti equally contributed to the work.

Introduction

Charcot–Marie–Tooth (CMT) disease is a spectrum of primary hereditary sensorimotor neuropathies with an overall prevalence of 1/1,200–2,500, making it the most common genetic neuromuscular disorder.¹ CMTs are

Abstract

Objective: This work aims to expand knowledge regarding the genetic spectrum of *HSPB1*-related diseases. *HSPB1* is a gene encoding heat shock protein 27, and mutations in *HSPB1* have been identified as the cause of axonal Charcot–Marie–Tooth (CMT) disease type 2F and distal hereditary motor neuropathy (dHMN). **Methods:** Two patients with axonal sensorimotor neuropathy underwent detailed clinical examinations, neurophysiological studies, and next-generation sequencing with subsequent bioinformatic prioritization of genetic variants and in silico analysis of the likely causal mutation. **Results:** The *HSPB1* p.S135F and p.R136L mutations were identified in homozygosis in the two affected individuals. Both mutations affect the highly conserved alpha-crystallin domain and have been previously described as the cause of severe CMT2F/dHMN, showing a strictly dominant inheritance pattern. **Interpretation:** Thus, we report for the first time two cases of biallelic *HSPB1* p.S135F and p.R136L mutations in two families.

classified according to their neurophysiological properties and inheritance pattern.¹ Motor nerve conduction velocity (MNCV) allows to distinguish demyelinating CMT type 1 (slow MNCV) from axonal CMT type 2 (preserved MNCV).¹ Both these forms mainly display autosomal dominant transmission, although recessive inheritance

have been described in several demyelinating and axonal CMT subtypes.¹ Moreover, six types of X-linked CMT have been described. While the link between CMT1 forms and mutations in genes affecting myelination is clear, the specific disease-causing mechanisms underlying mutations in genes associated with axonal forms is not.

Distal hereditary motor neuropathy (dHMN), presenting as a pure length-dependent motor nerve syndrome with no sensory involvement, might overlap CMT. Both diseases are characterized by wide clinical and genetic heterogeneity. As an example, mutations in *HSPB1* are associated with both CMT type 2F presentation and distal HMN2B phenotype. *HSPB1* encodes heat shock protein 27 (HSP27), a member of the small HSP family, which act as molecular chaperones, binding partially denatured proteins and preventing irreversible aggregation under stressful conditions.² HSP27 shares with other HSPs a highly conserved alpha-crystallin domain of approximately 90 amino acids near the C-terminus, and its beta-sheets conformation guarantees stable dimerization.² HSP27 has a role in thermotolerance, stress response, and activation of the proteasome. Moreover, HSP27 plays a role in several cellular processes, such as the modulation of intracellular redox state, cellular differentiation,

apoptosis inhibition, and assembly of cytoskeletal structures such as microfilaments, neurofilaments (NFLs), and microtubules (MTs). Its interactions with NFLs are important in order to maintain the integrity of cytoskeletal networks, which can be disrupted by disease-causing mutations in *HSPB1*.^{3,4}

Forty-four *HSPB1* mutations have been reported thus far, distributed along the whole gene sequence^{5–31} (Figure 1). Most *HSPB1* mutations are missense, although sporadic frameshift, nonsense, and splicing mutations have been described (Figure 1). The vast majority of cases show autosomal-dominant (AD) inheritance, while recessive inheritance has been reported in very few cases (Table 1).^{6,17,26,32}

Both the p.S135F and the p.R136L missense mutations have been previously reported as the cause of CMT2F or hereditary motor neuropathy 2B (dHMN2B). The mutations affect the highly conserved alpha-crystallin domain and in silico analyses and functional studies support their pathogenic role.^{4,33,34} The p.S135F mutation is by far the most frequent cause of *HSPB1*-related neuropathy, with 40 patients reported, while the p.R136L has been observed in 10 patients.³⁵ Notably, both mutations seem to segregate with disease according to a strictly dominant pattern of inheritance.^{5–7,22–24,26}

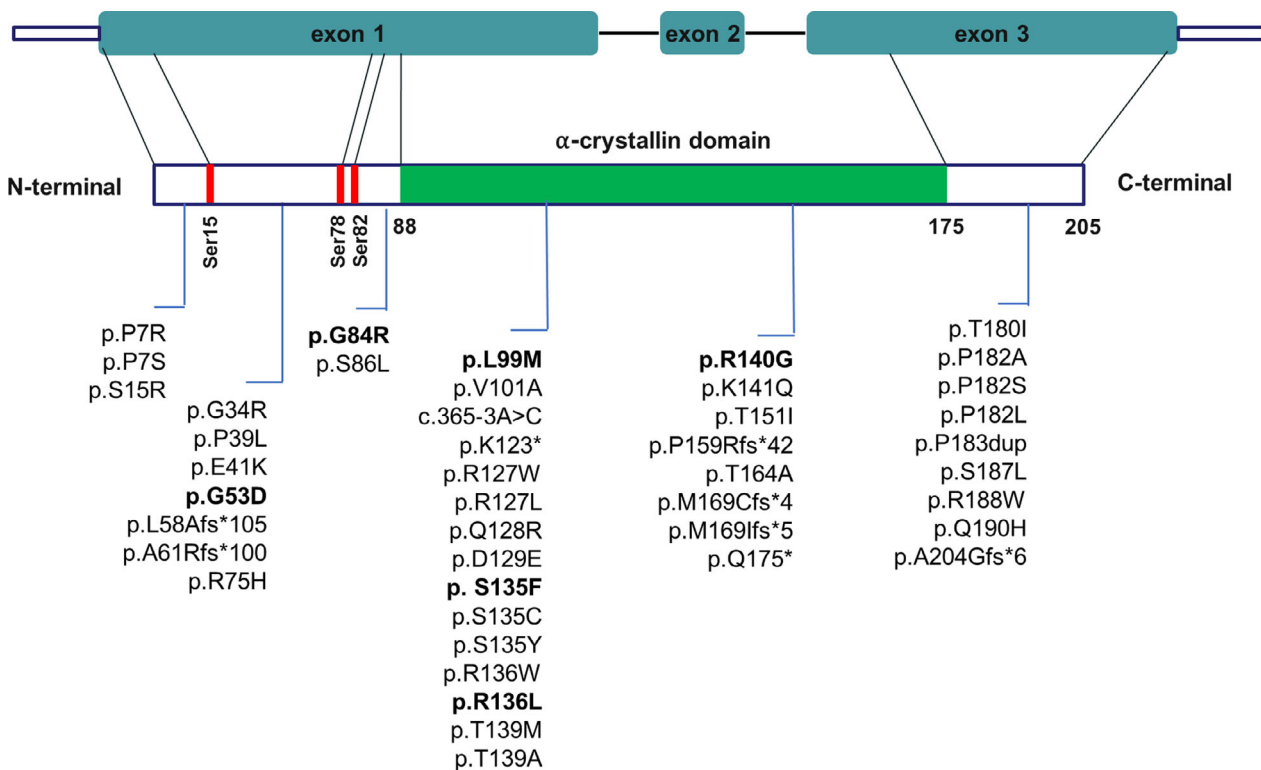


Figure 1. Domain architecture of *HSPB1* and localization of known *HSPB1* mutations. α -crystallin domain is shown in green. Sites of phosphorylation by MAPKAPK-2 are shown as red rectangles. All currently known mutations are listed. Biallelic *HSPB1* mutations are indicated in bold.

Table 1. Clinical and genetic features of known recessive *HSPB1* mutations.

Nucleotide change	Amino acid change	Domain	Clinical features	Parents' clinical features	Ref.
c.158G>A	p.G53D	N-terminal	dHMN + Cerebellar ataxia	Asymptomatic	Echaniz-Laguna et al. (2017)
c.250G>C	p.G84R	N-terminal	CMT2F	Mother: hyporeflexia (LLLL) Father: slowing of NCVs (LLLL)	Fischer et al. (2011)
c.295C>A	p.L99M	α -Crystallin domain	CMT2F	Asymptomatic	Houlden et al. (2008)
c.404C>T	p.S135F	α -Crystallin domain	CMT2F	Asymptomatic	This study
c.407G>T	p.R136L	α -Crystallin domain	CMT2F	Asymptomatic	This study
c.418C>G	p.R140G	α -Crystallin domain	dHMN + distal vacuolar myopathy	Mild distal weakness and areflexia (UULL + LLLL), mild hypoesthesia/hypopallesthesia (LLLL)	Bugiardini et al. (2017)

Abbreviations: CMT2F, Charcot–Marie–Tooth type 2F; dHMN, distal hereditary motor neuropathy; LLLL, lower limbs; NCV, nerve conduction velocities; UULL, upper limbs.

Here, we report the first description of biallelic *HSPB1* p.S135F and p.R136L mutations underlying CMT type 2F phenotype in two patients.

Materials and Methods

All the attendees provided written informed consent. The probands and assessed relatives (three asymptomatic sisters of Patient 1 and the patient's son) received a complete neurological evaluation and underwent neurophysiological assessment. The parents and one asymptomatic brother of Patient 1, who were unavailable for clinical review, were asked to complete a questionnaire in Portuguese language regarding the presence of motor and/or sensory symptoms suggesting the presence of neuropathy. The questionnaire was reviewed by a native Portuguese speaker. Personal and family history of the probands was obtained, and common causes of acquired axonal neuropathy were excluded. Patients' DNA was extracted from peripheral blood leukocytes and used to generate a library for the next-generation sequencing (NGS) of a panel of genes causing hereditary neuropathies. NGS was run on an Illumina MiSeq platform, according to the manufacturer instruction. Sanger sequencing was used to validate candidate variants and to test segregation in available family members.

Case Description and Results

Case 1

The first proband is a 44-year-old woman, born in the Republic of Cabo Verde, a small African archipelago with approximately 550,000 inhabitants. Her parents were

referred to be nonconsanguineous but originated from two nearby villages. They are asymptomatic, and their past medical history is unremarkable. The proband is the last of eight healthy siblings and has an asymptomatic 9-year-old son (Figure 2). She has no other relevant medical conditions. She had a normal psychomotor development. Her neurological symptoms started at the age of 25 with muscle cramps and progressive distal motor impairment in lower limbs, for which she was prescribed ankle foot orthoses (AFOs) at the age of 34. Distal upper limb muscle wasting and weakness occurred in the following years. She also complained of slight sensory loss and paresthesia in her feet. Neurological examination revealed severe distal muscle wasting and weakness in upper (intrinsic hand muscle MRC 1/5 on the left, 2–3/5 on the right) and lower limbs (MRC 0/5 for feet movements). Deep tendon reflexes (DTRs) were symmetrically reduced at upper limbs and absent at lower limbs. Light touch and pain sensations were slightly reduced up to mid-calf. CMTES was 12/28, and CMTNS was 13/36. Ambulation was possible with steppage gait, and the patient needed unilateral support when standing and occasionally when walking. The remaining neurological examination was normal, and she showed no foot deformity.

Nerve conduction studies (NCS), at age 42 years, showed normal sensory conduction velocities, preservation of sensory action potentials (SAPs), markedly decreased or absent compound muscle action potentials (CMAPs) in lower limbs (with greater involvement of posterior tibial than peroneal nerve) and left upper limb (Table 2). Needle EMG examination showed moderate signs of chronic and active denervation in distal limb muscles with complex repetitive discharges in lower limb muscles. Motor evoked potentials were normal. Sural nerve biopsy showed mild

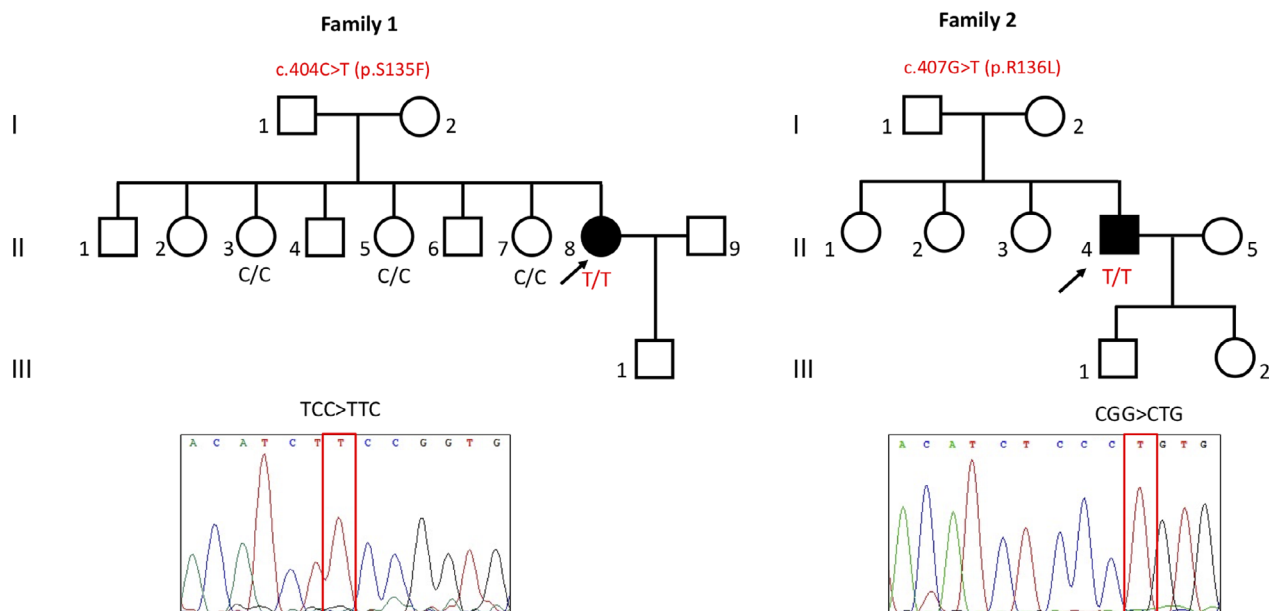


Figure 2. Family pedigree of the probands and Sanger electropherograms of the *HSPB1* p.S135F and p.R136L mutations.

reduction of myelinated fiber density, suggestive of slight sensory nerve damage. Cerebrospinal fluid (CSF) analysis was performed to exclude immune causes of axonal neuropathy, yielding normal results.

NGS analysis revealed homozygosity for the *HSPB1* (NM_001540) nucleotide transition c.404C > T resulting in the amino acid change p.S135F, subsequently confirmed by Sanger sequencing (Figure 2). *HSPB1* deletion

Table 2. Electrophysiological findings.

	Patient 1 (42 years)	Patient 2 (49 years)
Motor conduction		
DML ms, MNCV m/s, dCMAP mV		
R Median nerve	4.2 , 53.2, 18.4	3.5, 58, 18.6
L Median nerve	5.2, 48, 2.8	3.8, 62.5, 17
R Ulnar nerve	3.5, 54.7, 6.7	
L Ulnar nerve		2.8, 58.4, 13.2
L Peroneal nerve	NA, NA, 0	5, 50.7, 2.8
R Tibial nerve	NA, NA, 0	5, 44.3, 0.1
Sensory conduction		
SNCV m/s, SNAP μ V		
L Median nerve		58.3, 7.3
R Radial nerve	62.9, 24	
R Sural nerve	43.5, 22	54.5, 6.5
L Superficial peroneal nerve	56.8, 12	

Note: Abnormal values are in bold.

Abbreviations: dCMAP, distal compound muscular action potential amplitude; DML, distal motor latency; L, left; MNCV/SNCV, motor/sensory nerve conduction velocity; NA, not applicable; R, right; SNAP, sensory nerve action potential amplitude.

was excluded by quantitative PCR. The affected residue is the highly conserved serine-135 within the alpha-crystallin domain (Figure 1). DNA of asymptomatic parents was not available.

Three asymptomatic sisters—aged 59, 55, and 48, respectively—resulted negative at molecular testing (Figure 2). The 9-year-old son, an obligate carrier, has remained asymptomatic so far. Short tandem repeat (STR) haplotyping in available DNA samples does not support parental consanguinity or the occurrence of uniparental disomy (Table S1).

Case 2

The second proband is a 66-year-old man born to an Iranian family. His nonconsanguineous parents and his three sisters were reported to be asymptomatic (Figure 2). He had normal psychomotor development, and his past medical history is unremarkable. At the age of 48, he started complaining of gait disturbances, easy fatigability and muscle pain at lower limbs, which progressed slowly over the following years. When last seen in 2006, neurological examination revealed slight distal weakness, atrophy and fasciculations, and sensory loss in lower limbs. There was no muscle wasting nor weakness in upper limbs. Ankle jerks were absent.

NCS, performed at age 49 years in 2003, showed normal sensory conduction velocities, slightly reduced sural and median SAPs, markedly decreased or absent CMAPs in lower limbs (with greater involvement of posterior tibial than peroneal nerve) (Table 2). Needle EMG examination showed marked signs of chronic and active

denervation with complex repetitive discharges in lower limb muscles. Motor-evoked potentials were normal. Brain MRI was normal. Notably, CK levels were increased (699 U/l; n.v. <195). CSF analysis, performed to rule out immune causes of axonal neuropathy, showed moderately elevated proteins (83 mg/dl; n.v., 10–45).

NGS analysis showed homozygosity for the *HSPB1* (NM_001540) nucleotide transversion c.407G > T, resulting in the p.R136L amino acid change, subsequently confirmed by Sanger sequencing (Figure 2). *HSPB1* deletion was excluded by quantitative PCR. The affected residue is the highly conserved arginine-136 within the alpha-crystallin domain (Figure 1). DNA of parents was not available. STR haplotyping in this patient does not exclude parental consanguinity or segmental uniparental disomy (Table S1).

Discussion

HSPB1 pathogenic variants are responsible for both distal HMN and CMT type 2F. Although the vast majority of described mutations show dominant inheritance, four homozygous substitutions (p.G53D, p.G84R, p.L99M, and p.R140G) have been previously reported in patients with typical (dHMN/CMT2F) and atypical (distal vacuolar myopathy, cerebellar ataxia) presentations (Table 1).^{6,17,26,32} In two cases, parents were asymptomatic heterozygous carriers, while obligate carriers for p.G84R and p.R140G mutations displayed a subclinical phenotype with minor disturbances only detected by neurophysiological studies. The same mutations had been previously detected in several families with dHMN, displaying autosomal dominant inheritance with complete penetrance.^{6,9,31}

In this manuscript, we describe the first reports of *HSPB1*-related neuropathy presenting p.S135F or p.R136L genotype in homozygosis. Heterozygous p.S135F change was previously associated with severe clinical presentations.^{6,26} Reported age at onset for this mutation spans from 9 to 26 years.^{6,26,31} Heterozygous p.R136L change was previously reported in 10 patients from four families with phenotypic variability and atypical signs. Reported age at onset for this mutation spans from 42 to 60 years.^{7,22,23}

Several functional studies evaluated the impact of *HSPB1* mutations on cytoskeletal organization, axonal viability, and mitochondrial transport. In particular, *HSPB1*^{S135F} displays a dominant-negative effect on NFL assembly, inducing aggregation of wild-type NFLs and subsequent motor neuron degeneration and death.⁴ Interestingly, the presence of NFL protein is essential to manifest mutant *HSPB1* toxicity.⁴

In a subsequent study, chaperone activity was evaluated for several *HSPB1* mutants, including the p.S135F and a different amino acid substitution affecting arginine 136

(p.R136W).³³ Both mutants were associated with an abnormal increase in *HSPB1* chaperone activity that might promote toxic MT stabilization, engaging a deacetylating response which impairs the integrity of the MT network and axonal transport leading to nerve degeneration.³⁴

Finally, defects in axonal mitochondrial transport were observed in cellular and animal models expressing *HSPB1*-S135F^{36,37}; similar alterations were also observed in MFN2-related CMTs,³⁸ suggesting the existence of common pathogenic pathways in axonal CMTs. Remarkably, in 2011 d'Ydewalle and colleagues discovered that the use of a selective HDAC6 inhibitor successfully reversed the clinical phenotype in *HSPB1*^{S135F} rodent models.³⁶ Further preclinical studies of these compounds in other models of axonal CMT have revealed promising results,^{39,40} thereby paving the way for future clinical trials in patients.

Our first patient showed typical features of a length-dependent predominantly motor neuropathy, with onset during early adulthood, severe involvement of distal limb muscles, and minor sensory signs and symptoms with preserved SAP amplitudes at NCS but mild loss of myelinated fibers at nerve biopsy. She showed some asymmetry of clinical and electrophysiological involvement, a feature already reported in other subjects carrying *HSPB1* mutations.^{9,26-27} The second patient presented a later onset with distal motor and sensory involvement of lower limbs. In both cases, parents were asymptomatic but could be examined neither clinically nor electrophysiologically. Therefore, we cannot exclude subclinical abnormalities in obligate carriers. Nevertheless, it is puzzling that monoallelic p.S135F and p.R136L mutations are clinically silent in these families, considering the complete penetrance in the pedigrees reported so far. Moreover, the phenotype of the homozygous probands does not appear to be more severe than that previously reported in heterozygous patients.^{5-7,22,23,26} A limitation of the present study is the lack of DNA samples from the parents, that precludes the assessment of their actual genotype and a definite conclusion about the transmission pattern of the *HSPB1* locus in these families. However, our discovery of biallelic inheritance of the two most frequent dominant *HSPB1* mutations further expands the genetic complexity of *HSPB1*-associated neuropathies.

Acknowledgments

We thank “Associazione Amici del Centro Dino Ferrari” for its support. DP, CP, PS, FT, GC and SC are members of the European Reference Network for Rare Neuromuscular Diseases (ERN EURO-NMD). DP, CP, PS, FT, and SM are members of the Inherited Neuropathy Consortium RDCRN. This study received support by grant CP 20/2018 (Care4NeuroRare) from Fondazione Regionale per la

Ricerca Biomedica (FRRB) to FT and SC, and by Italian Ministry Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico Ricerca Corrente 2020 to NB and GC.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from authors FT, DP, and SC upon request.

References

- Laurá M, Pipis M, Rossor AM, Reilly MM. Charcot-marie-Tooth disease and related disorders: An evolving landscape. *Curr Opin Neurol* 2019;32(5):641–650.
- Sun Y, MacRae TH. Small heat shock proteins: molecular structure and chaperone function. *Cell Mol Life Sci* 2005;62(21):2460–2476.
- Perng MD, Cairns L, van den IJssel P, et al. Intermediate filament interactions can be altered by HSP27 and alphaB-crystallin. *J Cell Sci* 1999;112(Pt 13):2099–2112.
- Zhai J, Lin H, Julien J-P, Schlaepfer WW. Disruption of neurofilament network with aggregation of light neurofilament protein: a common pathway leading to motor neuron degeneration due to Charcot-Marie-Tooth disease-linked mutations in NFL and HSPB1. *Hum Mol Genet* 2007;16(24):3103–3116.
- Evgrafov OV, Mersianova I, Irobi J, et al. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat Genet* 2004;36(6):602–606.
- Houlden H, Laura M, Wavrant-De Vrièze F, et al. Mutations in the HSP27 (HSPB1) gene cause dominant, recessive, and sporadic distal HMN/CMT type 2. *Neurology* 2008;71(21):1660–1668.
- Capponi S, Geroldi A, Fossa P, et al. HSPB1 and HSPB8 in inherited neuropathies: study of an Italian cohort of dHMN and CMT2 patients. *J Peripher Nerv Syst* 2011;16(4):287–294.
- Kijima K, Numakura C, Goto T, et al. Small heat shock protein 27 mutation in a Japanese patient with distal hereditary motor neuropathy. *J Hum Genet* 2005;50(9):473–476.
- James PA, Rankin J, Talbot K. Asymmetrical late onset motor neuropathy associated with a novel mutation in the small heat shock protein HSPB1 (HSP27). *J Neurol Neurosurg Psychiatry* 2008;79(4):461–463.
- Fortunato F, Neri M, Geroldi A, et al. A CMT2 family carrying the P7R mutation in the N-terminal region of the HSPB1 gene. *Clin Neurol Neurosurg* 2017;163:15–17.
- Bacquet J, Stojkovic T, Boyer A, et al. Molecular diagnosis of inherited peripheral neuropathies by targeted next-generation sequencing: molecular spectrum delineation. *BMJ Open* 2018;8(10):e021632.
- Ikeda Y, Abe A, Ishida C, et al. A clinical phenotype of distal hereditary motor neuropathy type II with a novel HSPB1 mutation. *J Neurol Sci* 2009;277(1–2):9–12.
- Liu X, Duan X, Zhang Y, et al. Molecular analysis and clinical diversity of distal hereditary motor neuropathy. *Eur J Neurol* 2020;27(7):1319–1326.
- Taga A, Cornblath DR. A novel HSPB1 mutation associated with a late onset CMT2 phenotype: case presentation and systematic review of the literature. *J Peripher Nerv Syst* 2020;jns.12395.
- Scarlato M, Viganò F, Carrera P, et al. A novel heat shock protein 27 homozygous mutation: widening of the continuum between MND/dHMN/CMT2. *J Peripher Nerv Syst* 2015;20(4):419–421.
- DiVincenzo C, Elzinga CD, Medeiros AC, et al. The allelic spectrum of Charcot-Marie-Tooth disease in over 17,000 individuals with neuropathy. *Mol Genet Genomic Med* 2014;2(6):522–529.
- Fischer C, Trajanoski S, Papić L, et al. SNP array-based whole genome homozygosity mapping as the first step to a molecular diagnosis in patients with Charcot-Marie-Tooth disease. *J Neurol* 2012;259(3):515–523.
- Lašuthová P, Šafka Brožková D, Krůtová M, et al. Improving diagnosis of inherited peripheral neuropathies through gene panel analysis. *Orphanet J Rare Dis* 2016;11(1):118.
- Capponi S, Geuens T, Geroldi A, et al. Molecular chaperones in the pathogenesis of amyotrophic lateral sclerosis: the Role of HSPB1. *Hum Mutat* 2016;37(11):1202–1208.
- Antoniadi T, Buxton C, Dennis G, et al. Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. *BMC Med Genet* 2015;16:84.
- Karakaya M, Storbeck M, Strathmann EA, et al. Targeted sequencing with expanded gene profile enables high diagnostic yield in non-5q-spinal muscular atrophies. *Hum Mutat* 2018;39(9):1284–1298.
- Gaeta M, Mileto A, Mazzeo A, et al. MRI findings, patterns of disease distribution, and muscle fat fraction calculation in five patients with Charcot-Marie-Tooth type 2 F disease. *Skeletal Radiol* 2012;41(5):515–524.
- Stancanelli C, Fabrizi GM, Ferrarini M, et al. Charcot-Marie-Tooth 2F: phenotypic presentation of the Arg136Leu HSP27 mutation in a multigenerational family. *J Neurol Sci* 2015;36(6):1003–1006.
- Chung KW, Kim S-B, Cho SY, et al. Distal hereditary motor neuropathy in Korean patients with a small heat shock protein 27 mutation. *Exp Mol Med* 2008;40(3):304–312.

25. Dohrn MF, Glöckle N, Mulahasanovic L, et al. Frequent genes in rare diseases: panel-based next generation sequencing to disclose causal mutations in hereditary neuropathies. *J Neurochem* 2017;143(5):507–522.
26. Echaniz-Laguna A, Geuens T, Petiot P, et al. Axonal neuropathies due to mutations in small heat shock proteins: clinical, genetic, and functional insights into novel mutations. *Hum Mutat* 2017;38(5):556–568.
27. Amornvit J, Yalvac ME, Chen L, Sahenk Z. A novel p.T139M mutation in HSPB1 highlighting the phenotypic spectrum in a family. *Brain Behav* 2017;7(8):1–8.
28. Luigetti M, Fabrizi GM, Madia F, et al. A novel HSPB1 mutation in an Italian patient with CMT2/dHMN phenotype. *J Neurol Sci* 2010;298(1–2):114–117.
29. Rossor AM, Davidson GL, Blake J, et al. A novel p.Gln175X [corrected] premature stop mutation in the C-terminal end of HSP27 is a cause of CMT2. *J Peripher Nerv Syst* 2012;17(2):201–205.
30. Ylikallio E, Konovalova S, Dhungana Y, et al. Truncated HSPB1 causes axonal neuropathy and impairs tolerance to unfolded protein stress. *BBA Clin* 2015;3:233–242.
31. Rossor AM, Morrow JM, Polke JM, et al. Pilot phenotype and natural history study of hereditary neuropathies caused by mutations in the HSPB1 gene. *Neuromuscul Disord* 2017;27(1):50–56.
32. Bugiardini E, Rossor AM, Lynch DS, et al. Homozygous mutation in HSPB1 causing distal vacuolar myopathy and motor neuropathy. *Neurol Genet* 2017;3(4):1–3.
33. Almeida-Souza L, Goethals S, de Winter V, et al. Increased monomerization of mutant HSPB1 leads to protein hyperactivity in Charcot-Marie-Tooth neuropathy. *J Biol Chem* 2010;285(17):12778–12786.
34. Almeida-Souza L, Timmerman V, Janssens S. Microtubule dynamics in the peripheral nervous system: a matter of balance. *Bioarchitecture* 2011;1(6):267.
35. Adriaenssens E, Geuens T, Baets J, et al. Novel insights in the disease biology of mutant small heat shock proteins in neuromuscular diseases. *Brain* 2017;140(10):2541–2549.
36. D'Ydewalle C, Krishnan J, Chiheb DM, et al. HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. *Nat Med* 2011;17(8):968–974.
37. Kim JY, Woo SY, Bin HY, et al. HDAC6 inhibitors rescued the defective axonal mitochondrial movement in motor neurons derived from the induced pluripotent stem cells of peripheral neuropathy patients with HSPB1 mutation. *Stem Cells Int* 2016;2016:9475981.
38. Rizzo F, Ronchi D, Salani S, et al. Selective mitochondrial depletion, apoptosis resistance, and increased mitophagy in human Charcot-Marie-Tooth 2A motor neurons. *Hum Mol Genet* 2016;25(19):4266–4281.
39. Adalbert R, Kaieda A, Antoniou C, et al. Novel HDAC6 inhibitors increase tubulin acetylation and rescue axonal transport of mitochondria in a model of Charcot-Marie-Tooth Type 2F. *ACS Chem Neurosci* 2020;11(3):258–267.
40. Picci C, Wong VSC, Costa CJ, et al. HDAC6 inhibition promotes α -tubulin acetylation and ameliorates CMT2A peripheral neuropathy in mice. *Exp Neurol* 2020;328:113281.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. STR markers on chromosome 7 in the two patients and available family members. Haplotyping of *HSPB1* locus in Patient 1 (Family 1, II-8) suggested the existence of two different biparental alleles harboring the c.404C>T change. The extension of STR analysis to available family members support independent biallelic recombination occurring on chromosome 7 in a region between D7S672 and D7S2204. These findings do not support parental consanguinity or uniparental disomy in Family 1. Haplotyping in Patient 2 (Family 2, II-4) does not exclude parental consanguinity or segmental uniparental disomy and the lack of DNA from other relatives did not provide additional informative meioses to consolidate genetic findings.