SUPPLEMENTARY MATERIAL

Sanger sequencing

The coding regions and flanking intronic regions of *NEFL* (GenBank accession number NM_006158.4) were PCR-amplified using AmpliTaq Gold 360 Master Mix (Applied Biosystems). Primer sequences and PCR conditions are available upon request. PCR products were cleaned up using the MultiScreen-PCR96 Filter Plate (Millipore). Sequencing reactions were performed using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and products were cleaned up using the Dye Terminator Removal Kit (Thermo Scientific ABgene). DNA fragments were separated on an ABI3730XL automatic DNA sequencer (Applied Biosystems). The resulting sequences were analysed with SeqScape software v2.5 (Applied Biosystems).

Short tandem repeat analysis

DNA was amplified for 16 short tandem repeat (STR) loci using the PowerPlex 16 HS System kit (Promega Corp.) as per the manufacturers' instructions. PCR products for STR analysis were run on an ABI3730XL automatic DNA sequencer (Applied Biosystems) and analyzed by GeneMapper software v4.0 (Applied Biosystems) for automated molecular mass sizing.

Whole-exome sequencing

The exomes of patient 2 were enriched using the SureSelect Human All Exon V5 capture kit (Agilent) and sequenced on the HiSeq 2000 platform (Illumina) at the John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA. The resulting 100bp paired-end sequence reads were mapped against the human reference genome assembly 19 (GRCh37) with the Burrows-Wheeler Aligner (BWA) package.[1] Variant calling and indel realignment

were performed with FreeBayes.[2] All variants were submitted to SeattleSeq for annotation (http://snp.gs.washington.edu/).[3] VCF files were imported to the GEnomes management application (GEM.app)[4] for further analysis and candidate variant identification (supplementary table 1).

Bioinformatic analyses

NEFL sequence variants are described in accordance with the recommendations of the Human Genome Variation Society (http://varnomen.hgvs.org/) using GenBank NM_006158.4 as the reference sequence. Minor allele frequencies of all *NEFL* variants were obtained from the Exome Aggregation Consortium (ExAC) browser version 0.3 (http://exac.broadinstitute.org) using genomic coordinates from the human reference genome assembly 19 (GRCh37). Evolutionary conservation of nucleotides was assessed using phyloP (46 vertebrate basewise conservation) and GERP scores, which were accessed through the UCSC Genome Browser (https://genome.ucsc.edu/cgi-bin/hgGateway) using genomic coordinates from GRCh37. Grantham scores were used to assess the physicochemical nature of the amino acid substitutions.[5] *In silico* analyses of sequence variants were performed using SIFT (http://provean.jcvi.org/index.php; UniProt ID P07196), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/; UniProt ID P07196) and CADD (http://cadd.gs.washington.edu/).

Clinical details

Patient 1. This 40-year-old woman was diagnosed with mild Erb's palsy one month after birth. Flexion deformities of the thumbs were noted three months later. She started walking at age 2 years with marked unsteadiness and frequent falls. Hypotonia and areflexia were observed at age 3. Pure-tone audiometry confirmed bilateral sensorineural hearing loss at age 5 and hearing aids were placed. Hand weakness was present since the first decade of life and lower limb weakness

developed in her teens. In her 20s, she started noticing distal sensory loss. At age 39, on neurological examination, she had mild dysarthria, mild head tremor, mildly broken-up ocular pursuits, right tongue hemiatrophy, bilateral scapular winging, mild scoliosis, bilateral pes cavus, distal greater than proximal muscle weakness and atrophy, marked sensory impairment and marked limb ataxia with an element of proprioceptive deficit. She could stand unaided for a few seconds. Romberg's test was positive. She walked with bilateral foot drop and a broad-based ataxic gait.

Nerve conduction studies (NCS) were consistent with a length-dependent, motor and sensory neuropathy with asymmetric reduction in amplitude of the ulnar nerve compound muscle action potential (CMAP) and with motor nerve conduction velocities (MNCV) in the demyelinating range (supplementary table 2). Brainstem auditory evoked potentials (BAEP) were absent and cochlear responses were well preserved, consistent with bilateral VIII cranial nerve impairment. Visual evoked potentials were normal. Brain and cervical spine MRI at ages 31 and 37 revealed mild cerebellar, medullary and cervical spinal cord volume loss (figure 2). Quadriceps muscle biopsy at age 30 showed features of chronic denervation with reinnervation. Genetic analysis for *FXN* gene expansions was negative. Direct sequencing of *NEFL* revealed a heterozygous missense mutation c.293A>G; p.(Asn98Ser) that was not detected in either parent.

Patient 2. This 34-year-old man was noted to be hypotonic at age 6 months and to have walking difficulties by age 3 years. Bilateral sensorineural hearing loss was detected at age 8 and hearing aids were placed. Pes cavus and lower limb weakness were evident in his teens and he developed hand weakness and distal sensory loss in his 20s. At age 33, on neurological examination, he had mild dysarthria, mild head tremor, broken-up ocular pursuits, bilateral scapular winging, bilateral pes cavus, distal greater than proximal muscle weakness and atrophy, marked sensory impairment, mild limb sensory ataxia and pseudoathetosis. He

could stand unaided for a few seconds. Romberg's test was positive. He walked with bilateral foot drop and an element of proximal weakness. Neuro-otological evaluation revealed moderate bilateral sensorineural hearing loss with evidence of VIII cranial nerve involvement on BAEP, severe bilateral vestibular hypofunction (positive head impulse test and absent responses to caloric stimulation) and signs of cerebellar involvement (broken-up smooth pursuit eye movements, occasional spontaneous nystagmus with optic fixation, low gain of optokinetic nystagmus and failure of vestibulo-ocular reflex suppression).

NCS were consistent with a length-dependent, motor and sensory neuropathy with reduced CMAP amplitudes, MNCV in the demyelinating range and temporal dispersion on proximal stimulation of the right ulnar nerve (supplementary table 2). Brain and cervical spine MRI at ages 24 and 29 revealed mild cervical spinal cord volume loss (figure 2). CSF protein concentration was slightly increased at 0.55 g/L (reference values 0.13-0.40). Plasma creatine kinase levels at age 27 were raised at 644 IU/L (reference values 38-204). Genetic analysis for *FXN* gene expansions was negative. Whole-exome sequencing revealed a heterozygous pathogenic mutation c.293A>G; p.(Asn98Ser) in the *NEFL* gene that was confirmed by direct sequencing. The mutation was not detected in either parent.

Patient 3. This 15-year-old young girl was born to healthy, non-consanguineous parents of Austrian and Russian descent. Early motor developmental milestones were delayed and she walked at age 2 years with notable weakness and balance difficulties. She was diagnosed with left optic nerve hypoplasia at age 18 months and had strabismus surgery at age 2 years. Hypotonia, areflexia and progressive weakness were noted at age 4. She subsequently developed hand tremor and muscle cramps. Moderate bilateral sensorineural hearing loss was present by age 8 years and hearing aids were placed. By age 10 she had bilateral tendon transfers and heel cord lengthening for inverted feet. At age 15, on neurological examination,

she had bilateral pes cavus, distal greater than proximal muscle weakness and atrophy and marked proprioceptive sensory deficit. Finger tapping was slow and a fine hand tremor was present bilaterally. Romberg's test was positive. She walked with bilateral foot drop and with a broad-based ataxic gait.

NCS were consistent with a length-dependent, motor and sensory neuropathy with reduced CMAP amplitudes and MNCV in the demyelinating range (supplementary table 2). BAEP were absent bilaterally. Direct sequencing of *NEFL* revealed a heterozygous missense mutation c.293A>G; p.(Asn98Ser) that was not detected in either parent.

Patient 4. This 53-year-old man had normal early motor developmental milestones. In his teens, he had frequent ankle sprains and developed restless legs. In his early 20s, he noticed hand tremor and gait unsteadiness. Bilateral foot drop was evident in his 30s. In his late 40s he underwent osteotomies and tendon transfers on his feet and started using ankle-foot orthoses. He also complained of repetitive limb movements during sleep. At age 53, on neurological examination, he had normal speech, mild tremor of his head and hands, mildly broken-up ocular pursuits, bilateral pes cavus, distal muscle weakness and atrophy and marked sensory impairment. Romberg's test was positive. He walked with bilateral foot drop. Neuro-otological evaluation revealed mild bilateral high-frequency sensorineural hearing loss on pure tone audiometry and broken-up smooth pursuit eye movements on electronystagmography.

NCS (supplementary table 2) were consistent with a length-dependent, motor and sensory neuropathy with reduced CMAP amplitudes, mildly slowed MNCV and temporal dispersion on proximal stimulation of the right ulnar nerve. BAEP were normal. A polysomnography confirmed the diagnosis of periodic limb movement disorder. Brain and cervical spine MRI at ages 42 and 54 revealed no abnormalities

(figure 2). CSF analysis was unremarkable. Direct sequencing of *NEFL* revealed a heterozygous missense mutation c.23C>G; p.(Pro8Arg). DNA samples from relatives were not available for segregation analysis.

Patient 5. This 76-year-old man developed hand tremor in his mid-50s. Five years later he started noticing balance difficulties, unsteady gait and wasting of leg muscles. In his mid-60s he developed hand and leg weakness and started using ankle-foot orthoses and support for walking. He had no sensory symptoms. The disease course was slowly progressive. His family history was remarkable for his mother and sister having had similar symptoms of adult-onset balance problems and possible peripheral neuropathy. At age 70 years, on neurological examination, he had normal speech and normal eye movements, mild no-no head tremor, postural and intention tremor affecting the right more than the left hand, thickened ulnar nerves, distal muscle weakness and atrophy and distal sensory impairment in the lower limbs. Romberg's test was positive. He walked with a stamping and slightly wide-based gait.

NCS were consistent with a mild, length-dependent, motor and sensory neuropathy with asymmetric reduction in both CMAP amplitudes and MNCV and temporal dispersion on proximal stimulation of the left ulnar and left common peroneal nerves. CSF protein concentration was raised at 0.76 g/L. Brain and whole-spine MRI at age 69 were unremarkable (figure 2). Mutation analysis for the SCA1, SCA2, SCA3, SCA6 and SCA7 repeat expansions was negative. Direct sequencing of *MPZ*, *LITAF*, *ERG2*, *GDAP1* and *GJB1* revealed no pathogenic sequence variants. Direct sequencing of *NEFL* revealed a heterozygous missense mutation c.932T>C; p.(Leu311Pro). DNA samples from relatives were not available for segregation analysis.

References

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