SUPPLEMENTARY MATERIAL

CASE REPORTS

Clinical features are described below and summarized in Table 1 and Supplementary Table 1.

Family F

Patient F:II-3 was the eighth of 11 children, a boy, who was born full term after an uneventful pregnancy with a birth weight of 3.6 kg and height of 48 cm. He had mild laryngomalacia. He learned to walk independently at the age of 15 months. He was referred for pediatric neurological investigations at the age of 4 years due to delayed speech and motor development. He was in special education at school because of difficulties in language and visuomotor development.

At the age of 8 years he was investigated at Helsinki University Hospital under suspicion of a progressive neurodegenerative disorder. He had distal muscular atrophy both in his feet and hands, his gait was unsteady with valgus deformity in his feet, he was unable to walk on his heels, and had tremor in his hands. His patellar and Achilles reflexes were accelerated, and Babinski sign was positive. Cranial nerve functions were normal. Nerve conduction study (NCS) and electromyography (EMG) showed sensorimotor axonal neuropathy. Sural nerve biopsy showed moderate loss of myelinated axons, no hypertrophic onion bulbs were detected. Muscle biopsy showed severe type 1 fiber predominance. Mitochondrial investigations (immunohistochemical and biochemical analyses) were normal. Brain MRI showed mild unspecific increased signal intensity in the right temporal lobe in T2-weighted images, but proton magnetic spectroscopy revealed metabolic abnormality in supratentorial white matter and basal ganglia nuclei.
A wide-range of etiological investigations (including chromosomes, *PMP22, GJB1, MFN2, POLG*, urine organic acids, plasma and urine amino acids, lactate, pyruvate, ammonia, alpha fetoprotein, desialotransferrin, creatine kinase, liver transaminases) were normal.

His disease has been progressive. He has been using a wheelchair since the age of 10-11. He developed nocturnal hypoventilation and has used a noninvasive ventilator during sleep from the age of 13. He had difficulties with eating and weight loss. He underwent an operation for scoliosis at the age of 14. Some weeks after the operation he got severe pneumonia and his respiratory function did not recover. He therefore received a tracheostomy and has been ventilator-dependent ever since.

Patient F:II-4, the 10\(^{th}\) of 11 children, a girl, was born full term after an uneventful pregnancy at full term, birth weight was 3.9 kg and height 49.5 cm, head circumference 34 cm, and Apgar scores were 9/9. She had delayed motor development and hypotonia, and she learned to walk independently at the age of 23 months. She was investigated at the age of 2.5 years. She had a wide-based gait, tendon reflexes were normal, Babinski sign was negative. She had intermittent strabismus. Sensorimotor axonal neuropathy was diagnosed by NCS. Her brain MRI showed mild unspecific signal intensity in temporal lobes in T2-weighted images.

Her disease has been progressive. She has primarily used a wheelchair since the age of 10. At that time she had oedema of the hands and feet, and cavus deformity of the feet was detected. She had developed distal and facial muscle weakness, and she spoke with frail voice. Her tendon reflexes were absent. At the age of 12 she was unable to stand independently, had contractures in her fingers, and her weakness in hands was so severe that she hardly could use her electric chair. She had developed urinary incontinence. She had no respiratory problems, and polysomnography was normal. Family’s etiological
investigations were complemented with exclusion of NARP (neuropathy, ataxia, and retinitis pigmentosa) mutation. On sensory examination individual F:II-4 had hyperesthesia, cold feet and oedema in feet, her brother F:II-3 has reported similar symptoms. Endocrinologic testing was done at age 12 years 3 months of age, after menarche: follicle stimulating hormone (FSH) 68 IU/l (reference range 1-7 IU/l), luteinizing hormone (LH) 22 IU/l (0.5 - 9 IU/l), prolactin 146 mIU/l (50 - 500 mIU/l), thyrotropin 2.28 (0.5-3.6 mIU/l), growth hormone 0.43 µg/l (0-8 µg/l).

Patient F:II-5, the 11th and youngest child of the family, a boy, was born after uneventful pregnancy at full term, birth weight was 3.6 kg and height 47 cm, head circumference 35.5 cm, and Apgar scores were 9/9/10. Nevus flammeus-like skin lesions in the left arm and chest were noticed as newborn, as well as bicuspic aortic valve and coarctation of aorta, which was operated on at the age of three months. He has left-sided amblyopia and microtia with sensorineural hearing impairment.

His investigations started at the age of 1 year because of delayed motor development. Sensorimotor axonal neuropathy was detected at the age of 2 years by NCS. Because of global developmental delay he attends special education. Wechsler Preschool and Primary Scale of Intelligence third edition showed mild intellectual disability (ID). At the age of 7 he walked independently with broad-base, and used wheel chair with longer distances. He had valgus deformity in his feet, and could not walk on his heels. Deep tendon reflexes were diminished, and Babinski sign was positive. Etiological investigations were complemented with molecular karyotyping.
Family A

Patient A:II-2 is a 8-year old girl, second child of an Australian family out of two girls born to a family of non-consanguineous parents. She has a healthy unaffected older sister and two unaffected paternal half-brothers.

Patient was born at term following an unremarkable pregnancy. She was first noted to have developmental delay at 6 months; this was particularly marked in the area of speech and motor function. She first walked at almost 4 years of age. She is currently able to ambulate independently for very short distances, but requires a wheel chair to walk long distances. She attends a mainstream school with an aide, but has moderate cognitive impairment. Her IQ is approximately 70-75. She requires ongoing speech therapy input for difficulties with speech expression and swallowing. She has velopharyngeal weakness. A sleep study showed evidence of severe obstructive sleep apnea.

On examination her height is on the 25th centile and her head circumference is on the 50th centile. She was not dysmorphic but had puffy hands and feet with subtle second and third toe syndactyly. Her speech is very hypernasal and dysarthric. She has a marked thoracic kyphosis but no scoliosis. She is areflexic with pes planus and marked wasting of the intrinsic muscles of the hands and distal lower extremities. She has progressive flexor contractures of the fingers. She has decreased muscle strength in upper and lower limbs, which is more marked proximal than distal (Supplementary table 1). She is not able to walk on toes or heels. Strength testing is difficult since she has marked joint hypermobility with tendency to hyperextend at elbows, fingers and knees. Furthermore, she has ataxia and tremor, which are most likely sensory ataxia and neuropathic tremor. She has significant distal sensory loss. No nystagmus or
dysdiadochokinesis has been noted, past-pointing cannot be reliably estimated due to her severe weakness.

Her NCS were consistent with a moderately severe sensorimotor axonal neuropathy affecting upper and lower limbs. An MRI brain revealed no structural abnormalities, but she has progressive cervical myelomalacia of uncertain cause without symptomatic myelopathy. *MFN2* and *PLA2G6* sequencing were negative as was mitochondrial DNA mutation and deletion testing. *SMN1* deletion testing was also normal. Metabolic investigations including a urine metabolic screen, plasma amino acids, transferrin isoforms, CSF biochemistry and copper studies which were all within normal limits. A sural nerve biopsy was non-diagnostic with normal myelination, no axonal spheroids and no Wallerian degeneration.

**Gonadotrophin levels have not been measured.**

**Family N**

Patient N:II-1 is a 3-year old girl, first child of Dutch non-consanguineous healthy parents. She presented with strong delay in motor development, general hypotonia, areflexia, hypermobility, and atrophic distal extremities. A hereditary neuropathy was suspected. **EMG performed at the age of 2 years 8 months showed a severely lowered compound muscle action potential (CMAP) of the median and ulnar nerves with slowed nerve conduction velocity (NCV) in the distal arm. The sensory nerve action potential (SNAP) of the radial nerve was not reproducible. The NCS led to a conclusion of loss of motor and sensory axons suggesting demyelination and axonal degeneration.**

Neuropsychological testing at age 2 years and 6 months using Bayley Scales of Infant Development II Dutch version (BSID-II-NL), showed a developmental age of 19 months. At the age of 3.5 years she is
able to roll over. She cannot sit and is unable to walk. The patient presents also with facial weakness, proximal more than distal muscle weakness, and an infantile A-esotropia with congenital ptosis. Antigravity movements were only possible with proximal limb muscles. She also developed a scoliosis. On the CHOP INTEND (Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders) test of motoric function she received 46/64 points, and her Hammersmith Scale Expanded score was 13.

DNA analysis for GDAP1, KIF1B, MFN2, PMP22, RAB7A, TRPV4 and SMN gene mutations were negative. Gonadotropin levels have not been measured.

**Family C**

This is a non-consanguineous family with two affected sisters who are currently 28 and 27 years of age. They initially presented to Medical Genetics at 1 and 2 years of age with developmental delay, strabismus, and striking frizzy, curly hair. They were followed by a developmental team at the local children’s hospital and were diagnosed with mild intellectual disability. Both developed a progressive sensorimotor polyneuropathy in early childhood. They both presented with primary amenorrhea and elevated gonadotropins, and were diagnosed with primary ovarian failure in their late teens (C:II-1; FSH 52 IU/l, LH 15 IU/l and C:II-2; FSH 91 IU/l, LH 15 IU/l); they were started on estrogen to induce menses and are now on an oral contraceptive.

Patient C:II-1 had multiple surgeries throughout childhood for contractures of her feet and hands and became wheelchair bound at 15 years of age. Muscle biopsy demonstrated early neurogenic atrophy. A sural nerve biopsy was not diagnostic. Her brain MRI was normal at 12 years of age. She had NCS which
demonstrated a length dependent axonal sensorimotor polyneuropathy. With regard to cognition, she is able to read at a grade 1 level, is unable to do simple math and attended a life skills program.

Patient C:II-2 also had surgery for foot contractures and lost her ability to ambulate at age 24 years. She has more recently developed significant contractures in her hands for which surgery is being considered. She has dysarthric speech. Her brain MRI identified mild ventriculomegaly and two white matter cysts in the left posteriotemporal parietal region. She developed seizures in early childhood and was treated with surgery, to remove epileptogenic tissue, and is currently well controlled on Dilantin. She also has velopharyngeal insufficiency, and was recently diagnosed with corneal hydrops, and depression. Sural nerve biopsy showed chronic and axonal demyelinating features. With regard to cognition she is able to read at a grade 3 level, and is able to perform simple addition.

The patients were negative for mutations in GAN, FRAX and infantile onset spinocerebellar ataxia (TWNK). They both had normal karyotypes. IQ testing has not been done for either patient. They will not live independently and the level of functioning is consistent with a mild ID.

When assessed for strength at 18 and 17 years respectively, C:II-1 and C:II-2 were 4+/5 at the shoulder girdle, 4/5 at the forearm, 4/5 at the thigh, and 0-1/5 distally (bellows wrist and knees). Sensory testing was not possible due to their intellectual disability. Currently, both siblings show signs of a length dependent, severe atrophy, nearly complete for hand intrinsics and muscles below the knee, with global areflexia and contractures with reasonably preserved proximal strength.
Family T

The parents of the affected brothers are consanguineous. Their sister is clinically and electrophysiologically normal but has pes cavus. Patient T:II-1 was diagnosed with CMT1 at the age of onset 2-10 years, at 10 years he was confined to wheelchair, and presenting with distal and proximal weakness. His motor milestones were delayed, and he learned to sit at age of 1.5 years and walk with a walking aid at the age of 4 years. He has both proximal and distal weakness since the beginning with areflexia and ataxia. The last neurological exam in 2002 showed quadriplegiasis and he is using wheelchair. His EMG in 1997 showed severe motor and sensory neuropathy. Motor median NCVs of T:II-1 are unobtainable. At age 15 the first neurological examination showed a sensory median nerve action potential amplitude of 2 µV. Muscle strength was markedly decreased distally.

Patient T:II-2 is less severely affected. Motor milestones are not delayed. When first seen at age 7 for neurological examination, the patient had mild proximal and more severe distal weakness of all limbs, with steppage gait, areflexia and diminished vibration sensation. Median motor NCV in EMG is 23 m/s indicating demyelinating neuropathy. When last seen in 2002, distal weakness of all limbs were more prominent. Median SNAP was 3 µV. Muscle strengths were diminished with distal predominance.

Both patients had problems with cognitive ability, but no formal IQ tests have been performed. Stocking and glove type hypoalgesia and diminished vibration sensation were present in both patients.
SUPPLEMENTARY METHODS

Sequencing protocols

For Family F, target capture and next-generation sequencing was done at Oxford Gene Technology (Begbroke, UK). Samples preparation and enrichment were carried out according to Agilent SureSelect Protocol Version 1.2. Sequencing was performed on the Illumina HiSeq2000 platform using TruSeq v3 chemistry. Read alignment to the hg19 reference genome was done with the Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009) variant calling with SAMtools (Li et al., 2009) and annotation with Annovar (Wang et al., 2010). Sequencing data were analyzed by inclusion of variants that were protein damaging (nonsense, missense, frameshift, indel and splice variants), had a population frequency of less than $10^{-3}$ for both total population and the Finnish subset in ExAC, and a presence in <1% of samples in an in-house sequencing database of 255 exomes.

For Family A Whole exome sequencing (WES) was performed by the Melbourne Genomics Health Alliance and received Human Research Ethics Committee approval (13/MH/326). Peripherally-derived blood DNA was sequenced using Nextera v1.2 Rapid Exome Capture Kit and TruSeq Rapid SBS chemistry on a HiSeq 2500, and bioinformatics analysis was performed using Cpipe (Sadedin et al., 2015).

For Family C, WES was performed on the proband. Whole-exome library preparation, exon capture (Agilent SureSelect V5), and paired-end sequencing (Illumina HiSeq2000) were performed at Genome Quebec Innovation Center (Montreal, QC, Canada). Read alignment, variant calling, and annotation were done with a pipeline based on BWA (Li and Durbin, 2009), SAMtools (Li et al., 2009), Annovar (Wang et al., 2010), and custom annotation scripts. All sequences were aligned to Human genome Hg19.
We excluded variants with minor allele frequency greater than 5% in either the 1000 genomes project (http://browser.1000genomes.org/index.html) or the 6500 NHLBI EVS (http://evs.gs.washington.edu/EVS), and seen in more than 30 samples from our in-house database (containing approximately 2000 samples). Protein-damaging variants were prioritized for further analysis.

For Family N, after referral for routine diagnostic exome sequencing, exomes of child and parents were enriched using the SureSelect XT Human All Exon V5 kit (Agilent) and sequenced in rapid run mode on the HiSeq2500 sequencing system (Illumina) at a mean target depth of 85X. The target is defined as all coding exons of UCSC and Ensembl +/- 20bp intron flanks. At this depth >95% of the target is covered at least 10X. Reads were aligned to hg19 using BWA (BWA-MEM v0.7.5a) and variants were called using the GATK haplotype caller (v2.7-2). Detected variants were annotated, filtered and prioritized using the Bench NGS Lab platform (Cartagenia, Leuven, Belgium). Analysis is based upon a tiered analysis approach. In the first tier known intellectual disability genes were analyzed. Since this tier did not result in a diagnosis the second tier was performed to detect de novo variants. No de novo variants were detected. Finally the last tier, which filters for compound heterozygous, homozygous or hemizygous variants, was performed. This last tier detected compound heterozygous variants in the MCM3AP gene. Since variants in this gene were not previously described as causative for a genetic disease this candidate gene was submitted to the GeneMatcher database. This resulted in several matches.

For Family T, WES was performed on individuals II.1 and II.2. Exomes were captured by Agilent’s SureSelect Enrichment array followed by paired-end sequencing on Hiseq2000 Illumina platform performed at the Beijing Genomics Institute (Beijing, China). The sequencing yielded ~6 Gb of raw data
per exome, with 90% of the targets covered at least 20x. Primary analysis was performed using an in-
house developed software pipeline GenomeComb (Reumers et al., 2011). The pipeline used BWA (Li
and Durbin, 2009) for alignment of sequencing reads to the reference human genome (hg19). Duplicate
reads were marked using Picard (http://picard.sourceforge.net); GenomeAnalysisToolKit (GATK)
(McKenna et al., 2010) was used to perform realignment around indels. Variants were called using both
GATK and SAMtools (Li et al., 2009), and were consequently combined, annotated and compared to
different databases e.g. in-house exomes, 1000 Genome project
(http://browser.1000genomes.org/index.html), Exome Aggregation Consortium (ExAC -
http://exac.broadinstitute.org/). Variants with a minor allele frequency less than 5% in these databases
were selected. Homozygosity mapping was performed with SNPs extracted from WES data, as described
(Kancheva et al., 2016). After excluding all potential disease-causing variants in known CMT genes, the
novel homozygous variants within the homozygous regions were selected for further analysis.

**Cell culture and western blotting**

Skin biopsies were obtained from patients and control subjects and fibroblast cultures established. For
detection of GANP protein, after PBS wash, cells were lysed in RIPA buffer on ice for 5 min (#9806,
Cell Signaling), scraped and centrifuged for 10 min at 14,000 x g at +4 °C. Protein concentrations were
measured using the Bradford assay. Then 40 µg of protein was subjected to SDS-PAGE on a 10%
polyacrylamide gel (Bio Rad) at 150 V for 60 min. Membrane transfer was performed with Turbo Blot
(Bio Rad) on a Trans Blot Turbo Transfer Pack (#17-4159, Bio Rad). For blocking the membrane 5 %
milk/TBST was used for 1 hour at RT, followed by incubation with primary antibodies overnight at +4
° C. On the following day, the membrane was washed with TBST and incubated with secondary antibody
for 2 hours at RT. After washing with TBST, Luminol Detection Reagent (#18-59694, Thermo Fisher)
was used to detect the proteins for imaging with a Chemidoc XRS+ Molecular Imager (Bio-Rad). Anti-
sheep GANP antibody, contributed by Dr Vihandha Wickramasinghe, University of Cambridge, was
used at a concentration 1:1000 with secondary antibody donkey-anti-sheep (#A-3415, Sigma). MG132
proteasome inhibitor (Calbiochem, # 474791) was added to fibroblasts for 8 hours at concentration of 10
mM as described previously (Ylikallio et al., 2013).

cDNA synthesis and sequencing
Total RNA was isolated from cultured fibroblast cells using Trizol Reagent (#15596, Ambion, Life
Technologies). In brief, cells were washed with PBS twice, homogenizing from semi-confluent plates,
then centrifuged in 12,000 x g for 10 min at +4 ⁰C. Homogenate was incubated at RT for 10-15 min,
followed by separation with 200 µl of chloroform, then incubated in RT for 3 min and centrifuged at
12,000 x g for 15 min at +4 ⁰C. RNA was precipitated with 500 µl of isopropyl alcohol and incubated at
RT for 10 min. The sample was then further centrifuged at 12,000 x g for 10 min at +4 ⁰C. Pelleted
purified RNA was washed with 1 ml of 75% ethanol and centrifuged at 7,500 x g for 5 min at +4 ⁰C.
cDNA was synthesized with DyNAmo™ cDNA Synthesis Kit (Finnzymes, Thermo Fisher). RNA was
first denatured and cDNA synthesized with random hexamers for 5 min at 65 ⁰C and M-MuLV RNase
H+ reverse transcriptase (cDNA synthesis kit, Thermo Fisher) using cycles of 25 ⁰C for 10 min, then 37
⁰C for 60 min and 85 ⁰C 5 min. PCR reaction for sequencing was done with Phusion DNA Polymerase
(Finnzymes, Thermo Fisher) with 3.1 buffer and Big Dye. For sequencing of genomic DNA and cDNA,
the following primers were used: exons 9 and 10, primers 5-TGCTCAGTCTCAACAAGGGA-3
(forward) and 5-CTGGTTCCAGGAATGCAGAC-3 (reverse); exons 1 and 2, primers 5-
TGGACTTGAGCACACTTCC-3 (forward) 5-GTTACTCTGACGCGCCGG-3 (reverse); exons 16 to
18, primers 5-CGTTGCTGTGAGGATGTCTG-3 (forward) and 5-GTTTCTGAGCCGCGCTTAACC-3
(reverse). CHX treatment of 20 mg/ml (#C7698, Sigma) for 24 hours in DMEM cell culture media with serum was used to inhibit nonsense-mediated decay.

**Measurement of mRNA expression by qPCR**

cDNA synthesis of isolated RNA as described above, was done using Maxima Kit (#K1641, Thermo Scientific). 1 µg of total RNA was mixed with 5X reaction mix, 2 µl of Maxima Enzyme Mix and nuclease-free water up to 20 µl of total volume. Sample was mixed and vortexed. The sample was then incubated for 10 min at 25°C followed by 15 min at 50°C. Finally, reaction was terminated by incubating for 5 min at 85°C. Quantitative PCR (qPCR) was done using DyNAmo Flash SYBR Green qPCR Kit (#F-415L, Thermo Scientific). GAPDH was used as a control gene for normalization with primers 5-cgctctgtctctctctgtt-3 and 5- ccatggtctgagcgctgt-3. Results in manuscript file are shown for biological triplicates.

**Mixed Neuronal Cultures and Immunocytochemistry**

Mixed neuronal populations were differentiated from human iPSC. iPSCs reprogrammed of fibroblasts derived from a healthy control subject (Trokovic et al., 2013) were cultured in Essential 8 basal medium (# A1516901, Life Technology) with Essential 8 Supplement (50X, #1517101, Life Technology) on Matrigel matrix (#356231, BD) plates. Differentiation into neurons was done on Poly-D-lysine and Laminin coated plates using an established differentiation protocol with modifications (Hamilainen et al., 2013). In summary, iPSCs were dissociated with 0.5 mM EDTA (#15575, Life Technology) in PBS, transferred to low-attachment 6-well plates (Corning® 3471 Costar®) for suspension culture to initiate neurospheres. The medium contained DMEM/12, Neurobasal media, GlutaMax, B27 supplement (50X), N2 supplement (100X), and bFGF (40 µg/ml). After 7-9 weeks culturing, the neurospheres were plated
into monolayer by dissociating with TrypLE (#12605036, Thermo Fisher) on Poly-D-lysine (A-003-E, Merke Millipore) and Laminin (L2020, Sigma) coated plates. Differentiated neurons were cultured for a week in medium containing DMEM/12, Neurobasal media, GlutaMax, B27 supplement, and N2 supplement, and neuronal identity was confirmed with immunostaining using neuronal marker TUJ1 (#801201, Biosite, 1:500) with 1:1000 of chicken-anti-mouse IgG secondary antibody (#A21201, Invitrogen). GANP antibody was used at a concentration of 1:200 (#ab198173, Abcam) with 1:1000 of goat-anti-rabbit IgG secondary antibody (#A11008, Invitrogen). For immunocytochemistry the cells were first washed with PBS four times, then fixed with 4% paraformaldehyde for 15 min at RT, rinsed in PBS four times, incubated in 0.2% Triton in PBS for 15 min for permeabilization and finally rinsed in PBST (0.1% Tween-20 in PBS) four times. Cells were blocked for 2 hr at RT with 5% BSA/PBST and incubated in primary antibody overnight at +4°C. The cells were then washed with PBST for 15 min four times, then incubated in secondary antibody for 1 hr at RT. After repeating washing with PBST four times for 10 min, the cells were mounted with Vectashield with DAPI (#H-1200, Vector Laboratories). Fluorescence imaging was done with Zeiss Axio Observer microscope (Zeiss, Germany) with a 63X oil immersion objective and Apotome.2 with excitation/emission wavelengths (nm): blue (DAPI), 350/461; green (GFP), 498/516; and red (DsRed), Images were saved with Zeiss ZEN Pro software (Zeiss, Germany).

DNA damage

The following skin fibroblasts were used in the DNA damage experiments: patients F:II-3 and F:II-4, three unrelated normal fibroblast lines, fibroblasts derived from a Xeroderma Pigmentosum group C (XPC) patient (Coriell Institute Cell repository, GM16684) and fibroblasts derived from a DNA ligase 4 syndrome (LIG4) patient (Coriell Institute Cell repository, GM16088). To induce DNA damage,
fibroblasts were cultured in a 96-well plate and exposed to 10 Gy of ionizing radiation (IR) or to 10 J/m$^2$ of ultra-violet (UV) radiation. Non-irradiated cells served as baseline reference. Irradiated cells were allowed to recover for 4, 8 and 24 hours, prior to fixation with 4% paraformaldehyde. Primary antibodies against γ-H2Ax (ab22551, Abcam), Ki67 (ab15580, Abcam) and cleaved-Caspase 3 (#9661, Cell Signaling Technologies) were used to assess DNA damage, cellular proliferation and apoptosis, respectively, by immunocytochemistry. Cells were then incubated with fluorescently labeled secondary antibodies (donkey anti-mouse IgG-AlexaFluor 488, A21202, and donkey anti-rabbit IgG-AlexaFluor 647, A11057, Life Technologies), and nuclei were counterstained with DAPI. Images were acquired on a CellInsight high-content imaging system (Thermo Scientific) and analyzed and quantified with the Cellomics software (Thermo Scientific). Results are shown for duplicates.
SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure 1. DNA damage repair in patient and control fibroblasts.

Fibroblasts from affected individuals in Family F were exposed to ionizing radiation (IR) and to ultraviolet light (UV) for assessing the proficiency of DNA damage repair 4, 8 and 24 hours after the insult. Shown are the percentages for cell survival, γH2AX-positivity, Ki67 positivity, and cleaved-caspase 3 in cell lines from three healthy controls, patients F:II-3 and F:II-4, and positive controls [a patient with LIG4 (DNA ligase 4) syndrome as positive control for IR sensitivity, and a patient with Xeroderma Pigmentosum type C for UV sensitivity]. As expected, LIG4 cells were sensitive to IR and Xeroderma Pigmentosum type C for UV. No differences were seen in patient cells compared to healthy controls.
Supplementary Figure 2. pSPL3 minigene vector map and RT-PCR primers

Map of the pSPL3-vector (6031 bp) used for the minigene splicing assay. MCM3AP exon 5 (191 bp) and flanking intronic sequences were cloned into the multiple cloning site (MCS) of pSPL3 with restriction enzymes XhoI and NheI. The plasmid contained 3’ and 5’ coding exons SD and SA. Primers used for RT-PCR were SD6-F (5’-TCTGAGTCACCTGGACAACC-3’) (amplifying 92 bp of SD) and SA2-R (5’-ATCTCAGTGGTATTGTGAGC-3’) (amplifying 171 bp of SA). In total, the correctly spliced product including MCM3AP exon 5 was 454 bp, whereas the exon 5 skipping resulted in a product of 263 bp.
### Supplementary Table 1. Extended clinical data. The table summarizes the affected individuals’ ages of onset, distribution of muscle weakness, sensory involvement and cognitive function testing.

<table>
<thead>
<tr>
<th>Patient</th>
<th>AAO</th>
<th>Proximal muscle weakness (MRC)</th>
<th>Distal muscle weakness (MRC)</th>
<th>Sensory involvement</th>
<th>Cognitive function</th>
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<tbody>
<tr>
<td>F:II-3</td>
<td>4 years</td>
<td>Not specified</td>
<td>Severe (no grading available)</td>
<td>Decreased SNAP</td>
<td>Learning difficulty, no ID</td>
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<tr>
<td>F:II-4</td>
<td>2.5 years</td>
<td>Not specified</td>
<td>Severe (no grading available)</td>
<td>Distal hyperesthesia</td>
<td>Learning difficulty, no ID</td>
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<tr>
<td>F:II-5</td>
<td>1 year</td>
<td>-</td>
<td>Yes (no grading available)</td>
<td>Decreased SNAP</td>
<td>Mild ID (WPPSI III)</td>
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<tr>
<td>A:II-2</td>
<td>0.5 years</td>
<td>3/5 (UL) 3/5 (LL)</td>
<td>4/5 (UL) 4/5 (LL)</td>
<td>Significant distal sensory impairment</td>
<td>IQ 70-75</td>
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<td>N:II-1</td>
<td>&lt;3 years</td>
<td>Antigravity movement possible</td>
<td>Severe (MRC grading not possible)</td>
<td>Radial SNAP not reproducible</td>
<td>Impaired (BSID-II NL)</td>
</tr>
<tr>
<td>C:II-1</td>
<td>1 year</td>
<td>4/5 (UL) 4/5 (LL)</td>
<td>1/5 (UL) 1/5 (LL)</td>
<td>Unable to test due to cooperation</td>
<td>Intellectual Disability (mild)</td>
</tr>
<tr>
<td>C:II-2</td>
<td>2 years</td>
<td>4/5 (UL) 4/5 (LL)</td>
<td>1/5 (UL) 1/5 (LL)</td>
<td>Unable to test due to cooperation</td>
<td>Intellectual Disability (mild)</td>
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<tr>
<td>T:II-1</td>
<td>2-10 years</td>
<td>4/5 (UL) 0-1/5 (UL)</td>
<td>Decreased SNAP, distal hypoalgesia</td>
<td>Impaired (IQ not available)</td>
<td></td>
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<tr>
<td>T:II-2</td>
<td>&lt;7 years</td>
<td>5/5 (UL) 4/5 (LL)</td>
<td>4/5 (UL) 3/5 (LL)</td>
<td>Decreased SNAP, distal hypoalgesia</td>
<td>Impaired (IQ not available)</td>
</tr>
</tbody>
</table>

Supplementary Table 2. Summary of evidence for pathogenicity of MCM3AP variants.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>cDNA change</th>
<th>Protein change level</th>
<th>Protein change level</th>
<th>CADD</th>
<th>ExAC</th>
<th>Proposed pathogenic mechanism</th>
<th>Evidence in support of proposed pathogenic mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>F:II-3</td>
<td>c.3814G&gt;A</td>
<td>p. (Val1272Met)</td>
<td>10.54</td>
<td>3x10^-4</td>
<td>Protein instability</td>
<td>Low residual GANP protein in western blot</td>
<td></td>
</tr>
<tr>
<td>F:II-4</td>
<td>c.443delC</td>
<td>p. (Pro148fs*48)</td>
<td>-</td>
<td>0</td>
<td>Protein instability</td>
<td>Low residual GANP protein in western blot</td>
<td></td>
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<tr>
<td>F:II-5</td>
<td></td>
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<tr>
<td>A:II-2</td>
<td>c.2600C&gt;A</td>
<td>p. (Ala867Asp)</td>
<td>28.1</td>
<td>0</td>
<td>Potential effect on mRNA export or other function of GANP</td>
<td>Localisation to functionally important Sac3 domain, rare variant, high CADD-score</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.2667C&gt;A</td>
<td>p. (Tyr889*)</td>
<td>-</td>
<td>0</td>
<td>NMD</td>
<td>mRNA degradation confirmed by cDNA sequencing</td>
<td></td>
</tr>
<tr>
<td>N:II-2</td>
<td>c.1857A&gt;G</td>
<td>p. (Gln619=)</td>
<td>-</td>
<td>0</td>
<td>Splice abnormality leading to frameshift</td>
<td>Exon skipping demonstrated by Minigene splicing assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.3949_3950insG</td>
<td>p. (Asn1317fs*18)</td>
<td>-</td>
<td>0</td>
<td>NMD likely</td>
<td>Nonsense variant in exon 18/28</td>
<td></td>
</tr>
<tr>
<td>C:II-1</td>
<td>c.4433C&gt;A</td>
<td>p. (Ser1478*)</td>
<td>-</td>
<td>0</td>
<td>NMD likely</td>
<td>Nonsense variant in exon 21/28</td>
<td></td>
</tr>
<tr>
<td>C:II-2</td>
<td>c.4729G&gt;A</td>
<td>p. (Glu1577Lys)</td>
<td>24.4</td>
<td>1.653x10^-5</td>
<td>Potential effect on mRNA export or other function of GANP</td>
<td>Changes a conserved amino acid, rare variant, high CADD-score</td>
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<tr>
<td>T:II-1</td>
<td>c.2285T&gt;C</td>
<td>p. (Met762Thr)</td>
<td>25.4</td>
<td>0</td>
<td>Potential effect on mRNA export or other function of GANP</td>
<td>Localisation to functionally important Sac3 domain, rare variant, high CADD-score</td>
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<tr>
<td>T:II-2</td>
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</table>

Mutation nomenclature is based on HGVS guidelines and use of transcript NM_003906.4. CADD, combined annotation dependent depletion; NMD, nonsense mediated mRNA decay. CADD scores are only shown for missense variants.
**Supplementary Table 3.** Oligonucleotide primers used for sequencing of *MCM3AP*. gDNA, genomic DNA; cDNA, complementary DNA; qPCR, quantitative polymerase chain reaction.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Direction</th>
<th>Sequence</th>
<th>Use</th>
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<tbody>
<tr>
<td>1</td>
<td>Forward</td>
<td>5’-TGGGAAGAGGCTCGGGATTTT-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>1</td>
<td>Reverse</td>
<td>5’-AAGCAGGGGTAAAGGCAGAT-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>8</td>
<td>Forward</td>
<td>5’-GAAATTTGAGGTCGTCATGATGAC-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>8</td>
<td>Reverse</td>
<td>5’-CTACTGCCACTCTCTGAAAGC-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>17</td>
<td>Forward</td>
<td>5’-CTTCCCCATGCTGAGTTCT-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>17</td>
<td>Reverse</td>
<td>5’-AAAATGCAACCCTGTGCCTG-3’</td>
<td>gDNA sequencing</td>
</tr>
<tr>
<td>1-2</td>
<td>Forward</td>
<td>5’-TGGGACTTGAGAGGACACTTCC-3’</td>
<td>cDNA sequencing</td>
</tr>
<tr>
<td>1-2</td>
<td>Reverse</td>
<td>5’-GTTACTCTGACGCGCGGG-3’</td>
<td>cDNA sequencing</td>
</tr>
<tr>
<td>16-18</td>
<td>Forward</td>
<td>5’-CGTTGCGTGTGAGGATGTCTG-3’</td>
<td>cDNA sequencing</td>
</tr>
<tr>
<td>16-18</td>
<td>Reverse</td>
<td>5’-GTTTCTGAGCCCGCTTAACC-3’</td>
<td>cDNA sequencing</td>
</tr>
<tr>
<td>9-10</td>
<td>Forward</td>
<td>5’-TGCTCAGTCTCAACAAGGGA-3’</td>
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</tr>
<tr>
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<td>5’-GATCAGAGGCCTGAGTGAA-3’</td>
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<td>gDNA sequencing</td>
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<td>23</td>
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</table>
ORIGINAL BLOTS

GANP 208 kDa

GAPDH 37kDa


pSPL3 minigene assay electrophoresis

1 kb ladder Gene Ruler DNA ladder (#SM0311, Thermo Scientific).
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