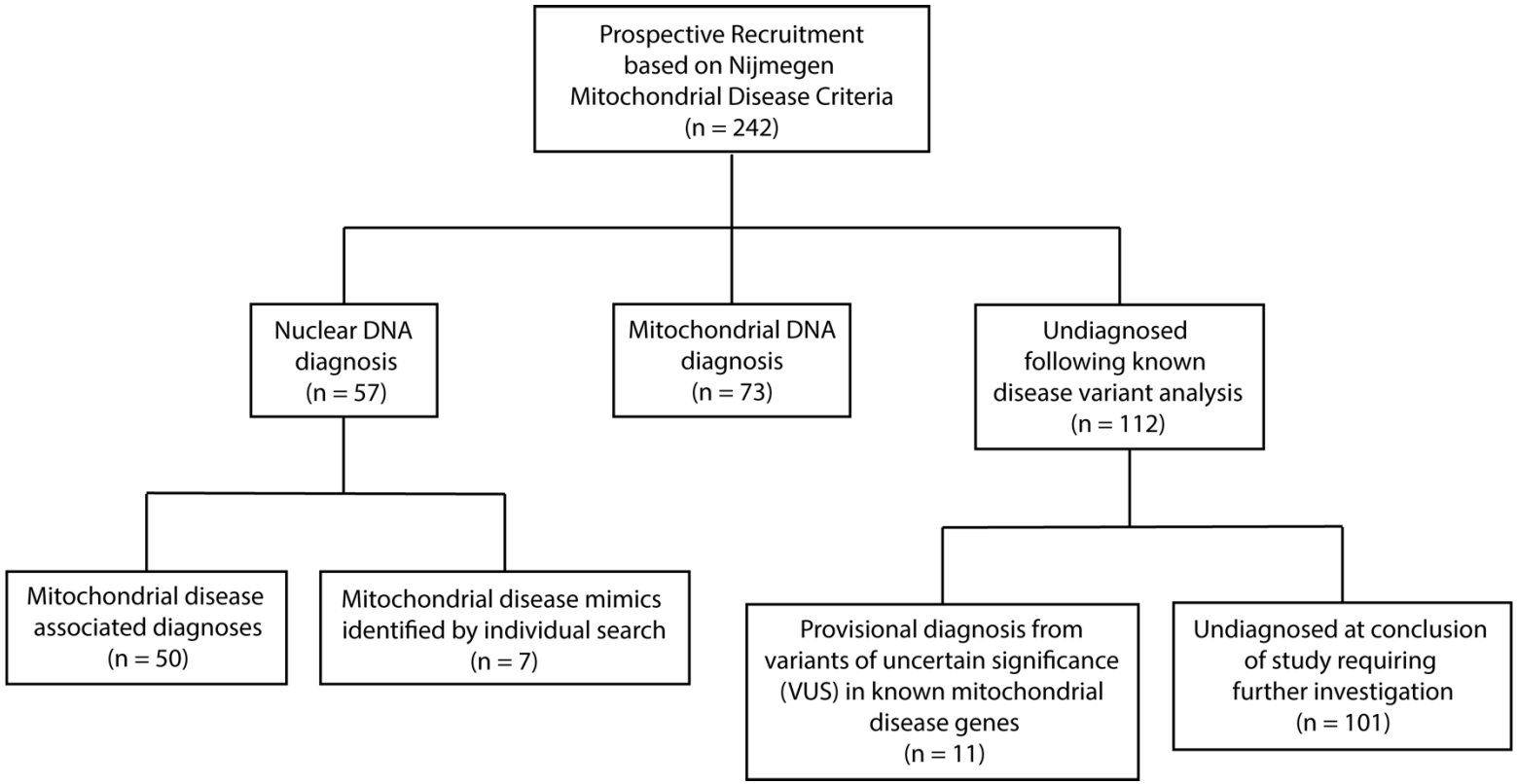
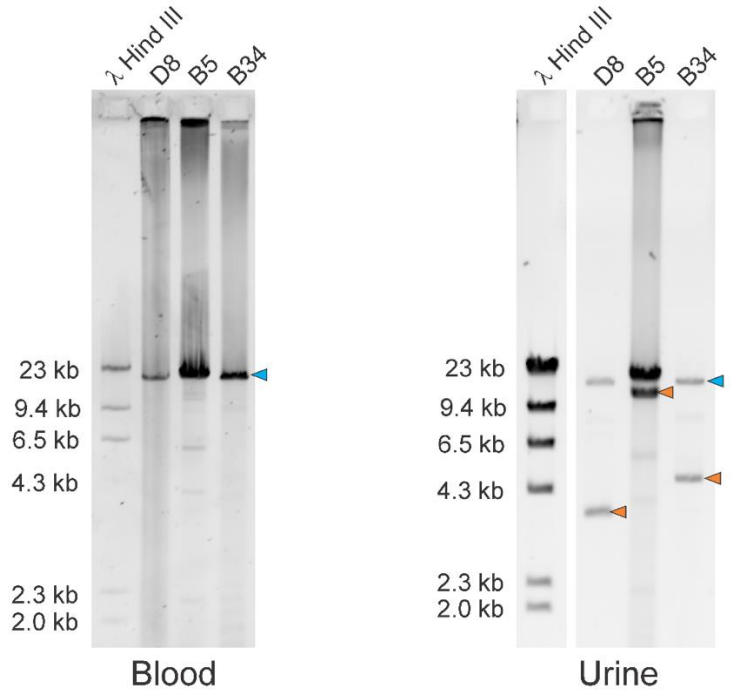
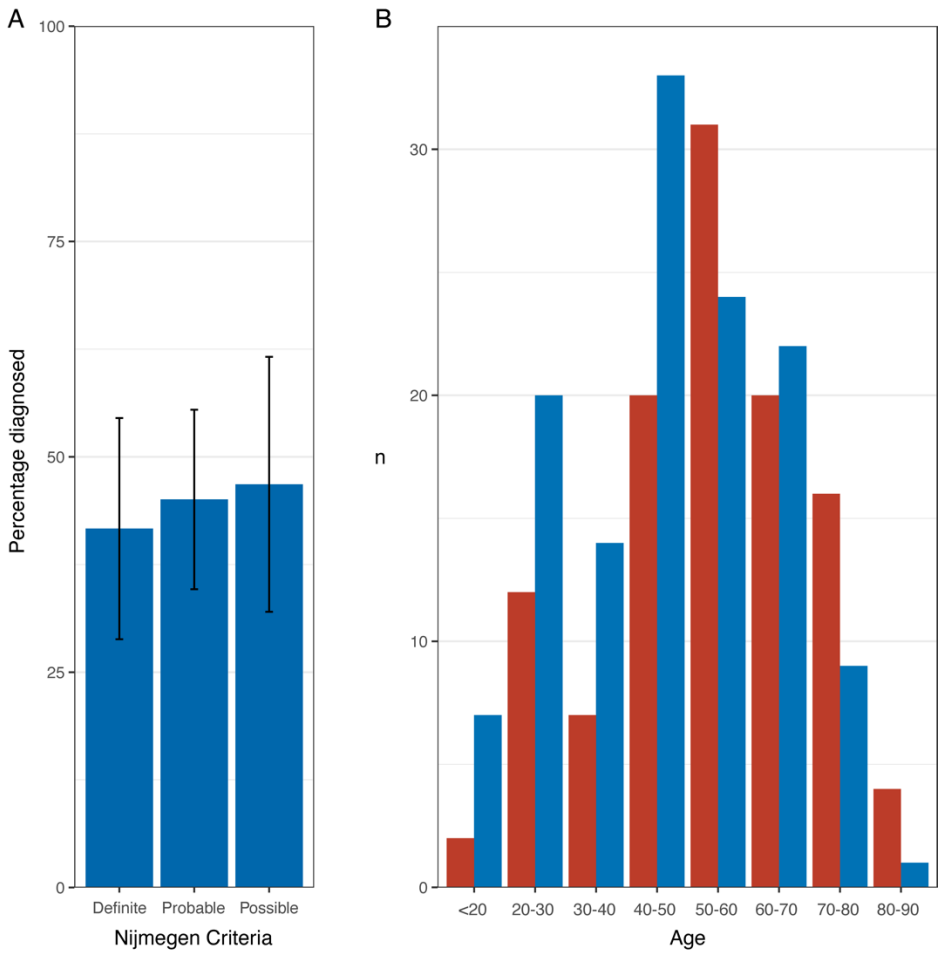
SUPPLEMENTARY FIGURES



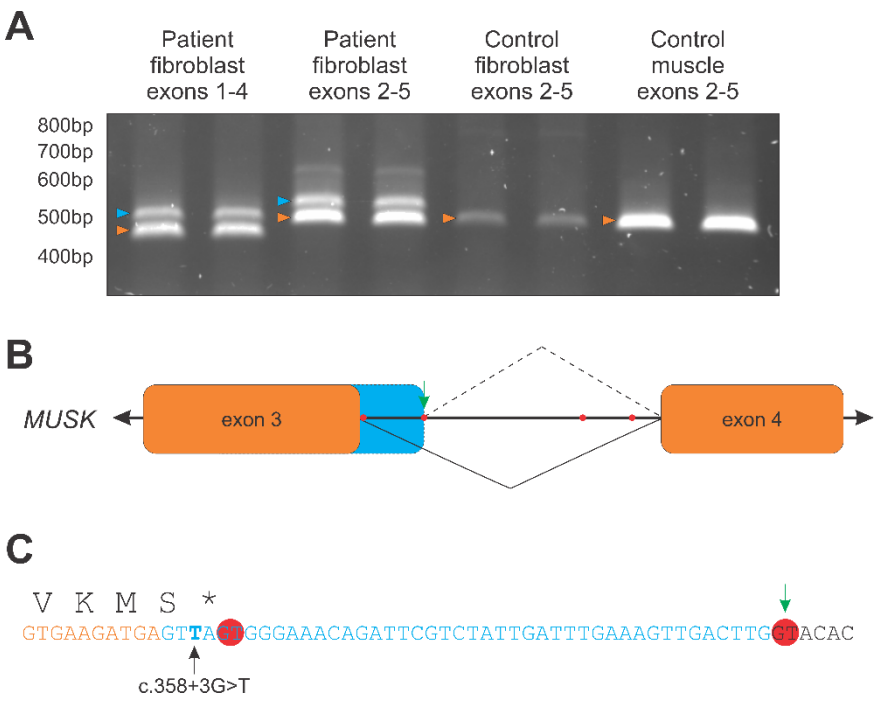
eFigure 1: STARD diagram of participants. 242 participants were recruited to the study. Following initial analysis for variants known to cause mitochondrial disease and individual searches for mitochondrial disease mimics, 57 patients were found to have nuclear DNA variants and 73 patients were found to have mitochondrial DNA variants that could explain their disease. Of the 57 patients with nuclear DNA variants, 7 had variants in genes not associated with mitochondrial disease, but with phenotypes that mimic mitochondrial disease. Of the 112 participants remaining without a diagnosis, 11 were found to have variants of uncertain significance in genes known to cause mitochondrial disease that required functional genetic confirmation and 101 participants had no candidate genetic diagnosis and required further investigation.



eFigure 2: Long-range polymerase chain reaction amplification of single deletions in mitochondrial DNA from blood versus urine. A single amplicon amplification of mitochondrial DNA from blood showed no single deletions in three chronic progressive external ophthalmoplegia (CPEO) patients (left panel; blue arrow indicates full-length mitochondrial DNA at 16.5kb). D8 and B5 had nuclear mutations identified by whole genome sequencing (WGS) associated with CPEO but no mitochondrial DNA deletion in blood. Whereas B34 had a single deletion in mitochondrial DNA associated with chronic progressive external ophthalmoplegia that was not detected by WGS in blood. When comparing to urine (right panel), single deletions were observed for all three patients (orange arrows; blue arrow indicates full-length mitochondrial DNA at 16.5kb), confirming the absence of deletions in blood not the inability of WGS to detect deletions in blood.



eFigure 3: Clinical classification of mitochondrial disease criteria and number of molecular diagnoses. **(A)** The percentage of patients diagnosed by whole genome sequencing for each of the Nijmegen criteria, showing that a diagnosis by WGS is not determined by Nijmegen criteria classification (error bars = 95% confidence intervals). **(B)** The number of patients diagnosed by WGS compared to patient age at presentation. Patients <50 years old were more likely to obtain a diagnosis by WGS compared to those >50 years old. Blue denotes diagnosed by WGS, red denotes undiagnosed after WGS.



eFigure 4: Altered splicing in congenital myasthenic patient-derived fibroblasts with a homozygous c.358+3G>T variant. (A) Polymerase chain reaction amplification of *MUSK* exons 1 to 4 and exons 2 to 5 of cDNA from cycloheximide-treated patient fibroblasts showed wild type (orange arrowhead) and variant transcripts (blue arrowhead) for the patient only. **(B)** Sequencing of cDNA confirmed normal splicing out of intron 3 from wild type transcripts (solid line) with intronic read-through of 44 base pairs (blue exon 3 extension) to an alternate donor splice site (red dot and green arrow) before splicing out of the remaining intron 3 in the variant transcripts (dotted line). Possible alternate donor splice sites are shown as red dots along the intron. **(C)** The *MUSK* c.358+3G>T variant abolishes the existing splice site (transition from orange to blue text), causing read-through into the intron (blue nucleotide sequence) for 44 base pairs to an alternate donor splice site (red dot and green arrow). The intronic read-through would result in a coding frameshift, introduction of a serine residue, before premature termination of translation at a stop codon (*) created by the c.358+3T variant.

SUPPLEMENTARY TABLES

eTable 1: Positive controls used to assess the capability of WGS to identify known variants in nDNA and mtDNA extracted from blood or autopsy samples

Sample #	Age	Sex	Gene	Mode	Nucleotide and amino acid change
1	26	F	*POLG*	AR	c.2551A>G p.T851A
				AR	c.487C>T; p.P163S
2	61	F	*POLG*	AR	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile
3	65	M	*POLG*	AR	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile
4	49	F	*POLG*	AR	c.2551A>G; p.Thr851Ala
				AR	c.1402A>G; p.Asn468Asp
5	40	F	*POLG*	AR	c.1399G>A; p.Ala467Thr
6	54	F	*POLG*	AR	c.1684C>T; p.Arg562Trp
				AR	c.1399G>A; p.Ala467Thr
7	26	F	*POLG*	AR	c.2243G>C; p.Trp748Ser
				AR	c.1399G>A; p.Ala467Thr
8	43	M	*TYMP*	AR	c.1228_1247delGACGGCCCCGCGCTCAGCGG; p.Asp410Profs?
9	30	M	*TYMP*	AR	c.1228_1247delGACGGCCCCGCGCTCAGCGG; p.Asp410Profs?
10	24	F	*TYMP*	AR	c.931G>T; p.Gly311Cys
				AR	c.1311delG; p.Trp437Cysfs?
11	31	F	*WFS1*	AR	c.1243_1245delGTC; p.Val415del
				AR	c.2100G>T, p.Trp700Cys
12	25	F	*YARS2*	AR	c.156C>G; p.Phe52Leu
13	39	F	*TWNK*	AD	c.1106C>A; p.Ser369Tyr
14	62	F	*TWNK*	AD	c.1106C>A; p.Ser369Tyr
15	23	F	*OPA1*	AD	c.1334G>A; p.Arg445His
16	54	F	*OPA1*	AD	c.1334G>A; p.Arg445His
17	74	F	*OPA1*	AD	c.1313A>G; p.Asp438Gly
18	44	F	*OPA1*	AD	c.1313A>G; p.Asp438Gly
19	14	M	*OPA1*	AD	c.655C>T; p.Gln219Ter
20	53	F	*OPA1*	AD	c.655C>T; p.Gln219Ter
21	40	M	*OPA1*	AD	c.1325A>G; p.Asp442Gly

22	61	F	*OPA1*	AD	c.1325A>G; p.Asp442Gly
23	67	M	*OPA1*	AD	c.888T>A; p.Asp296Glu
24	72	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
25	70	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
26	41	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
27	42	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
28	41	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
29	39	M	*OPA1*	AD	c.888T>A; p.Asp296Glu
30	69	F	*OPA1*	AD	c.888T>A; p.Asp296Glu
31	64	M	*MT-RNR1*	Mat	m.1555A>G
32	47	F	*MT-TL1*	Mat	m.3243A>G
33	16	M	*MT-TL1*	Mat	m.3243A>G
34	12	F	*MT-TL1*	Mat	m.3243A>G
35	51	M	*MT-TL1*	Mat	m.3243A>G
36	38	F	*MT-TL1*	Mat	m.3243A>G
37	53	F	*MT-TL1*	Mat	m.3243A>G
38	45	M	*MT-TL1*	Mat	m.3243A>G
39	57	F	*MT-TL1*	Mat	m.3302A>G
40	23	F	*MT-ND1*	Mat	m.3460G>A; p.Ala52Thr
41	20	F	*MT-TK*	Mat	m.8344A>G
42	27	M	Single deletion [M]	Spo	4.8kb single deletion

Sample # = Positive control sample identification number for this study; F = Female; M = Male; Mode = Mode of inheritance; AD = Autosomal Dominant; AR = Autosomal Recessive; Mat = Maternal; Spo = Sporadic. ter? = downstream termination site not able to be predicted. [M]All samples were blood DNA except for Sample #42, which was from muscle obtained at autopsy.

eTable 2: Curated Mitochondrial Disease Nuclear Gene Panel Generated from Literature Review

Autosomal Dominant	Autosomal Recessive	X-Linked
AARS2;AFG3L2;ATAD3A; CHCHD10;CYCS;DNA2;DNM1L; HSPD1;MFN2;OPA1;OPA3; POLG;POLG2;RRM2B;SDHAF2; SDHB;SDHC;SDHD;SLC25A4; STXBP1;TWNK	*AARS2;ABAT;ACAD9;ACSM2A;ACSM5;ADCK3;AFG3L2;AGK;ALDH1L1;ANO10;APOA1BP;APOPT1;APTX;ARHGEF5;ATAD3A;ATP5A1;ATP5E;ATPAF2;BCKDHB;BCS1L;BOLA3; C12orf65;CARS2;CEP89;CISD2;CLPB;CLPP;COA3;COA5;COA6;COA7;COQ2;COQ4;COQ6;COQ9;COX4I2;COX6A1;COX6B1;COX8A;COX10;COX14;COX15;COX20;CYC1;DARS2; DGUOK;DLAT;DLD;DNAJC19;DNM1L;EARS2;ECHS1;ELAC2;ERCC6L2;ETFA;ETFDH;ETHE1;FAAH2;FARS2;FASN;FASTKD2;FBXL4;FDX1L;FH;FLAD1;FNDC1;FOXRED1;FXN; GARS;GFER;GFM1;GFM2;GLRX5;GPT2;GTPBP3;HARS2;HERC2;HIBCH;HKDC1;HSPD1;HTRA2;IARS2;IBA57;IREB2;ISCA2;ISCU;KARS;LARS2;LIAS;LIPT1;LONP1;LRPPRC; LYRM4;LYRM7;MAGI1;MARS2;MECR;MEF2A;METAP1D;MFF;MFN2;MGME1;MICU1;MIPEP;MPC1;MPV17;MRPL3;MRPL12;MRPL44;MRPS7;MRPS16;MRPS22;MRPS23;MTFMT; MTO1;MTPAP;NARS2;NAXE;NDRG3;NDUFA2;NDUFA4;NDUFA9;NDUFA10;NDUFA11;NDUFA12;NDUFA13;NDUFAF1;NDUFAF2;NDUFAF3;NDUFAF4;NDUFAF5;NDUFAF6; NDUFB3;NDUFB8;NDUFB9;NDUFS1;NDUFS2;NDUFS3;NDUFS4;NDUFS6;NDUFS7;NDUFS8;NDUFV1;NDUFV2;NFS1;NFU1;NUBPL;OPA1;OPA3;PARS2;PC;PDHB;PDHX;PDP1; PDPR;PDSS1;PDSS2;PERP;PET100;PMPCA;PNPLA8;PNPT1;POLG;POLRMT;PPL;PTCD1;PUS1;QRSL1;RARS2;RMND1;RMRP;RNASEH1;RRM2B;RTN4IP1;SARS2;SCO1;SCO2; SDHA;SDHAF1;SDHD;SERAC1;SFXN4;SIGMAR1;SLC5A10;SLC19A3;SLC25A3;SLC25A4;SLC25A12;SLC25A19;SLC25A26;SLC25A42;SLC25A43;SLC25A46;SMCR7;SPG7; STARD13;STAT2;SUCLA2;SUCLG1;SURF1;TACO1;TAF9;TANGO2;TARS2;TFAM;TIMM50;TK2;TMEM70;TMEM126B;TPK1;TPX2;TRIT1;TRMT10C;TRMT5;TRMU;TRNT1;TSFM; TTC19;TUFM;TWNK;TXN2;TYMP;UQCC2;UQCC3;UQCRB;UQCRC2;UQCRQ;VARS2;YARS2;YME1L1*	*ABCB7;AIFM1;COX7B;HCCS; HSD17B10;NDUFA1;NDUFB11; PDHA1;PDK3;PNPLA4;TAZ; TIMM8A*

eTable 3: Demographic, Clinical and Genetic Information for Subjects with Disease-relevant Variants or Variants of Uncertain Significance in the Nuclear Genome

ID	Age	Sex	Family History	Clinical features	Nijmegen rating	Muscle biopsy	Changes on muscle biopsy	Gene	Mode	Reference sequence	Genomic change (GRCh37)	Nucleotide and amino acid change	Zygosity	Variant previously reported [e ref]	ACMG
Disease Relevant Variants															
B45	47	F	Y	Stroke-like episodes, migraines, generalized tonic-clonic seizures	Probable	N	N/A	*AMACR*	AR	NM_014324.5; NP_055139.4	chr5:g.34007971A>G	c.154T>C; p.Ser52Pro	Hom	Y [e 1]	Pathogenic, PS1, PS3, PP3
E19	46	F	Y	Stroke-like episodes, migraines, seizures, encephalopathy, myoclonic jerks, retinitis pigmentosa, myopathy	Probable	N	N/A	*AMACR*	AR	NM_014324.5; NP_055139.4	chr5:g.34007971A>G	c.154T>C; p.Ser52Pro	Hom	Y [e 1]	Pathogenic, PS1, PS3, PP3
E28	47	F	N	Stroke-like episodes, migraines, seizures, muscle weakness, retinitis pigmentosa	Probable	Y	N	*AMACR*	AR	NM_014324.5; NP_055139.4	chr5:g.34007971A>G	c.154T>C; p.Ser52Pro	Het	Y [e 1]	Pathogenic, PS1, PS3, PP3
											chr5:g.34005988GAGTTTCTCC>G	c.255_263delGGAGAAACT; p.Met85_Leu88delinsIle	Het	N	Likely pathogenic, PS3, PM2, PM4
F3	61	M	N	Myopathy, fasciculations, lipomatosis, mild axonal neuropathy on nerve biopsy, dystrophic changes with rimmed vacuoles on muscle biopsy	Possible	Y	N	*MFN2*	AR	NM_014874.3, NP_055689.1	chr1:g.12069698C>T	c.2119C>T; p.Arg707Trp	Hom	Y [e 2]	Likely pathogenic, PS1, PP2, PP3
B44	48	M	N	Myopathy, axonal sensorimotor neuropathy, lipodystrophy around the neck, scoliosis	Possible	N	N/A	*MFN2*	AR	NM_014874.3, NP_055689.1	chr1:g.12057436C>CT	c.558dupT, p.Lys187Ter	Het	N	Pathogenic, PVS1, PM1, PM2, PP3
											chr1:g.12069698C>T	c.2119C>T; p.Arg707Trp	Het	Y [e 2]	Likely pathogenic, PS1, PP2, PP3
B42	57	F	Y	CPEO, proximal muscle weakness, bulbar involvement	Possible	N	N/A	*MUSK* ‡	AR	NC_000009.12 (NM_005592.3), NP_005583.1	chr9:g.113449551G>T	c.358+3G>T	Hom	N	Likely pathogenic, PS3, PM2, PP3
B43	72	M	Y	CPEO, proximal muscle weakness, bulbar involvement	Possible	Y	Y	*MUSK* ‡	AR	NC_000009.12 (NM_005592.3), NP_005583.1	chr9:g.113449551G>T	c.358+3G>T	Hom	N	Likely pathogenic, PS3, PM2, PP3
B21	61	F	N	CPEO, sensory neuropathy, migraines, neuropathy, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89868870G>A: in cis g.89873415G>A	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile	Hom	Y [e 3, 4]	Likely pathogenic, PS1, PP3, PP5
E8	65	M	N	CPEO, proximal muscle weakness, dysphagia, neuropathy, diabetes, hearing loss	Probable	N	N/A	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89868870G>A: in cis g.89873415G>A	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile	Hom	Y [e 3, 4]	Likely pathogenic, PS1, PP3, PP5

E81	70	M	N	CPEO, myopathy, diabetes, neuropathy, elevated lactate on MRS, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89868870G>A: in cis g.89873415G>A	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile	Hom	Y [e 3, 4]	Likely pathogenic, PS1, PP3, PP5
B28	60	F	N	CPEO, proximal myopathy, diabetes, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89870432C>T	c.1399G>A; p.Ala467Thr	Het	Y [e 5]	Likely pathogenic, PS1, PM3, PP3
											chr15:g.89872174C>T	c.1023G>A (splice region)	Het	N	Likely pathogenic, PS3, PM2, PP3
B46	69	F	N	CPEO, hearing impairment, proximal myopathy, COX negative fibers on muscle biopsy	Probable	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89868870G>A: in cis g.89873415G>A	c.1760C>T; p.Pro587Leu: in cis c.752C>T; p.Thr251Ile	Het	Y [e 3, 4]	Likely pathogenic, PS1, PP3, PP5
											chr15:g.89866669A>ATGTCCACGTCGTTG	c.2217_2230dupCAACGACGTGGACA; p.Ile744ThrfsTer59	Het	N	Pathogenic, PVS1, PM2, PP5
B51	49	F	N	CPEO, proximal and distal weakness, GI dysmotility, sensorimotor polyneuropathy, COX negative, SDH positive, RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89865014T>C	c.2551A>G; p.Thr851Ala	Het	Y [e 6] and published previously [e 7]	Likely pathogenic, PS1, PM2, PP3
											chr15:g.89870429T>C	c.1402A>G; p.Asn468Asp	Het	Y [e 8] and previously published [e 7]	Likely pathogenic, PS1, PM3, BP4
B59	40	F	N	SANDO	Probable	N	N/A	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89870432C>T	c.1399G>A; p.Ala467Thr	Hom	Y [e 9]	Likely pathogenic, PS1, PM3, PP3
C17	54	M	Y	CPEO, dysphagia, hearing impairment, proximal myopathy, exercise intolerance, left anterior hemiblock, COX negative and RRFs on muscle biopsy	Probable	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89864238T>G	c.2740A>C; p.Thr914Pro	Het	Y [e 10]	Likely pathogenic, PS1, PM3, PP3
											chr15:g.89866637T>G	c.2263A>C; p.Lys755Gln	Het	Reported missense at the same position [e 11]	VUS – favor pathogenic, PM2, PM3, PP3
C27	54	F	N	CPEO, mild sensory axonal neuropathy, encephalopathy, paranoia, hallucinations, GI dysmotility, cognitive impairment, mitochondrial myopathy with COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89869871G>A	c.1684C>T; p.Arg562Trp	Het	N	Likely pathogenic, PM2, PM3, PM5, PP3
											chr15:g.89870432C>T	c.1399G>A; p.Ala467Thr	Het	Y [e 9]	Likely pathogenic, PS1, PM3, PP3
F5	26	F	N	SANDO, seizures, tremor, visual hallucinations, GI dysmotility	Probable	N	N/A	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89866657C>G	c.2243G>C; p.Trp748Ser	Het	Y [e 12]	Likely pathogenic, PS1, PP3, PP5
											chr15:g.89870432C>T	c.1399G>A; p.Ala467Thr	Het	Y [e 9]	Likely pathogenic, PS1, PM3, PP3

E73	59	F	N	CPEO, hearing loss, myopathy	Probable	N	N/A	*POLG*	AR	NM_002693.2, NP_002684.1	chr15:g.89865001A>G	c.2564T>C; p.Val855Ala	Hom	Y [e 13]	Likely pathogenic, PS1, PM2, PP3
C9	53	F	Y	CPEO, spastic paraplegia, dysarthria, ataxia, Oxidative enzymes normal on muscle biopsy	Possible	Y	N	*SPG7*	AR	NC_000016.9(NM_003119.2), NP_003110.1	chr16:g.89623293A>G	c.2182-2A>G	Hom	N	Pathogenic, PVS1, PM2, PP3
E41	60	F	Y	CPEO, spastic paraplegia, cerebellar ataxia, blepharospasm, cervical dystonia	Probable	Y	N	*SPG7*	AR	C_000016.9(NM_003119.2), NP_003110.1	chr16:g.89623293A>G	c.2182-2A>G	Hom	N	Pathogenic, PVS1, PM2, PP3
E75	64	M	Y	CPEO, ataxia, spastic paraplegia, neuropathy, muscle biopsy consistent with a denervation process	Definite	Y	N	*SPG7*	AR	NM_003119.2, NP_003110.1	chr16:g.89595397-89596343del	946 bp deletion	Hom	N	Likely pathogenic, PVS1, PM2
B57	43	M	Y	CPEO, neuropathy, gastric dysmotility, weight loss, myopathy	Possible	N	N/A	*TYMP*	AR	NM_001113755.1, NP_001107227.1	chr22:g.50964300GCCGCTGAGCGCGGGGCCGTC>G	c.1228_1247delGACGGCCCCGCGCTCAGCGG; p.Asp410ProfsTer?	Hom	Y [e 14]	Likely pathogenic, PVS1, PM2
E45	24	F	N	CPEO, neuropathy, gastric dysmotility, weight loss, myopathy, SDH positive and RRFs on muscle biopsy	Probable	Y	Y	*TYMP*	AR	NM_001113755.1, NP_001107227.1	chr22:g.50964903C>A	c.931G>T; p.Gly311Cys	Het	Y [e 15] and reported previously [e 14]	Pathogenic, PS1, PS3, PP3
											chr22:g.50964336GC>G	c.1311delG; p.Trp437Cysfs?	Het	Y [e 29] and previously reported [e 14]	Pathogenic, PVS1, PS1, PP3
E86	30	M	Y	CPEO, neuropathy, myopathy, ataxia, COX negative, SDH positive and RRFs on muscle biopsy	Definite	Y	Y	*TYMP*	AR	NM_001113755.1, NP_001107227.1	chr22:g.50964300GCCGCTGAGCGCGGGGCCGTC>G	c.1228_1247delGACGGCCCCGCGCTCAGCGG; p.Asp410ProfsTer?	Hom	Y [e 14]	Likely pathogenic, PVS1, PM2
E55	31	F	N	Wolfram syndrome; diabetes mellitus, diabetes insipidus, optic atrophy, hearing loss	Probable	N	N/A	*WFS1* ‡	AR	NM_006005.3, NP_005996.2	chr4:g.6302761TTCG>T	c.1243_1245delGTC; p.Val415del	Het	Y [e 16]	Likely pathogenic, PS1, PM2, PM4
											chr4:g.6303622G>T	c.2100G>T, p.Trp700Cys	Het	Y [e 16]	Likely pathogenic, PS1, PM2, PP3, PP5
B60	25	F	N	Myopathy, exercise intolerance, sideroblastic anemia, lactic acidosis, GI dysmotility, (MLASA), COX negative fibers on muscle biopsy	Definite	Y	Y	*YARS2*	AR	NM_001040436.2, NP_001035526.1	chr12:g.32908653G>C	c.156C>G; p.Phe52Leu	Hom	Y [e 17]	Likely pathogenic, PS1, PM2, PP3, PP5
C44	33	F	N	Ptosis, optic atrophy, proximal myopathy, fatigue, respiratory muscle weakness, cardiomyopathy, mitochondrial myopathy on muscle biopsy (respiratory chain enzyme analysis showed significant deficit in complex I and IV and moderate deficit in complex III, sparing of complex II)	Definite	Y	Y	*YARS2*	AR	NM_001040436.2, NP_001035526.1	chr12:g.32908711G>T	c.98C>A; p.Ser33Ter	Het	N	Pathogenic, PVS1, PM2, PP3
											chr12:g.32903808C>A	c.948G>T; p.Arg316Ser	Het	N	VUS-favor pathogenic, PM2, PM3
B20	41	F	N	CPEO, gestational diabetes, spasticity, mitochondrial myopathy, COX positive and RRFs on muscle biopsy	Definite	Y	Y	*AFG3L2*	AD	NC_000018.10 (NM_006796.2); NP_006787.2	chr18:g.12370846A>G	c.292+2T>C	Het	Likely pathogenic on ClinVar	Pathogenic, PVS1, PM2, PP3

B7	64	F	Y	CPEO, neuropathy, insulin resistance, myalgias, fatigue, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102748874C>T	c.907C>T; p.Arg303Trp	Het	Y [e 18]	Pathogenic, PS1, PM2, PM5, PP2, PP3
B29	58	F	Y	CPEO, RRFs on muscle biopsy	Probable	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749589T>G	c.1432T>G; p.Phe478Val	Het	Missense at the same position reported [e 19]	Likely pathogenic, PM1, PM2, PM5, PP2, PP3
C1	51	M	Y	CPEO, muscle fatigue, gastric dysmotility, proteinuria, neuropathy, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749610T>A	c.1453T>A; p.Phe485Ile	Het	N	Likely pathogenic, PM1, PM2, PP2, PP3
C10	68	F	Y	CPEO, proximal myopathy, COX negative and RRFs on muscle biopsy	Probable	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749610T>A	c.1453T>A; p.Phe485Ile	Het	N	Likely pathogenic, PM1, PM2, PP2, PP3
C29	69	F	N	CPEO, proximal myopathy, dysphagia, fatigue, mitochondrial myopathy, COX negative and RRFs on muscle biopsy	Probable	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749037G>C	c.1070G>C; p.Arg357Pro	Het	Y [e 20]	Likely pathogenic, PS1, PM2, PP2, PP3
C33	48	F	N	CPEO, proximal myopathy, muscle fatigue	Possible	N	N/A	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749535G>C	c.1378G>C; p.Ala460Pro	Het	Y [e 21]	Pathogenic, PS1, PM1, PM2, PP2, PP3
C40	39	F	Y	CPEO, migraine, hearing loss, gastric dysmotility, gestational diabetes	Probable	N	N/A	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749073C>A	c.1106C>A; p.Ser369Tyr	Het	Y [e 22]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3
C41	62	F	Y	CPEO, proximal myopathy, GI dysmotility, hearing loss, migraines	Probable	N	N/A	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102749073C>A	c.1106C>A; p.Ser369Tyr	Het	Y [e 22]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3
D3	64	F	Y	CPEO, hearing impairment, insulin resistance, mitochondrial myopathy COX negative and RRFs on muscle biopsy	Definite	Y	Y	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102748923A>G	c.956A>G; p.Lys319Arg	Het	Missense at the same position reported [e 23]	Likely pathogenic, PM2, PM5, PP2, PP3
D8	46	F	Y	CPEO, exercise intolerance, Grave's disease	Possible	N	N/A	*TWNK*	AD	NM_021830.4; NP_068602.2	chr10:g.102748923A>G	c.956A>G; p.Lys319Arg	Het	Missense at the same position reported [e 23]	Likely pathogenic, PM2, PM5, PP2, PP3
C24	23	F	Y	CPEO, dominant optic atrophy plus, hearing impairment, mild ataxia	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361785G>A	c.1334G>A; p.Arg445His	Het	Y [e 23]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3
C46	54	F	Y	CPEO, myopathy, dominant optic atrophy plus, hearing impairment, neuropathy, cognitive impairment	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361785G>A	c.1334G>A; p.Arg445His	Het	Y [e 24]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3

E1	74	F	Y	Dominant optic atrophy, ovarian failure, hearing loss	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361764A>G	c.1313A>G; p.Asp438Gly	Het	Y [e 25]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3
E25	44	F	Y	Dominant optic atrophy, ovarian failure	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361764A>G	c.1313A>G; p.Asp438Gly	Het	Y [e 26]	Pathogenic, PS1, PM1, PM2, PM5, PP2, PP3
E10	14	M	Y	Dominant optic atrophy, mild spasticity with clonus	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193349431C>T	c.655C>T; p.Gln219Ter	Het	Y [e 26]	Pathogenic, PVS1, PS1, PM2, PP3
E11	53	F	Y	Dominant optic atrophy	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193349431C>T	c.655C>T; p.Gln219Ter	Het	Y [e 26]	Pathogenic, PVS1, PS1, PM2, PP3
E14	40	M	Y	Dominant optic atrophy, diabetes	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361776A>G	c.1325A>G; p.Asp442Gly	Het	Likely pathogenic on ClinVar	Likely pathogenic, PM1, PM2, PM5, PP2, PP3
E22	61	F	Y	Dominant optic atrophy, muscle weakness and neuropathy	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193361776A>G	c.1325A>G; p.Asp442Gly	Het	Likely pathogenic on ClinVar	Likely pathogenic, PM1, PM2, PM5, PP2, PP3
E87	67	M	Y	CPEO, dominant optic atrophy, ataxia, neuropathy	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E88	72	F	Y	CPEO, dominant optic atrophy plus, hearing loss, sensory neuropathy, ataxia, migraine	Definite	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E89	70	F	Y	CPEO, dominant optic atrophy plus, proximal myopathy, ataxia	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E90	41	F	Y	Dominant optic atrophy, neuropathy	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E91	42	F	Y	Dominant optic atrophy	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E94	41	F	Y	CPEO, dominant optic atrophy plus, mild cerebellar ataxia, neuropathy	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
E95	39	M	Y	CPEO, dominant optic atrophy plus, hearing impairment, proximal myopathy	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3

E97	69	F	Y	CPEO, dominant optic atrophy plus, diabetes, hearing loss, ataxia, proximal myopathy	Probable	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193355758T>A	c.888T>A; p.Asp296Glu	Het	Previously published [e 27]	Likely pathogenic, PM1, PM2, PP1, PP2, PP3
C36	47	F	Y	CPEO, dysphagia, proximal myopathy, peripheral neuropathy	Possible	N	N/A	*POLG*	AD	NM_002693.2, NP_002684.1	chr15:g.89864114T>C	c.2864A>G; p.Tyr955Cys	Het	Y [e 28]	Likely pathogenic, PS1, PM2, PP3
B39	58	M	N	McLeod neuroacanthocytosis syndrome; bilateral ptosis, proximal myopathy, seizures, multiple frequent tics, acanthocytes, COX negative and RRFs on muscle biopsy	Probable	Y	Y	*XK* ‡	XLR	NM_021083.2, NP_066569.1	chrX:g.37553559CT>C	c.268delT; p.Tyr90ThrfsTer40	Het	N	Pathogenic, PVS1, PM2, PP3
B3	38	F	N	Mild CPEO, proximal weakness, seizures, colonic pseudo-obstructions, diabetes, cerebellar ataxia, elevated lactate	Probable	N	N/A	Numerous ‡	De novo	GRCh37/hg19	chr4:g.119266501_135689400del		Het	N	Pathogenic PVS1, PM2
Variant of Uncertain Significance (VUS)															
D11	43	F	N	Stroke-like episodes, seizures, ptosis, COX negative fibers on muscle biopsy	Definite	Y	Y	*ACAD9*	AR	NC_000003.12 (NM_014049.4), NP_054768.2	chr3:g.128628253C>T	c.1552C>T p.Arg518Cys	Het	Y [e 29]	Likely pathogenic, PS1, PM2, PP3, PP5
											chr3:g.128614267A>G	c.453+8A>G	Het	N	VUS, BP4
E12	44	F	Y	CPEO, myopathy, dysphagia, neuropathy, multiple intracranial meningiomas	Possible	N	N/A	*POLG*	AR	NM_002693.2, NP_056375.2	chr15:g.89864968C>G	c.2597G>C p.Arg866Pro	Het	VUS on ClinVar	VUS, PM2, PP3
											chr15:g.89872242T>C	c.955A>G p.Lys319Glu	Het	N	VUS, PM2, PP3
B11	44	M	Y	Myalgias, myopathy, COX negative and RRFs on muscle biopsy	Possible	Y	Y	*ALDH18A1* ‡	AD	NM_002860.3, NM_002860.3	chr10:g.97387286T>G	c.991A>C p.Thr331Pro	Het	Uncertain significance on ClinVar	VUS, PM2
B2	46	F	N	Stroke-like episodes, myopathy, migraines, cyclic neutropenia, complex I deficiency on muscle biopsy	Probable	Y	Y	*TWNK*	AD	NM_021830.4, NP_068602.2	chr10:g.102748082C>T	c.115C>T p.Arg39Cys	Het	N	VUS, PM2, PP2
B25	63	F	N	CPEO, proximal myopathy, ataxia, diabetes mellitus, gastrointestinal dysmotility, neuropathy, scattered fibers with oxidative enzyme abnormalities	Probable	Y	N	*TWNK*	AD	NM_021830.4, NP_068602.2	chr10:g.102748208C>G	c.241C>G p.Leu81Val	Het	Uncertain significance on ClinVar	VUS, PP2
E37	41	F	N	CPEO, myopathy, ataxia, spastic paraplegia	Possible	N	N/A	*KIF5A* ‡	AD	NM_004984.2, NP_004975.2	chr12:g.57965888G>T	c.1407G>T p.Glu469Asp	Het	N	VUS, PP2
C43	68	M	N	Ptosis, muscle weakness and fatigue, seizures	Possible	N	N/A	*OPA1*	AD	NM_015560.2, NP_056375.2	chr3:g.193332585TTTCACGAAGCATTTATCA>T	c.113_130delGAAGCATTTATCATTCAC; p.Arg38_Ser43del	Het	Y [e 30] Uncertain significance on ClinVar	VUS, PM4

B33	65	M	N	Stroke-like episodes, encephalopathy, migraine, hearing loss, gastric dysmotility, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AD	NM_002693.2, NP_056375.2	chr15:g.89861908T>C	c.3346A>G p.Met1116Val	Het	VUS on ClinVar	VUS, PM2, PM5, PP3
C34	68	F	Y	Ptosis, muscle weakness, migraines, hearing loss, neuropathy, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AD	NM_002693.2, NP_056375.2	chr15:g.89861859C>T	c.3395G>A p.Ser1132Asn	Het	N	VUS, PM2, PP3
C39	76	F	N	Proximal myopathy, hearing impairment, insulin resistance, raised lactate, COX negative, COX positive and RRFs on muscle biopsy	Definite	Y	Y	*POLG*	AD	NM_002693.2, NP_056375.2	chr15:g.89862265A>T	c.3170T>A p.Met1057Lys	Het	N	VUS, PM2, PP3
C11	41	M	N	Myalgias, fatigue, spastic paraplegia, hearing impairment, short stature, seizures, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*POLG2*	AD	NM_007215.3, NP_009146.2	chr17:g.62481897G>A	c.1058C>T p.Ser353Phe	Het	N	VUS, PP3

ID = Patient identification number for this study; F = Female; M = Male; Y = Yes; N = No; N/A = Not Applicable; COX =Cytochrome Oxidase; CPEO = Chronic Progressive External Ophthalmoplegia; GI = Gastrointestinal; MRS = Magnetic Resonance Spectroscopy; RRF = Ragged Red Fibers; SANDO = Sensory Ataxia, Neuropathy, Dysarthria and Ophthalmoplegia; SDH = Succinate Dehydrogenase; Nijmegen Rating = Classifications according to the Nijmegen criteria – Definite = score of 8-12, Probable = score of 5-7, Possible = score of 2-4; ‡ = Mitochondrial mimic; Mode = Mode of inheritance; AD = Autosomal Dominant; AR = Autosomal Recessive; Het = Heterozygous; Hom = Homozygous; VUS = Variant of Uncertain Significance; ACMG Classification = Classification according to the American College of Medical Genetics criteria.

eTable 4: Mitochondrial DNA variants identified using Whole Genome Sequencing

ID	Age	Sex	Family History	Clinical features	Nijmegen rating	Muscle biopsy	Changes on muscle biopsy	Gene	Mode	Nucleotide and amino acid change (GRCh37)	WGS plasmy (Pyroseq plasmy)
B32	64	M	Y	Hearing impairment, myalgia, generalized large fiber neuropathy, COX negative, SDH positive and RRFs on muscle biopsy (atypical presentation)	Probable	Y	Y	*MT-RNR1*	Mat	m.1555A>G	85%
E27	41	F	Y	Migraines	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	19% (19%)
E29	80	F	Y	Proximal myopathy, retinal pigmentary changes	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	1% (1%)
E30	29	M	Y	Migraines, diabetes, hearing loss, Wolf-Parkinson's-White syndrome	Definite	N	N/A	*MT-TL1*	Mat	m.3243A>G	32% (32%)
E33	37	F	Y	Muscle weakness, migraines, GI dysmotility, mitral valve prolapse	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	23% (23%)
F11	57	M	Y	Myopathy, hearing loss, GI dysmotility, hypothyroidism	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	11% (9%)
E34	40	M	Y	Proximal myopathy, CPEO, dysphagia, lactic acidosis, hearing loss, diabetes, cardiac conduction block	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	28% (27%)
B49	56	M	Y	Diabetes, hearing loss	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	12% (12%)
E35	49	M	Y	MELAS syndrome	Definite	Y	Y	*MT-TL1*	Mat	m.3243A>G	20% (22%)
E36	78	F	Y	hearing loss, retinal pigmentary changes, exercise intolerance	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	4% (4%)
B50	66	M	Y	MIDD, CPEO, cardiomyopathy	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	12% (12%)
F1	48	F	Y	Muscle fatigue	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	6% (6%)
E39	31	F	Y	Hearing loss, migraines, GI dysmotility	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	28% (26%)
D9	50	M	Y	Muscle pain, hearing loss, insulin resistance, elevated lactate on MRS	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	8% (10%)
E43	47	F	Y	MELAS syndrome, hearing loss, diabetes	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	17% (16%)
B52	61	F	Y	Migraines, mild encephalopathy, myoclonus, hearing loss, GI dysmotility, insulin resistance	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	2% (2%)
E100	16	M	Y	MELAS syndrome	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	53% (50%)
E101	12	F	Y	Migraines, short stature	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	54% (53%)

D10	51	M	Y	Exercise intolerance, hearing loss, diabetes mellitus, atrial fibrillation, left ventricular function abnormalities	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	6% (6%)
E46	48	M	Y	Proximal myopathy, hearing loss, diabetes mellitus, peripheral neuropathy, GI dysmotility, cardiac arrhythmia, lactic acidosis	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	13% (14%)
D5	20	M	N	MELAS syndrome	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	24% (25%)
B56	38	F	Y	Migraines, hearing loss, diabetes, elevated lactate on MRS	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	21% (22%)
E52	44	F	Y	Migraines, insulin resistance, exercise intolerance	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	3% (3%)
E53	57	F	Y	Mild proximal weakness	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	0.35% (1%)
E56	56	F	Y	Ptosis, proximal weakness, fatigue, myalgias, hearing loss, myopathy, RRFs on muscle biopsy	Definite	Y	Y	*MT-TL1*	Mat	m.3243A>G	2% (2%)
E57	24	M	Y	Myalgias, migraines	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	11% (11%)
E58	22	F	Y	Ptosis, hearing impairment, fatigue, GI dysmotility, mild peripheral neuropathy	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	42% (39%)
E59	53	F	Y	MELAS syndrome, hearing loss, gastric dysmotility, COX negative, SDH positive fibers on muscle biopsy	Definite	Y	Y	*MT-TL1*	Mat	m.3243A>G	16% (16%)
E60	50	F	Y	Mild proximal weakness, diabetes	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	13% (12%)
E65	74	F	Y	MELAS syndrome, hearing loss, GI dysmotility	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	48% (46%)
E9	45	M	Y	MELAS syndrome, myoclonus, hearing loss, COX negative and RRFs on muscle biopsy	Definite	Y	Y	*MT-TL1*	Mat	m.3243A>G	34% (32%)
E66	37	M	Y	Mild ptosis	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	26% (17%)
D2	45	F	Y	Muscle fatigue, hearing loss, GI dysmotility	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	18% (18%)
F10	42	F	Y	Muscle fatigue, hearing loss, GI dysmotility	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	18% (20%)
E68	42	F	Y	Muscle fatigue, hearing loss, insulin resistance	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	14% (16%)
E72	26	F	Y	Hearing loss, focal and segmental glomerulonephritis	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	37% (35%)
C45	53	F	Y	Myopathy, stroke-like episodes, migraines, hearing impairment, GI dysmotility, diabetes, renal impairment, lactic acidosis	Definite	N	N/A	*MT-TL1*	Mat	m.3243A>G	20% (18%)
A7	45	M	Y	MELAS syndrome, diabetes, GI dysmotility, elevated lactate on MRS	Possible	N	N/A	*MT-TL1*	Mat	m.3243A>G	24% (14%)

F8	78	F	Y	Stroke-like episodes, diabetes, hearing loss, GI dysmotility, Parkinsonism	Definite	N	N/A	*MT-TL1*	Mat	m.3243A>G	0.45% (1%)
F9	40	M	Y	Hearing loss, GI dysmotility, migraines	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	18% (18%)
D17	27	M	Y	Myalgias, impaired glucose tolerance	Definite	N	N/A	*MT-TL1*	Mat	m.3243A>G	34% (33%)
E20	61	F	Y	Myopathy, exercise intolerance, elevated lactate	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	2% (2%)
E79	19	M	Y	Short stature, exercise intolerance	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	21% (21%)
E80	47	F	Y	Diabetes, myopathy, exercise intolerance, hearing loss, GI dysmotility, neuropathy	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	17% (17%)
D20	60	F	Y	Myopathy, migraines, hearing loss, GI dysmotility, insulin resistance	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	9% (10%)
D4	35	F	Y	Migraines, seizures, myoclonic jerks, hearing loss, GI dysmotility	Probable	N	N/A	*MT-TL1*	Mat	m.3243A>G	31% (25%)
E85	55	F	N	MELAS syndrome, hearing loss, GI dysmotility, COX positive and RRFs on muscle biopsy	Definite	Y	Y	*MT-TL1*	Mat	m.3243A>G	4% (3%)
C23	51	M	Y	MELAS syndrome, hearing loss, cardiomyopathy	Definite	N	N/A	*MT-TL1*	Mat	m.3243A>G	19% (20%)
E105	21	F	N	Myopathy, migraines, optic atrophy, hearing loss, GI dysmotility, diabetes mellitus	Probable	Y	Y	*MT-TL1*	Mat	m.3243A>G	3% (4%)
F2	70	F	Y	Hearing impairment, proximal myopathy, mild dysphagia, respiratory muscle weakness, mitochondrial myopathy on muscle biopsy	Probable	Y	Y	*MT-TL1*	Mat	m.3251A>G	7%
A4	29	M	Y	Oligosymptomatic, migraines	Possible	N	N/A	*MT-TL1*	Mat	m.3256C>T	24%
E82	31	M	Y	Myopathy, migraines, COX positive and RRFs on muscle biopsy	Definite	Y	Y	*MT-TL1*	Mat	m.3256C>T	4%
E83	25	F	Y	Exercise intolerance	Possible	N	N/A	*MT-TL1*	Mat	m.3256C>T	2%
E78	57	F	Y	Ptosis, proximal myopathy, ataxia, tremor, myoclonus, peripheral neuropathy, hearing loss, RRFs on muscle biopsy	Probable	Y	Y	*MT-TL1*	Mat	m.3302A>G	19%
E5	20	M	Y	LHON	Possible	N	N/A	*MT-ND1*	Mat	m.3460G>A; p.Ala52Thr	100%
E6	23	F	Y	Migraines	Possible	N	N/A	*MT-ND1*	Mat	m.3460G>A; p.Ala52Thr	100%
A6	52	M	Y	CPEO, myopathy, GI dysmotility, sensorimotor axonal neuropathy	Probable	N	N/A	*MT-TI*	Mat	m.4269A>G	26%
A1	66	F	Y	Proximal muscle weakness, migraines, myoclonus, early onset hearing loss, neuropathy, elevated lactate on MRS	Definite	N	N/A	*MT-TK*	Mat	m.8344A>G	70%

E23	20	F	Y	MERRF syndrome	Possible	N	N/A	*MT-TK*	Mat	m.8344A>G	92%
E103	69	F	Y	MERRF syndrome, renal impairment, lipomas, hearing loss, RRFs on muscle biopsy	Definite	Y	Y	*MT-TK*	Mat	m.8344A>G	95%
E51	37	M	Y	NARP	Probable	N	N/A	*MT-ATP6*	Mat	m.8993T>G; p.Leu156Arg	90%
C7	28	M	N	Migraines, seizures, stroke-like episodes, hearing loss, lactic acidosis, complex 1 deficiency on muscle biopsy, abnormal mitochondria on EM	Probable	Y	Y	*MT-ND3*	Mat	m.10191T>C; p.Ser45Pro	32%
E3	57	F	Y	Oligosymptomatic, cervical dystonia, head tremor	Possible	N	N/A	*MT-ND4*	Mat	m.11778G>A; p.Arg340His	75%
E4	22	M	Y	LHON, postural tremor, myoclonus	Possible	N	N/A	*MT-ND4*	Mat	m.11778G>A; p.Arg340His	100%
A2	17	M	N	CPEO, optic atrophy, spasticity, hearing loss (atypical presentation)	Probable	N	N/A	*MT-ND4*	Mat	m.11778G>A; p.Arg340His	100%
A8	39	F	Y	LHON-MS (Harding's disease)	Possible	N	N/A	*MT-ND4*	Mat	m.11778G>A; p.Arg340His	100%
D18	27	M	N	MELAS syndrome, migraines, diabetes, hearing loss, Wolff-Parkinson-White syndrome, Complex I and IV deficiency on muscle biopsy	Probable	Y	Y	*MT-ND5*	Mat	m.13042G>A; p.Ala236Thr	6%
E2	45	F	Y	Bilateral optic neuropathy, hearing impairment, diabetes mellitus	Possible	N	N/A	*MT-ND5*	Mat	m.13513G>A; p.Asp393Asn	13%
E99	28	M	N	Leigh-like disease, mitochondrial disease on muscle biopsy ("parking lot" intracristal inclusions on electron microscopy)	Probable	Y	Y	*MT-ND5*	Mat	m.13528A>G; p.Thr398Ala	100%
A5	40	F	N	Proximal myopathy, RRFs on muscle biopsy	Probable	Y	Y	*MT-TE*	Mat	m.14674T>C	100%
E77	43	F	Y	Myalgia, fatigue, GI dysmotility, exercise intolerance, impaired glucose tolerance	Probable	N	N/A	*MT-TE*	Mat	m.14709T>C	65%
A10	18	F	N	KSS	Possible	N	N/A	Single deletion	Spo	Single deletion	0.29%
C16	16	F	N	KSS, COX negative and RRFs on muscle biopsy	Probable	Y	Y	Single deletion	Spo	Single deletion	0.60%

ID = Patient identification number for this study; F = Female; M = Male; Y = Yes; N = No; COX = Cytochrome Oxidase; CPEO = Chronic Progressive External Ophthalmoplegia; EM = Electron Microscopy; GI = Gastrointestinal; KSS = Kearns-Sayre Syndrome; LHON = Leber's Hereditary Optic Neuropathy; LHON-MS = Leber's Hereditary Optic Neuropathy with concurrent Multiple Sclerosis-like disease; MELAS = Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like episodes; MERRF = Myoclonic Epilepsy with Ragged Red Fibers; MIDD = Maternally Inherited Diabetes and Deafness; MRS = Magnetic Resonance Spectroscopy; NARP = Neuropathy, Ataxia and Retinitis Pigmentosa; RRF = Ragged Red Fibers; SANDO = Sensory Ataxia, Neuropathy, Dysarthria and Ophthalmoplegia; SDH = Succinate Dehydrogenase; Nijmegen Rating = Classifications according to the Nijmegen criteria – Definite = score of 8-12, Probable = score of 5-7, Possible = score of 2-4; Mode = Mode of inheritance; Mat = Maternal inheritance; Spo = Sporadic inheritance; WGS Plasmy = Level of heteroplasmy from whole genome sequencing analysis; Pyroseq plasmy = Level of heteroplasmy from pyrosequencing analysis; ND = Not Determined. * = Not included in diagnostic analysis due to autopsy muscle being used and not blood. Note that some phenotypic features are relatively common and may not be directly related to mitochondrial disease (e.g. migraine).

eTable 5: Clinical details of patients with CPEO that had mitochondrial DNA deletions previously identified in urine or muscle that were not detectable using whole genome sequencing on blood DNA

ID	Age	Sex	Family History	Clinical features	Nijmegen rating	Muscle biopsy	Changes on muscle biopsy	Mitochondrial DNA deletion (tissue previously identified in)
B34	36	F	N	CPEO, myopathy	Probable	Y	Y	Single deletion (U)
E50	78	M	N	CPEO, myopathy	Possible	Y	Y	Single deletion (M)
E62	46	F	Y	CPEO, myopathy, COX negative and RRFs on muscle biopsy	Probable	Y	Y	Single deletion (M)
E63	38	M	N	CPEO, COX negative and SDH positive fibers on muscle biopsy	Probable	Y	Y	Single deletion (M)
B9	54	F	N	KSS, mitochondrial myopathy, SDH positive fibers on muscle biopsy	Definite	Y	Y	Single deletion (M)
B13	71	M	Y	CPEO, myopathy, cardiac arrhythmia, COX negative and RRFs on muscle biopsy	Definite	Y	Y	Single deletion (M)
A9	55	F	N	CPEO, myopathy, COX negative fibers on muscle biopsy	Definite	Y	Y	Multiple deletions (M)

ID = Patient identification number for this study; F = Female; M = Male; Y = Yes; N = No; COX = Cytochrome Oxidase; CPEO = Chronic Progressive External Ophthalmoplegia; KSS = Kearns-Sayre Syndrome; RRF = Ragged Red Fibers; SDH = Succinate Dehydrogenase; Nijmegen Rating = Classifications according to the Nijmegen criteria – Definite = score of 8-12, Probable = score of 5-7, Possible = score of 2-4; Tissue deletion previously detected in: M = muscle, U = urine.

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