Diagnosing mitochondrial disorders remains challenging in the omics era

Patrick Forny *et al.*

Supplemental Material

Figure S1. Histograms of continuous variables

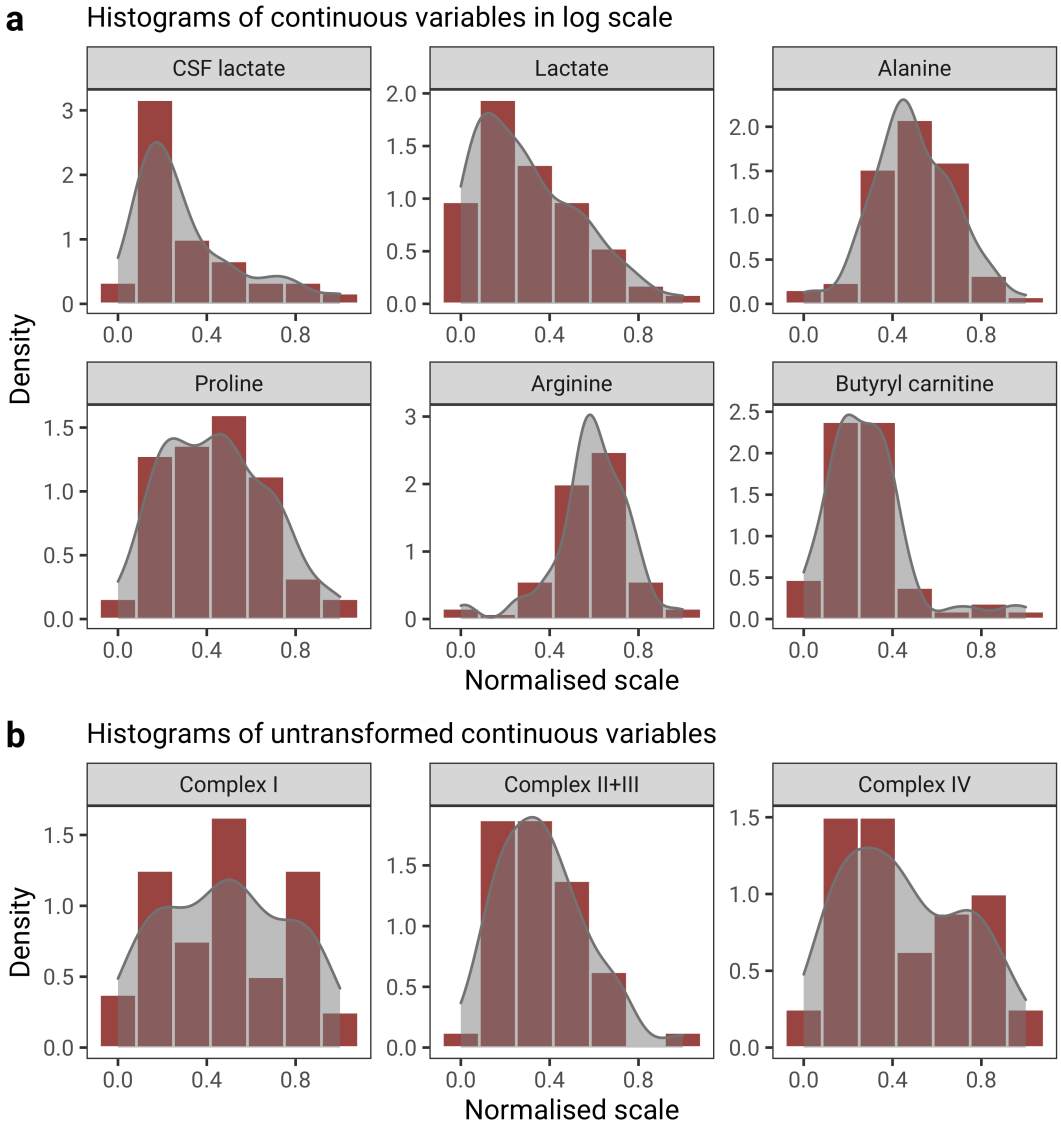

Fig S1. Combined histograms and density plots to illustrate distribution of variables. Some specific variables were log transformed prior to logistic regression analysis to achieve close to normal distribution. (**a**) Variables were log transformed and underwent min-max normalization for illustration purposes. (**b**) Variables were not log transformed but underwent min-max normalization for illustration purposes in this panel.

Figure S2. Turnaround times

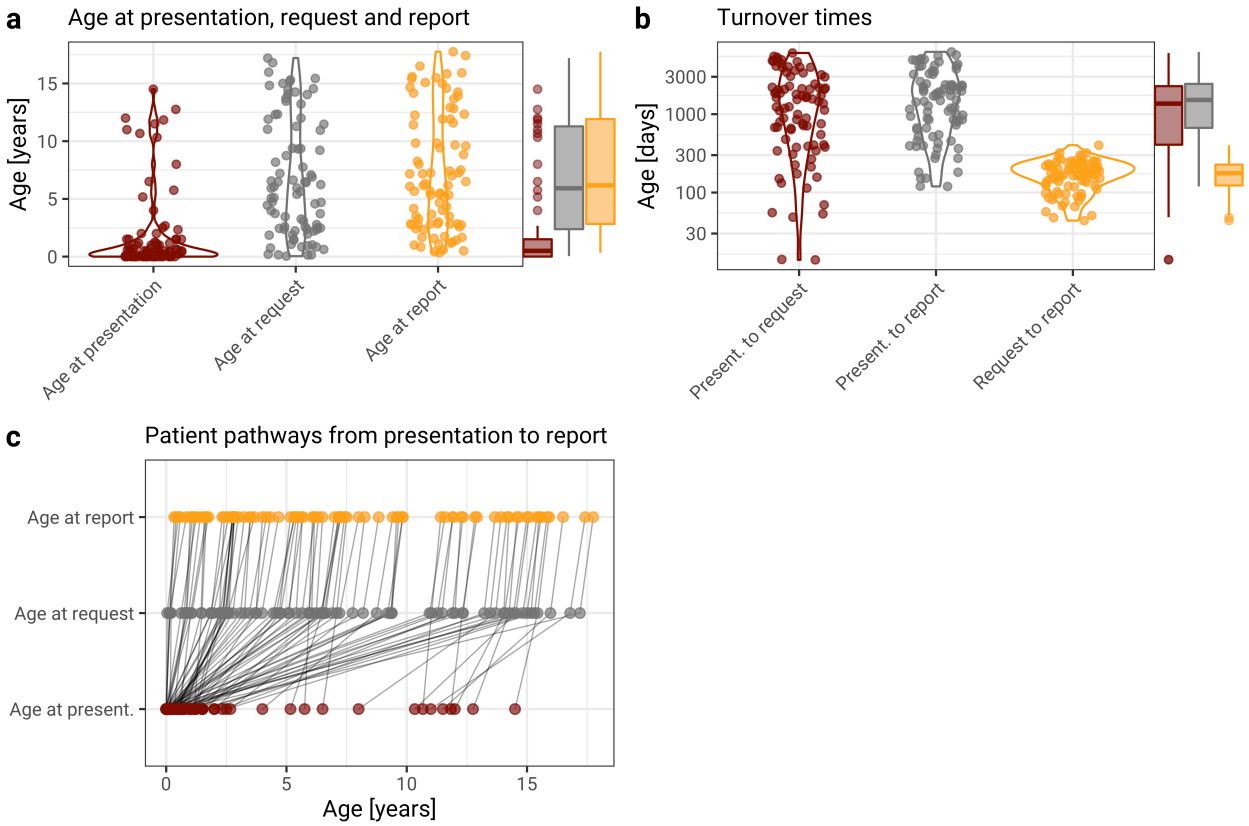

Fig. S2. Delay of diagnosis and turnaround times. (**a**) Ages of patients at different stages of the diagnostic process of clinical exome sequencing (age at request means time point when clinical exome sequencing was initiated; age at report means time point when final diagnostic report was completed. (**b**) Durations of three different periods during clinical exome sequencing as indicated by the labels on the x-axis. (**c**) Schematic representation of individual patient pathways (as connected by grey lines) from clinical presentation to completion of clinical exome analysis report. Present. = presentation.

Figure S3. Performed tests and outcomes

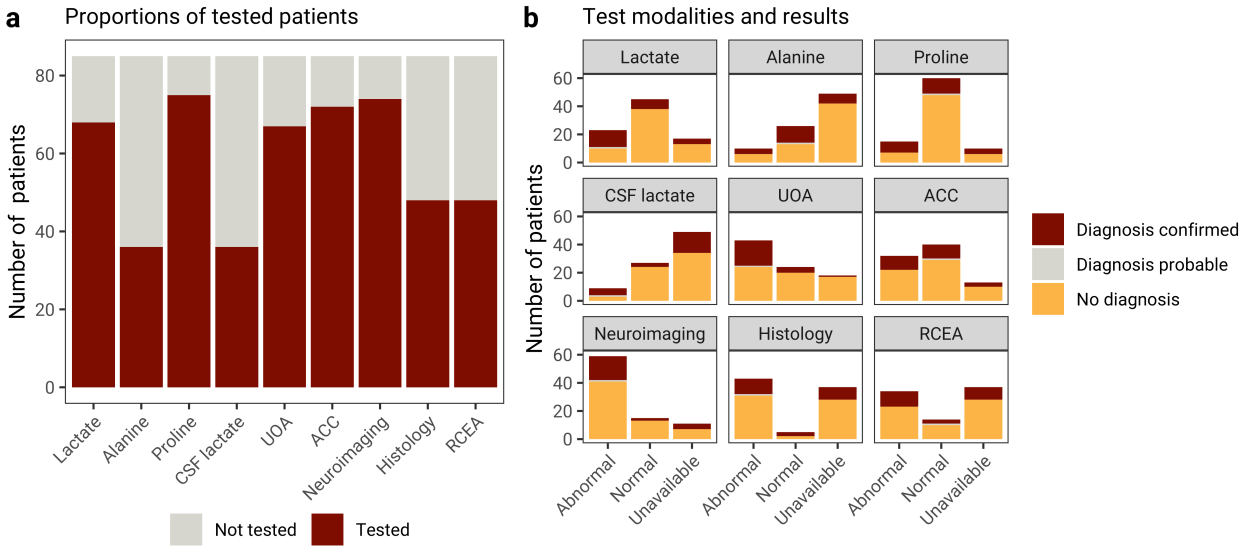

Fig. S3. Investigations in patients. (**a**) Number of patients (y-axis) which were investigated/tested with a specific method (x-axis). (**b**) Bar charts depicting number of patients which were investigated with different tests, separated into the categories of the test outcome (abnormal and normal). A third bar represents the number patients in whom the test was not performed. Abnormal in case of lactate, alanine, and proline means elevated above reference range. Urine organic acid (UOA) analysis and acylcarnitine (ACC) analysis results were designated abnormal, if any of the measured species was detected above reference range. Patients with confirmed or probable mitochondrial diagnosis and patients with no diagnosis are colour coded. CSF, cerebrospinal fluid; RCEA, respiratory enzyme activities.

Figure S4. Correlogram

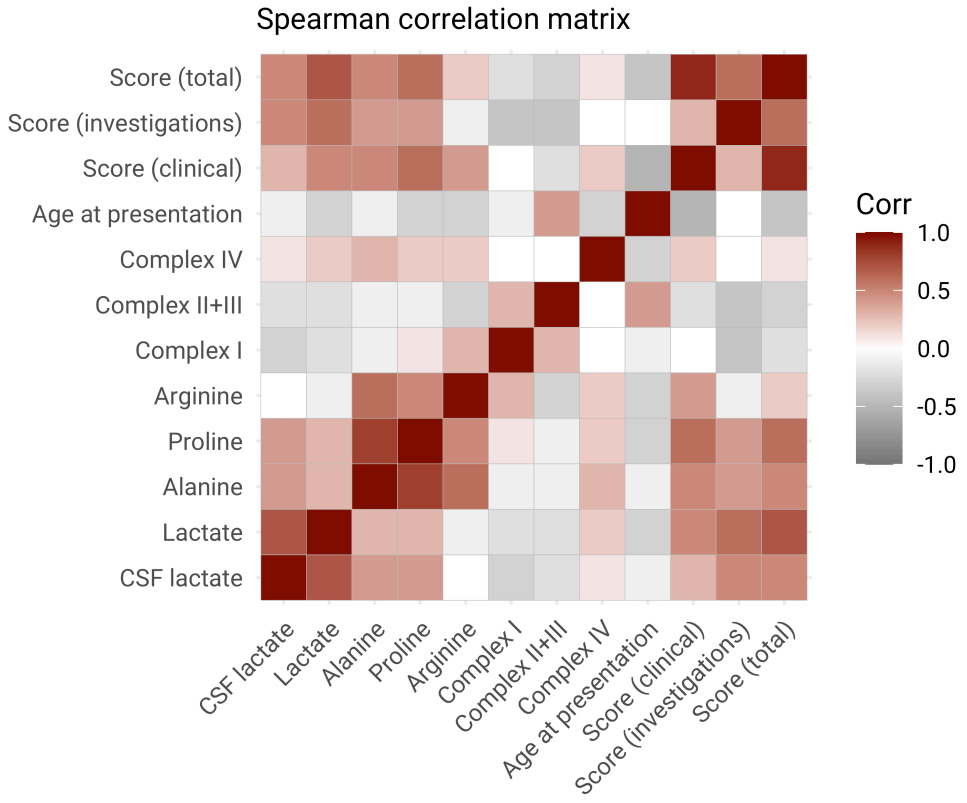

Fig S4. Spearman correlation matrix of continuous variables in the data set. Only complete observations were used.

Figure S5. Relationship of lactate to alanine and proline

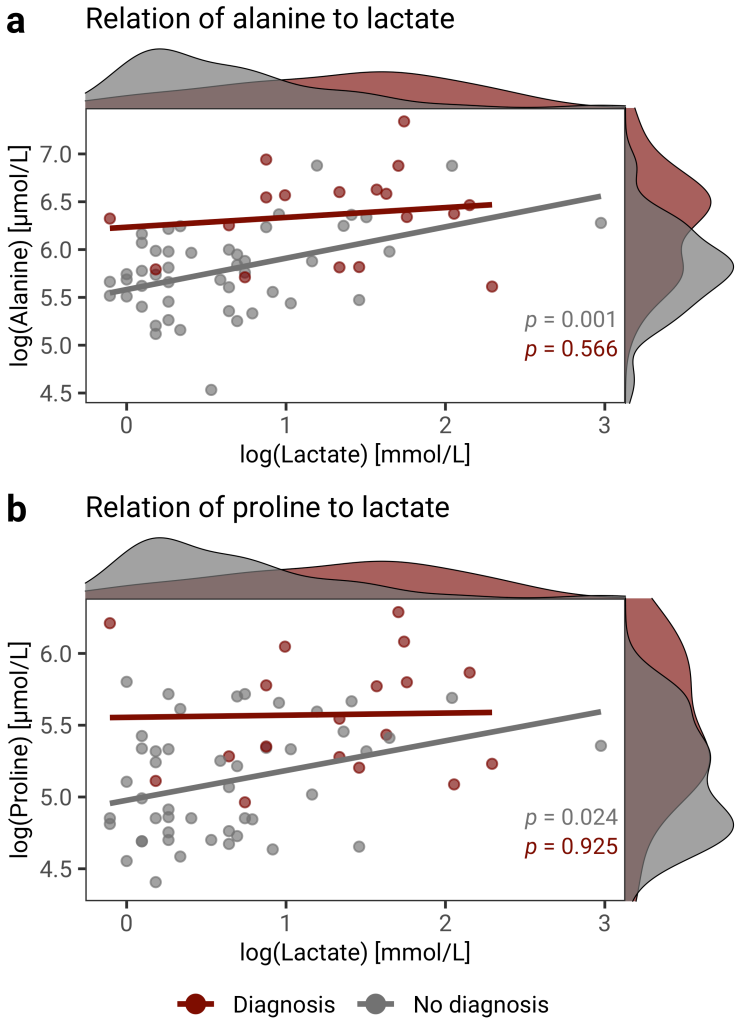

Fig S5. Relationship of lactate and its related metabolites alanine and proline. Scatter plots with marginal density plots depicting log transformed lactate levels on the x-axis and (**a**) alanine, and (**b**) proline levels on the y-axis. Dots and linear regression lines are colour coded according to two groups: (i) patients with a mitochondrial diagnosis (red), and (ii) patients without a mitochondrial diagnosis (grey).

Figure S6. ROC curves

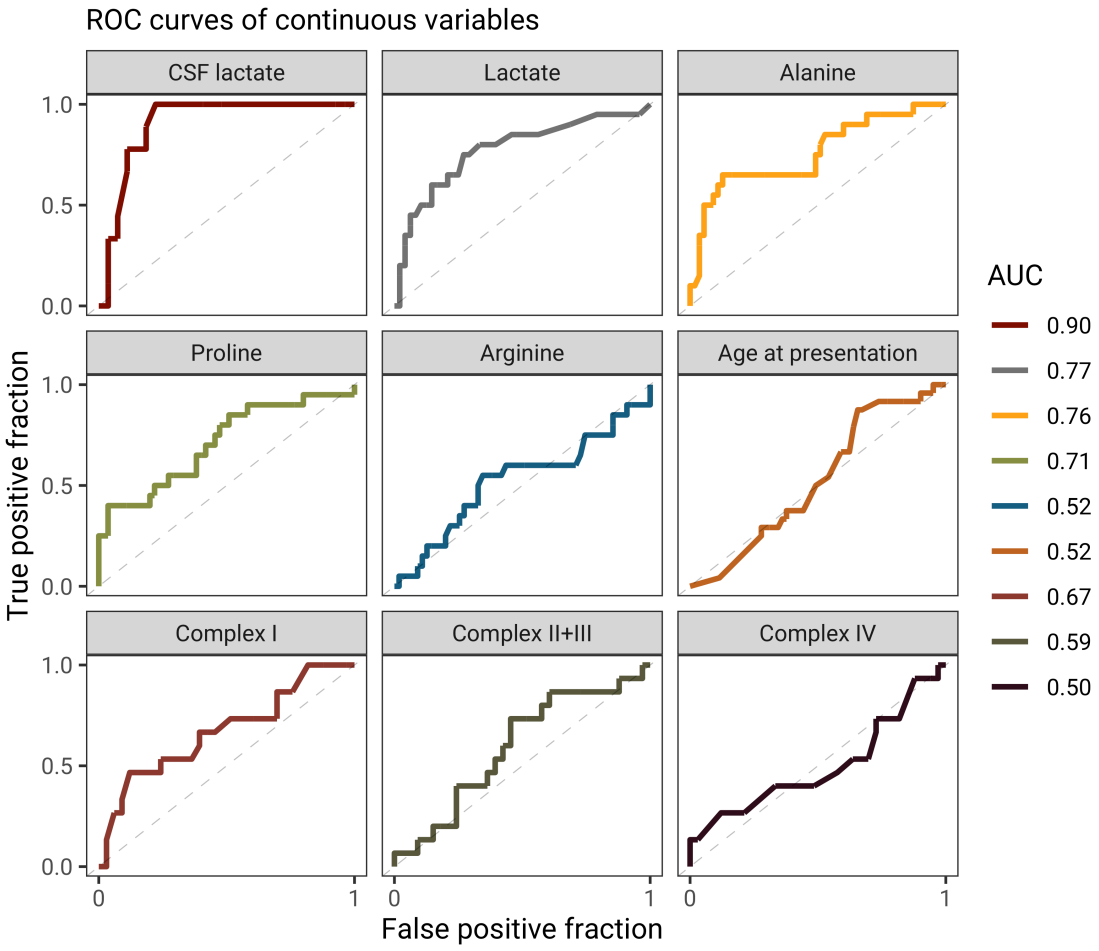

Fig S6. ROC curves of continuous variables. AUC, area under the curve.

Figure S7. Mitochondrial disease score

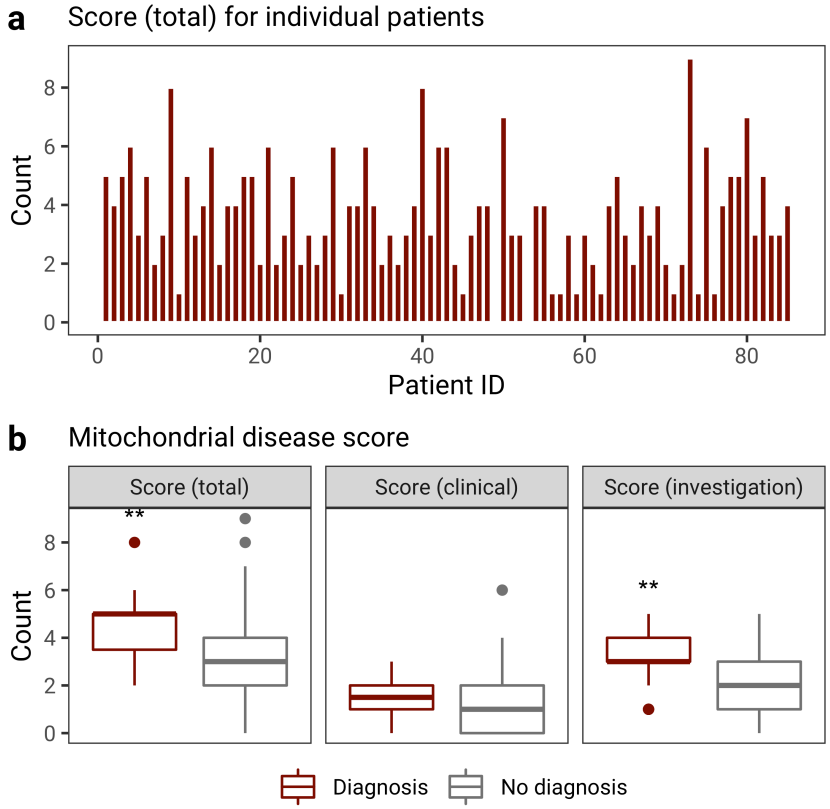

Fig. S7. Assessment by clinical and biochemical mitochondrial disease score. (**a**) Total score per individual patient. (**b**) Boxplot depicting total score, clinical sub-score and investigation sub-score, divided in two groups: (i) patients with a mitochondrial diagnosis, and (ii) patients without mitochondrial diagnosis.

Table S1

Gene symbol	HGNC ID
AARS2	21022
ABCB7	48
ACAD9	21497
ACO2	118
COQ8A	16812
COQ8B	19041
AFG3L2	315
AGK	21869
AIFM1	8768
ANO10	25519
COA8	20492
APTX	15984
ATPAF2	18802
BCS1L	1020
BOLA3	24415
BTD	1122
TWNK	1160
C12orf65	26784
C19orf12	25443
CHCHD10	15559
CHKB	1938
CLPB	30664
CLPP	2084
COQ2	25223
COQ4	19693
COQ6	20233
COQ9	25302
COX10	2260
COX14	28216
COX15	2263

COX20	26970
COX6A1	2277
COX6B1	2280
COX7B	2291
CYC1	2579
CYCS	19986
DARS1	2678
DARS2	25538
DGUOK	2858
DHTKD1	23537
DLAT	2896
DLD	2898
DNA2	2939
DNAJC19	30528
ECHS1	3151
ELAC2	14198
ETFDH	3483
ETHE1	23287
FARS2	21062
FASTKD2	29160
FBXL4	13601
FH	3700
FOXRED1	26927
G6PC	4056
GARS1	4162
GATM	4175
GDAP1	15968
GFM1	13780
GLRX5	20134
GLUD1	4335
HCCS	4837
HIBCH	4908
HLCS	4976

HSPA9	5244
HSPD1	5261
IBA57	27302
KARS1	6215
LARS2	17095
LIAS	16429
LIPT1	29569
LRPPRC	15714
MFN2	16877
MGME1	16205
MPV17	7224
MRPS22	14508
NDUFA1	7683
NDUFA10	7684
NDUFA11	20371
NDUFA2	7685
NDUFAF1	18828
NDUFAF2	28086
NDUFAF3	29918
NDUFAF4	21034
NDUFAF5	15899
NDUFB11	20372
NDUFS1	7707
NDUFS2	7708
NDUFS3	7710
NDUFS4	7711
NDUFS6	7713
NDUFS7	7714
NDUFS8	7715
NDUFV1	7716
NDUFV2	7717
NFU1	16287
NUBPL	20278

OPA1	8140
PC	8636
PDHA1	8806
PDHB	8808
PDHX	21350
PDP1	9279
PDSS1	17759
PDSS2	23041
PET100	40038
PMPCA	18667
PNPT1	23166
POLG	9179
POLG2	9180
PYCR1	9721
QARS1	9751
RARS2	21406
RMND1	21176
RRM2B	17296
SACS	10519
SAMHD1	15925
SCO1	10603
SCO2	10604
SDHA	10680
SDHAF1	33867
SDHB	10681
SLC19A2	10938
SLC19A3	16266
SLC25A19	14409
SLC25A22	19954
SLC25A26	20661
SLC25A3	10989
SLC25A38	26054
SLC25A4	10990

SLC25A46	25198
SPG7	11237
SUCLA2	11448
SUCLG1	11449
SURF1	11474
TACO1	24316
TAZ	11577
TIMM8A	11817
TK2	11831
TMEM70	26050
TPK1	17358
TRMU	25481
TRNT1	17341
TTC19	26006
TUFM	12420
TYMP	3148
UQCRB	12582
UQCRQ	29594

Table S2

Gene symbol	HGNC ID
AARS2	21022
ABAT	23
ABCB7	48
ACAD9	21497
ACO2	118
COQ8A	16812
COQ8B	19041
AFG3L2	315
AGK	21869
AIFM1	8768
ANO10	25519
COA8	20492
APTX	15984
ATPAF2	18802
BCS1L	1020
BOLA3	24415
BTD	1122
TWNK	1160
C12orf65	26784
C19orf12	25443
CHCHD10	15559
CHKB	1938
CLPB	30664
CLPP	2084
COQ2	25223
COQ4	19693
COQ6	20233
COQ9	25302
COX10	2260
COX14	28216

COX15	2263
COX20	26970
COX6A1	2277
COX6B1	2280
COX7B	2291
CYC1	2579
CYCS	19986
DARS1	2678
DARS2	25538
DGUOK	2858
DHTKD1	23537
DLAT	2896
DLD	2898
DNA2	2939
DNAJC19	30528
DNM1L	2973
EARS2	29419
ECHS1	3151
ELAC2	14198
ETFDH	3483
ETHE1	23287
FARS2	21062
FASTKD2	29160
FBXL4	13601
FH	3700
FLAD1	24671
FOXRED1	26927
G6PC	4056
GARS1	4162
GATM	4175
GDAP1	15968
GFER	4236
GFM1	13780

GLRX5	20134
GLUD1	4335
HCCS	4837
HIBCH	4908
HLCS	4976
HSPD1	5261
IARS2	29685
IBA57	27302
KARS1	6215
LARS2	17095
LIAS	16429
LIPT1	29569
LRPPRC	15714
MARS2	25133
MFN2	16877
MGME1	16205
MPV17	7224
MRPS22	14508
MTO1	19261
NARS2	26274
NDUFA1	7683
NDUFA10	7684
NDUFA11	20371
NDUFA2	7685
NDUFA4	7687
NDUFAF1	18828
NDUFAF2	28086
NDUFAF3	29918
NDUFAF4	21034
NDUFAF5	15899
NDUFB11	20372
NDUFB3	7698
NDUFB9	7704

NDUFS1	7707
NDUFS2	7708
NDUFS3	7710
NDUFS4	7711
NDUFS6	7713
NDUFS7	7714
NDUFS8	7715
NDUFV1	7716
NDUFV2	7717
NFU1	16287
NUBPL	20278
OPA1	8140
OPA3	8142
PARS2	30563
PC	8636
PDHA1	8806
PDHB	8808
PDHX	21350
PDP1	9279
PDPR	30264
PDSS1	17759
PDSS2	23041
PET100	40038
PMPCA	18667
PNPT1	23166
POLG	9179
POLG2	9180
PYCR1	9721
QARS1	9751
RARS2	21406
RMND1	21176
RRM2B	17296
SACS	10519

SAMHD1	15925
SARS2	17697
SCO1	10603
SCO2	10604
SDHA	10680
SDHAF1	33867
SDHB	10681
SDHD	10683
SERAC1	21061
SLC19A2	10938
SLC19A3	16266
SLC25A19	14409
SLC25A22	19954
SLC25A26	20661
SLC25A3	10989
SLC25A38	26054
SLC25A4	10990
SLC25A46	25198
SPG7	11237
SUCLA2	11448
SUCLG1	11449
SURF1	11474
TACO1	24316
TARS2	30740
TAZ	11577
TIMM8A	11817
TK2	11831
TMEM70	26050
TPK1	17358
TRMU	25481
TRNT1	17341
TTC19	26006
TUFM	12420

TYMP	3148
UQCRB	12582
UQCRQ	29594
VARS2	21642
XPNPEP3	28052
YARS2	24249

Table S3

Gene symbol	HGNC ID
AARS2	21022
ABAT	23
ABCB7	48
ACAD9	21497
ACO2	118
COQ8A	16812
COQ8B	19041
AFG3L2	315
AGK	21869
AIFM1	8768
ANO10	25519
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COQ9	25302
COX10	2260

COX14	28216
COX15	2263
COX20	26970
COX6A1	2277
COX6B1	2280
COX7B	2291
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GATM	4175
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GFM1	13780
GLRX5	20134

GLUD1	4335
HCCS	4837
HIBCH	4908
HLCS	4976
HMGCL	5005
HSPD1	5261
HTRA2	14348
IBA57	27302
IER3IP1	18550
KARS1	6215
LARS2	17095
LIAS	16429
LIPT1	29569
LRPPRC	15714
MARS2	25133
MFF	24858
MFN2	16877
MGME1	16205
MPV17	7224
MRPS22	14508
MTO1	19261
NARS2	26274
NDUFA1	7683
NDUFA10	7684
NDUFA11	20371
NDUFA2	7685
NDUFAF1	18828
NDUFAF2	28086
NDUFAF3	29918
NDUFAF4	21034
NDUFAF5	15899
NDUFAF6	28625
NDUFB11	20372

NDUFB3	7698
NDUFS1	7707
NDUFS2	7708
NDUFS3	7710
NDUFS4	7711
NDUFS6	7713
NDUFS7	7714
NDUFS8	7715
NDUFV1	7716
NDUFV2	7717
NFU1	16287
NUBPL	20278
OPA1	8140
OPA3	8142
PC	8636
PDHA1	8806
PDHB	8808
PDHX	21350
PDP1	9279
PDSS1	17759
PDSS2	23041
PET100	40038
PMPCA	18667
PNPT1	23166
POLG	9179
POLG2	9180
PYCR1	9721
RARS2	21406
RMND1	21176
ROBO3	13433
RRM2B	17296
SACS	10519
SAMHD1	15925

SARS2	17697
SCO1	10603
SCO2	10604
SDHA	10680
SDHAF1	33867
SDHB	10681
SDHD	10683
SERAC1	21061
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SLC25A26	20661
SLC25A3	10989
SLC25A38	26054
SLC25A4	10990
SLC25A46	25198
SPG7	11237
SUCLA2	11448
SUCLG1	11449
SURF1	11474
TACO1	24316
TAZ	11577
TIMM8A	11817
TK2	11831
TMEM70	26050
TPK1	17358
TRMU	25481
TRNT1	17341
TSFM	12367
TTC19	26006
TYMP	3148
UQCRQ	29594

VARS2	21642
YARS2	24249

Table S4

Patient identifier (ID)	Defective gene	CI (0.104 - 0.268)	CII+III (0.040 - 0.204)	CIV (0.014 - 0.034)
1	No diagnosis	0.120	0.111	**0.011**
2	*GRIN2A*	0.112	0.119	**0.005**
4	*FBXL4*	0.205	0.068	0.015
5	*VPS13B*	0.222	0.122	0.024
7	No diagnosis	**0.054**	0.078	**0.012**
8	No diagnosis	0.136	0.122	**0.005**
9	No diagnosis	0.123	0.055	**0.011**
11	*ELAC2*	**0.076**	0.060	**0.011**
12	No diagnosis	**0.080**	0.049	**0.013**
13	No diagnosis	0.190	0.131	**0.008**
14	No diagnosis	**0.078**	0.056	**0.005**
15	No diagnosis	0.197	0.288	0.018
16	No diagnosis	0.117	0.137	0.018
17	*FBXL4*	**0.068**	0.110	**0.008**
21	No diagnosis	0.147	0.139	**0.007**
23	No diagnosis	0.209	0.102	**0.007**
24	*FBXL4*	0.179	0.103	0.023
25	*NDUFB11*	0.171	0.198	0.019
26	*SDHA*	0.197	**0.026**	**0.005**
28	*ELAC2*	**0.098**	0.120	0.019
29	*FOXRED1*	**0.047**	0.113	0.015
31	*AARS2*	**0.042**	0.138	**0.002**
33	No diagnosis	0.146	0.089	**0.006**
34	*NKX6-2*	**0.086**	0.075	**0.010**
35	No diagnosis	0.166	0.207	0.015
37	No diagnosis	0.129	0.161	**0.006**
40	*MT-ND5*	**0.054**	0.088	**0.012**
41	No diagnosis	0.114	0.174	0.018
42	No diagnosis	0.155	0.076	**0.009**
44	No diagnosis	0.214	0.215	**0.008**
47	No diagnosis	0.208	0.157	**0.010**
48	No diagnosis	0.205	0.168	0.022
49	*MT-ND3*	**0.076**	0.217	0.019
50	No diagnosis	0.215	0.062	**0.012**
60	No diagnosis	**0.034**	0.204	0.014
61	No diagnosis	0.186	0.170	0.019
63	*MYL3*	**0.065**	0.065	**0.011**
64	*C12orf65*	0.128	0.121	**0.005**
67	No diagnosis	0.143	0.158	0.021
68	No diagnosis	**0.085**	0.146	**0.006**
69	No diagnosis	0.148	0.092	**0.010**

73	No diagnosis	0.236	0.127	0.019
75	*ECHS1*	0.123	0.081	0.016
77	*AARS2*	0.143	0.135	**0.007**
79	No diagnosis	0.146	0.105	0.020
80	No diagnosis	**0.076**	0.170	**0.010**
81	*RMND1*	**0.054**	0.083	**0.003**
85	No diagnosis	0.143	0.090	**0.004**

Table. S4. Available results of RCEA measurements. Ranges in brackets are reference values. Values below the reference range are indicated in bold. For details of the biochemical method refer to the manuscript. For each patient with a known genetic diagnosis, the affected gene is indicated in the second column in bold print for better visibility.

Table S5

Variable	Odds ratio	p value	sig	McFadden pseudo R^2
Clinical variables				
Sex	0.874	0.781		0.00
Early-onset (<29 days)	0.784	0.643		0.00
Developmental Delay	1.652	0.302		0.01
Seizures	1.520	0.498		0.00
Hypotonia	0.251	0.202		0.02
Cardiomyopathy	4.750	0.026	*	0.05
Encephalopathy	0.288	0.253		0.02
Visual impairment	5.088	0.036	*	0.04
Neurological regression	0.399	0.407		0.01
Neuropathy	0.399	0.407		0.01
Hearing impairment	0.487	0.522		0.00
Tubulopathy	5.455	0.175		0.02
Discrete metabolite elevations				
Elevated lactate	7.057	0.001	*	0.30
Elevated alanine	6.000	0.002	*	0.25
Elevated proline	4.571	0.013	*	0.20
Elevated CSF lactate	16.000	0.003	*	0.70
Urine organic acid analysis				
Urine lactate	2.301	0.001	*	0.28
Urine pyruvate	2.121	0.002	*	0.25
2-oxoisocaproate	1.451	0.200		0.16
3-hydroxybutyrate	1.088	0.691		0.15
2-hydroxybutyrate	1.431	0.191		0.17
Acetoacetate	1.136	0.563		0.15
3-hydroxyisovalerate	1.612	0.185		0.17
2-hydroxyisovalerate	1.659	0.242		0.16
2-oxoglutarate	3.328	0.003	*	0.28
4-hydroxyphenyllactate	1.165	0.720		0.15
4-hydroxyphenylpyruvate	1.205	0.749		0.15

3-methylglutaconate	3.618	0.222		0.17
Methylmalonate	2.324	0.397		0.16
Succinate	2.000	0.503		0.15
Citrate	3.136	0.009	*	0.24
Isocitrate	1.955	0.641		0.15
Fumarate	3.012	0.002	*	0.28
Malate	2.342	0.020	*	0.22
Adipate	0.678	0.434		0.16
Homovanillate	0.395	0.320		0.16
Neuroimaging				
Abnormal neuroimaging	2.854	0.196		0.17
Bilateral abnormalities	3.178	0.033	*	0.19
Cerebellar hypoplasia	0.557	0.404		0.15
Cerebral atrophy	0.263	0.220		0.17
White matter abnormality	2.444	0.094		0.17
Any other abnormality	4.000	0.026	*	0.20
Muscle tissue studies				
Abnormal histology	0.258	0.164		0.43
Excess lipids	1.342	0.630		0.00
Varied fibre size and type	0.683	0.484		0.00
Abnormal mitochondrial morphology	1.758	0.551		0.00
Abnormal RCEA	1.196	0.797		0.41
Complex I	0.000	0.081		0.44
Complex II+III	0.001	0.300		0.42
Complex IV	0.839	0.997		0.41
Mitochondrial disease score				
Score (clinical)	1.190	0.386		0.01
Score (investigation)	2.324	0.001	*	0.14
Score (total)	1.504	0.005	*	0.09

Table. S5. Logistic regression of discrete variables and continuous variables, which have not been log transformed prior to analysis.

Table S6

Variable	Odds ratio	p value	sig	McFadden pseudo R^2
Metabolites				
CSF Lactate	19.082	0.008	*	0.70
Lactate	4.180	0.001	*	0.31
Alanine	8.982	0.001	*	0.28
Proline	6.747	0.004	*	0.23
Arginine	0.802	0.655		0.14
Acylcarnitines				
Free carnitine	1.858	0.277		0.22
Acetyl carnitine	2.795	0.246		0.22
Propionyl carnitine	2.167	0.181		0.23
Butyryl carnitine	3.014	0.039	*	0.26
Isovaleryl carnitine	2.183	0.217		0.22
Hexanoyl carnitine	2.178	0.169		0.23
Octanoyl carnitine	1.730	0.516		0.21
Tetradecenoyl carnitine	1.325	0.605		0.21
Palmitoyl carnitine	0.482	0.295		0.22

Table. S6. Logistic regression of continuous variables, which have been log transformed prior to analysis.

Table S7

Table S7. Detailed variant description, assessment of pathogenicity and novelty of genetic variants for patients with confirmed (*n*=30) or probable (*n*=1) diagnoses. Table includes all patients, who received a genetic diagnosis via mitochondrial panel testing, alternative panel testing, or other methods; for details see manuscript section "mitochondrial disease genes". Inheritance patterns are all autosomal recessive, except for ID 25 and ID 51 (X-linked dominant), ID 2 and ID 66 (autosomal dominant (AD) disorders). Homozygous mutations are indicated by a comment in the column of the second allele. Gene names are according to HGNC nomenclature. n/a, not applicable.

Patient identifier (ID)	Gene	Allele 1			Allele 2			Parental testing
		Variant	Pathogenicity (ACMG evidences) [a]	Novelty	Variant	Pathogenicity (ACMG evidences) [a]	Novelty	
2	*GRIN2A*	NM_001134407.2:c.3706_3709dupTGCG p.(Asp1237ValfsTer12)	Likely pathogenic (PVS1_strong, PM2)	Novel	AD	n/a	n/a	None
3	*IBA57*	NM_001010867.2:c.802C>T p.(Arg268Cys)	Likely pathogenic (PS4_supporting, PM2, PM3, PP3)	PubMed: 29353736	NM_001010867.2:c.826C>T p.(Arg276Cys)	Likely pathogenic (PS4_supporting, PM2, PM3, PP3)	ClinVar: VCV000433546.1	None (but shown *in trans* in sequencing data)
4	*FBXL4*	NM_001278716.1:c.1304G>A p.(Arg435Gln)	Likely pathogenic (PS4_moderate, PM2, PM3)	PubMed: 25868664	Homozygous	n/a	n/a	None (unaffected sibling is heterozygous)
5	*VPS13B*	NM_017890.4:c.6713T>G p.(Leu2238Ter)	Pathogenic (PVS1, PM2)	Novel	Multi exon deletion [b]	Pathogenic	Novel	None
6	*LRPPRC*	NG_008247.1:g.104373G>T	Likely pathogenic (PS4_moderate, PM2, PM3, PP1_moderate, PVS1_moderate)	PubMed: 26510951	Homozygous	n/a	n/a	None
11	*ELAC2*	NM_018127.6:c.1126_1127dup p.(Val377GlnfsTer72)	Likely pathogenic (PM2, PM3, PVS1_strong)	Novel	NM_018127.6:c.1283G>A p.(Arg428His)	Likely pathogenic (PM2, PM3, PP3, PP4)	Novel	Compound heterozygous
17	*FBXL4*	NM_001278716.1:c.1703G>C p.(Gly568Ala)	Likely pathogenic (PS3_moderate, PM2, PM3, PP3)	PubMed: 23993194, 23993193	Homozygous	n/a	n/a	None
24	*FBXL4*	NM_001278716.1:c.1288C>T p.(Arg430Ter)	Pathogenic (PVS1, PM2, PM3)	Novel	NM_001278716.1:c.1750delT p.(Cys584ValfsTer21)	Likely pathogenic (PVS1_moderate, PM3, PM2)	Novel	Compound heterozygous

25	*NDUFB11*	NM_019056.6:c.183dupC p.(Glu62ArgfsTer7)	Pathogenic (PVS1, PM2)	Novel	Heterozygous (X-linked dominant)	n/a	n/a	*De novo*
26	*SDHA*	NM_004168.3:c.403G>C p.(Asp135His)	Uncertain significance (PM2, PP3)	Novel	NM_004168.3:c.1787A>G p.(Asp596Gly)	Uncertain significance (PM2, PP3)	Novel	None
28	*ELAC2*	NM_018127.6:c.2009del p.(Cys670SerfsTer14)	Pathogenic (PVS1, PM2, PM3)	PubMed: 31045291	NM_018127.6:c.2245C>T p.(His749Tyr)	Likely pathogenic (PS4_supporting, PM2, PM3_strong)	PubMed: 31045291	Compound heterozygous
29	*FOXRED1*	NM_017547.3:c.277C>T p.(Arg93Ter)	Pathogenic (PVS1, PM2)	Novel	NM_017547.3:c.1261G>A p.(Val421Met)	Likely pathogenic (PS3_supporting, PS4_supporting, PM2, PM3, PP3)	PubMed: 27215383	Mother heterozygous, father not tested
31	*AARS2*	NM_020745.3:c.302G>A p.(Arg101His)	Likely pathogenic (PM2, PP3, PM3_supporting, PP4_moderate)	Novel	Homozygous	n/a	n/a	Homozygosity confirmed (both parents heterozygous)
32	*CA5A*	single exon deletion	Likely pathogenic	Novel	Homozygous	n/a	n/a	Mother heterozygous, father not tested
36	*MMAB*	NM_052845.3:c.196G>A p.(Gly66Arg)	Likely pathogenic (PS3_supporting, PM2, PM3, PP3)	Novel	NM_052845.3:c.521C>T p.(Ser174Leu)	Likely pathogenic (PS4_supporting, PM2, PM3, PP3)	PubMed: 16410054	Compound heterozygous
43	*EARS2*	NM_001083614.1:c.184A>T p.(Ile62Phe)	Likely pathogenic (PM2, PS4_supporting, PM3, PS3_moderate)	PubMed: 27571996	Homozygous	n/a	n/a	None
51	*PDHA1*	NM_001173454.1:c.1256_1259dup p.(Trp421SerfsTer6)	Pathogenic (PS3, PS4_moderate, PM2, PVS1_moderate)	PubMed: 1779625	Heterozygous (X-linked dominant)	n/a	n/a	Absent from mother, likely *de novo*, but paternal testing not conducted
55	*LRPPRC*	NM_133259.3:c.3900+1G>T	Likely pathogenic (PS4_moderate, PM2, PM3, PP1_moderate, PVS1_moderate)	PubMed: 26510951	Homozygous	n/a	n/a	Homozygosity confirmed (both parents heterozygous)
62	*EXOSC3*	NM_016042.3:c.238G>T p.(Val80Phe)	Likely pathogenic (PS4_moderate, PM2, PM3, PP3)	PubMed: 23975261	Homozygous	n/a	n/a	Homozygosity confirmed (both parents heterozygous)
64	*C12orf65*	NM_001194995.1:c.210delA p.(Gly72AlafsTer13)	Pathogenic (PVS1, PS4_moderate, PM3)	PubMed: 20598281	NM_001194995.1:c.258_270dupCATCCCCTCAGGC p.(Ile91HisfsTer16)	Pathogenic (PVS1, PM2, PM3)	Novel	Compound heterozygous
66	*CACNA1A*	NM_023035.2:c.5006G>A p.(Arg1669Gln)	Likely pathogenic (PS2, PS4_moderate, PM2, PP3)	PubMed: 16325861	AD	n/a	n/a	None

77	*AARS2*	NM_020745.3:c.1774C>T p.(Arg592Trp)	Likely pathogenic (PS3_supporting, PS4_moderate, PM3_strong)	PubMed: 21549344	Homozygous	n/a	n/a	None
78	*FBXL4*	NM_001278716.1:c.1444C>T p.(Arg482Trp)	Likely pathogenic (PS4_moderate, PM2, PP1_strong)	PubMed: 23993194	Homozygous	n/a	n/a	Homozygosity confirmed (parents heterozygous)
81	*RMND1*	NM_017909.3:c.713A>G p.(Asn238Ser)	Likely pathogenic (PS3, PM3)	PubMed: 25058219	NG_033031.1:g.34644A>C	Likely pathogenic (PM2, PM3, PP3, PP4)	Novel	Compound heterozygous
83	*LAMA2*	NM_000426.3:c.3085C>T p.(Arg1029Ter)	Pathogenic (PVS1, PM2)	PubMed: 11938437	Homozygous	n/a	n/a	Homozygosity confirmed (parents heterozygous)
75	*ECHS1*	NM_004092.3:c.748dupA p.(Met250AsnfsTer8)	Pathogenic (PVS1, PM2)	Novel	Heterozygous [c]	n/a	n/a	None
18	*MT-ND1*	m.3955G>A p.(Ala217Thr)	n/a	Novel	mtDNA mutation	n/a	n/a	Mutation not detected in maternal blood and urine samples
22	*TBC1D24*	NM_001199107.1:c.901C>T p.(Gln301Ter)	Pathogenic (PVS1, PM2)	Novel (but patient reported in PubMed: 31922275)	NM_001199107.1:c.605C>T p.(Ser202Leu)	Uncertain significance (PM2, PM3)	Novel (but patient reported in PubMed: 31922275)	Compound heterozygous
34	*NKX6-2*	NM_177400.2:c.121A>T p.(Lys41Ter)	Pathogenic (PVS1, PM2)	PubMed: 28575651	Homozygous	n/a	n/a	None
40	*MT-ND5*	m.13514A>G p.(Asp393Gly)	Pathogenic	PubMed: 18332249	mtDNA mutation	n/a	n/a	None
49	*MT-ND3*	m.10197G>A p.(Ala47Thr)	Pathogenic	PubMed: 17152068	mtDNA mutation	n/a	n/a	None

[a] Pathogenicity assessed using ACMG/AMP (American College of Medical Genetics/Association for Molecular Pathology) guidelines (Richards *et al.* 2015, PMID: 25741868), with additional interpretative and evidence weighting guidance following ACGS (Association for Clinical Genomic Science) best practice guidelines (Ellard *et al.* 2020: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf, accessed 17th October 2020). Evidence weighting is supplied where divergent from original evidence level.

[b] Multi-exon deletion was detected by read depth analysis using ExomeDepth (see methods) and was confirmed using qPCR.

[c] Deficiency of ECHS1 confirmed by enzyme activity studies.