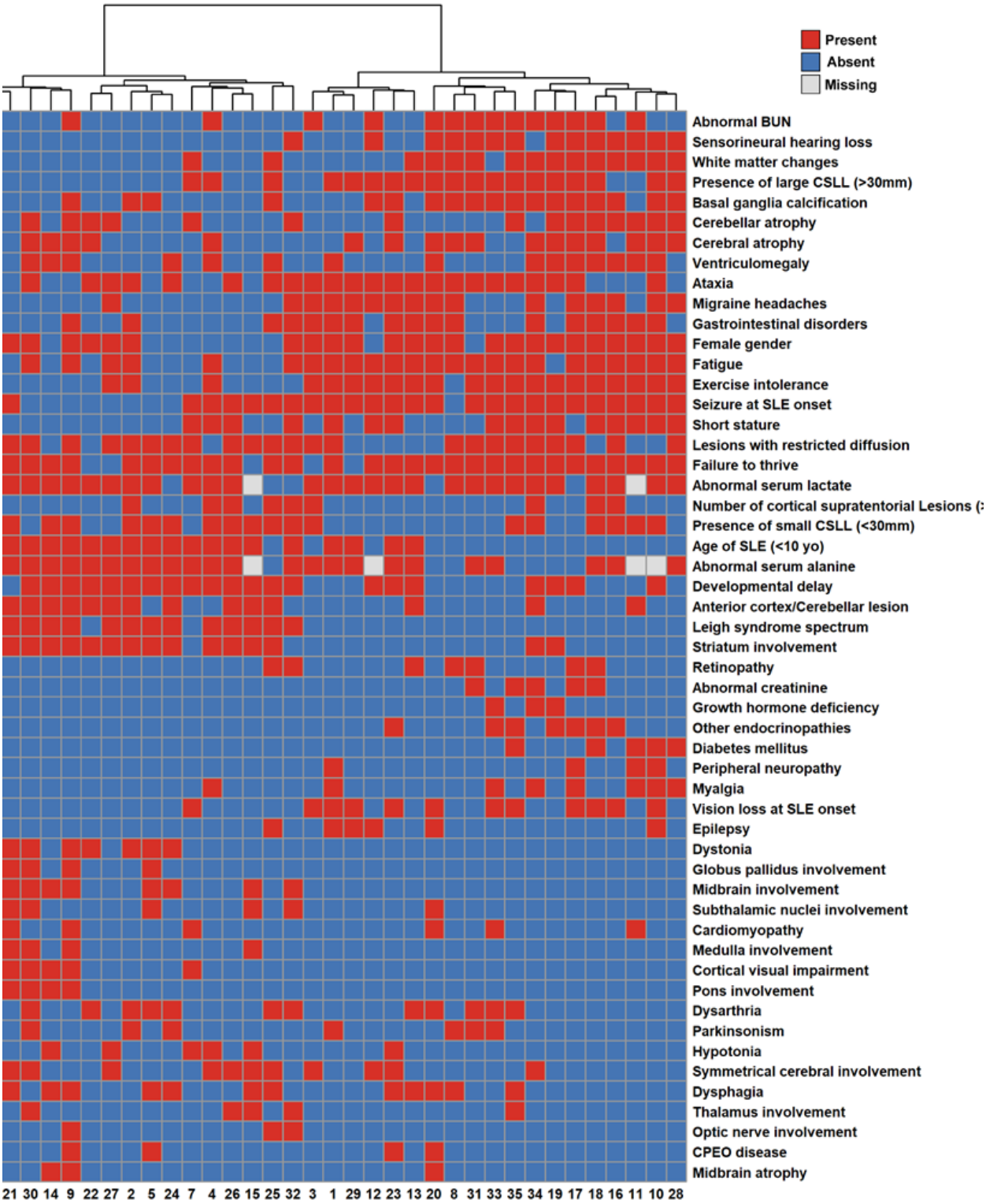
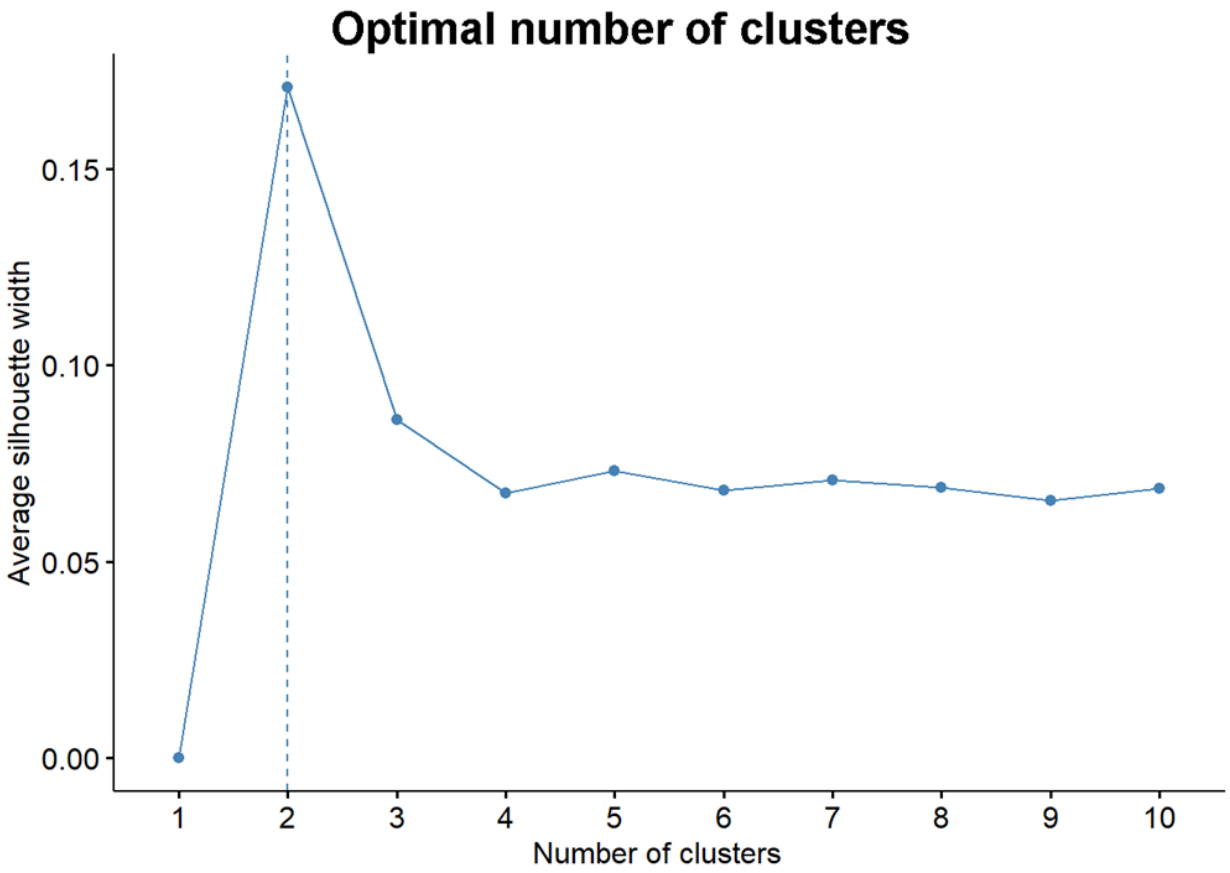
Online-Only Figures

eFigure 1. Unsupervised cluster analysis of the binary data of thirty-five patients based on fifty-three variables (y-axis) including demographic, clinical, neuroimaging and laboratory findings using Ward hierarchical clustering method. Each column is representative of one patient (x-axis). Red and blue squares show the presence and absence of the findings in each patient respectively. At the top of the heatmap, the height of dendrogram for main clusters demonstrates the high distance between clusters which confirms the two distinct patterns of disease. SLE: stroke-like episode; CPEO: chronic progressive external ophthalmoplegia; CSLL: cortical stroke-like lesion; BUN: blood urea nitrogen.


Present
Absent
Missing
Abnormal BUN
Sensorineural hearing loss
White matter changes
Presence of large CSLL (>30mm)
Basal ganglia calcification
Cerebellar atrophy
Cerebral atrophy
Ventriculomegaly
Ataxia
Migraine headaches
Gastrointestinal disorders
Female gender
Fatigue
Exercise intolerance
Seizure at SLE onset
Short stature
Lesions with restricted diffusion
Failure to thrive
Abnormal serum lactate
Number of cortical supratentorial Lesions (
Presence of small CSLL (<30mm)
Age of SLE (<10 yo)
Abnormal serum alanine
Developmental delay
Anterior cortex/Cerebellar lesion
Leigh syndrome spectrum
Striatum involvement
Retinopathy
Abnormal creatinine
Growth hormone deficiency
Other endocrinopathies
Diabetes mellitus
Peripheral neuropathy
Myalgia
Vision loss at SLE onset
Epilepsy
Dystonia
Globus pallidus involvement
Midbrain involvement
Subthalamic nuclei involvement
Cardiomyopathy
Medulla involvement
Cortical visual impairment
Pons involvement
Dysarthria
Parkinsonism
Hypotonia
Symmetrical cerebral involvement
Dysphagia
Thalamus involvement
Optic nerve involvement
CPEO disease
Midbrain atrophy
21 30 14 9 22 27 2 5 24 7 4 26 15 25 32 3 1 29 12 23 13 20 8 31 33 35 34 19 17 18 16 11 10 28

eFigure 2. Average Silhouette plot to determine the optimal number of clusters based on the similarity of patients within clusters compared to the other clusters. The maximal average silhouette of two demonstrates the optimal number of clusters



Online-Only Tables

eTable 1. Demographic and genetic characteristics of MELAS subjects

eTable 2. Comparing pathogenic variants of the study cohort based on the unsupervised cluster analysis.

eTable 1. Demographic and genetic characteristics of MELAS subjects

Case number	Sex	Clinical Diagnosis	Genome origin of disease	Metabolic pathophysiologic mechanism	Specific mitochondrial pathway defect	Causal disease gene	Pathogenic Variant(s) (if mtDNA, heteroplasmy % and tissue included)	Age at first SLE	Age at first MRI with lesion (years)
1	F	MELAS	mtDNA	Respiratory chain subunit	Complex III	*MT-CYB*	m.14864T>C (32%, blood; 57%, urine; 39%, muscle; 42%, fibroblasts)	10	10
2	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10191T>C (76%, blood; 48%, muscle; 86% fibroblasts)	3	7.5
3	F	MELAS	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10158T>C (35%, blood; 81%, buccal; 77% urine)	12	12
4	M	MELAS/Leigh	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (78%, blood)	4	5.5
5	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10191T>C (65%, blood)	4	7.5
6	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10191T>C (100%, blood)	2	2
7	M	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (77%, blood)	7	7.5
8	M	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (N/A)	57	57
9	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND5*	m.13513G>A (46%, blood; 35%, buccal; 43%, urine; 66.5%, fibroblasts)	2	10
10	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (45%, buccal; 84%, urine)	24	24
11	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (36%, buccal)	42	42
12	M	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (54%, blood; 50%, buccal; 96%, urine)	19	24
13	F	MELAS	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP6*	m.8609dupC, p.L29sfsX36 (67%, blood)	10	10
14	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP6*	m.8993T>G (100%, blood)	0.5	0.5
15	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10158T>C (94%, blood)	0.2	0.3
16	F	MELAS	mtDNA	Respiratory chain subunit	Complex I	*MT-ND5*	m.13513G>A (22%, blood, 67%, urine)	19	19
17	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (35%, blood)	29	30
18	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (58%, blood; 55%, saliva)	11	15
19	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (57%, blood; 61%, buccal; 90%, urine)	18	18
20	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (8%, blood; 60%, urine)	42	42
21	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP8*	m.8430T>C	4	4
22	F	MELAS	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP6*	m.8993T>G (99%, blood)	8	11
23	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (52%, blood; 53%, saliva; 68%, muscle)	10	12
24	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10191T>C (44%, blood; 78%, buccal; 42%, fibroblast; 78%, urine; 90%, muscle)	7	15
25	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10197G>A (90%, blood)	11	11
26	M	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND3*	m.10158T>C (71%, blood; 80%, muscle)	7	7
27	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP6*	m.8993T>G (100%, blood; 95%, buccal)	7	7
28	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (12.4%, blood; 22%, buccal; 78%, urine)	31	40
29	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (84%, blood; 98%, urine)	6	7
30	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex V	*MT-ATP6*	m.8993T>C (92%, blood; 96%, urine)	1.5	17
31	M	MELAS	mtDNA	Respiratory chain subunit	Complex I	*MT-ND5*	m.13513G>A (73%, muscle)	39	40
32	F	MELAS/Leigh	mtDNA	Respiratory chain subunit	Complex I	*MT-ND5*	m.13513G>A (45%, muscle)	6	6
33	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TV*	m.1630A>G (75%, blood; 95%, urine; 60%, fibroblast)	15	15
34	F	MELAS	mtDNA	Respiratory chain subunit	Complex I	*MT-ND1*	m.3946G>A (28%, blood; 51%, saliva; 78%, urine; 78%, muscle)	14	16
35	F	MELAS	mtDNA	DNA translation	Mitochondrial tRNAs	*MT-TL1*	m.3243A>G (39%, buccal)	32	33

M: male; F: female; mtDNA: mitochondrial DNA; DNA: Deoxyribonucleic acid; tRNA: Transfer ribonucleic acid; MRI: Magnetic resonance imaging; MELAS: Mitochondrial Encephalomyopathy Lactic Acidosis; SLE: Stroke-like episodes.

eTable 2. Comparing pathogenic variants of the study cohort based on the unsupervised cluster analysis

	Characteristics	Cluster 1 (N=19)	Cluster 2 (N=16)	P value	Odds ratio (95% CI)
Demographic and clinical features	Female gender	17	8	0.022	0.12 (0.02-0.68)
	Age of SLE (< 10 Y)	4	15	<0.001*	56 (5.6-564)
	Seizure at SLE onset	18	8	0.005*	0.06 (0.006-0.5)
	Vision loss at SLE onset	11	1	0.002*	0.05 (0.005-0.4)
	Failure to thrive	17	13	0.642	0.5 (0.07-3.5)
	Short stature	12	5	0.092	0.3 (0.06-1.1)
	Sensorineural hearing loss	13	1	<0.001*	0.03 (0.003-0.29)
	Ataxia	15	8	0.09	0.27 (0.06-1.17)
	Migraine headaches	14	2	<0.001*	0.05 (0.008-0.31)
	Gastrointestinal disorders [#]	13	5	0.044	0.21 (0.05-0.88)
	Fatigue	18	6	0.001*	0.04 (0.004-0.32)
	Exercise intolerance	18	3	<0.001*	0.013 (0.001-0.14)
	Developmental delay	7	15	0.001*	25.7 (2.8-239)
	Overlap with Leigh syndrome spectrum	0	14	<0.001*	226 (10-5079)
	Retinopathy	5	2	0.415	0.4 (0.07-2.4)
	Growth hormone deficiency	3	0	0.234	0.14 (0.007-3)
	Diabetes mellitus	5	0	0.049	0.08 (0.004-1.6)
	Other endocrinopathies [§]	7	0	0.009*	0.05 (0.003-0.97)
	Peripheral neuropathy	4	0	0.109	0.1 (0.005-2.1)
	Myalgia	7	1	0.047	0.11 (0.01-1.06)
	Epilepsy	5	2	0.415	0.4 (0.07-2.4)
	Dystonia	0	8	0.001*	39 (2-755)
	Cardiomyopathy	3	4	0.677	1.8 (0.33-9.5)
	Cortical visual impairment	0	6	0.005*	24 (1.2-472)
	Dysarthria	5	7	0.311	2.2 (0.5-9)
	Parkinsonism	4	3	1	0.86 (0.16-4.6)
	Hypotonia	1	6	0.032	10.8 (1.13-103)
	Dysphagia	5	7	0.311	2.18 (0.53-9)
	CPEO disease	2	2	1	1.2 (0.15-9.8)
Neuroimaging findings [†]	White matter changes	13	2	0.002*	0.07 (0.01-0.4)
	Presence of large CSLL (>30 mm)	16	3	<0.001*	0.04 (0.007-0.25)
	Basal ganglia calcification	14	4	0.072	4.7 (1-21)
	Cerebral atrophy	12	5	0.092	0.26 (0.06-1.1)
	Cerebellar atrophy	9	6	0.734	0.67 (0.17-2.6)
	Ventriculomegaly	9	7	1	0.86 (0.23-3.3)
	Lesions with restricted diffusion	11	13	0.167	3.15 (0.67-14.9)
	Number of cortical supratentorial lesions (>3)	4	6	0.454	2.2 (0.5-10)
	Presence of small CSLL (<30 mm)	7	12	0.041	5.1 (1.2-22.3)
	Anterior cortex/cerebellar lesion	3	11	0.002*	11.7 (2.3-59.5)
	Striatum involvement	2	14	<0.001*	59.5 (7.4-476)
	Globus pallidus involvement	0	5	0.013	18 (0.94-369)
	Midbrain lesions	0	9	<0.001*	49.4 (2.5-959)
	Subthalamic nuclei involvement	1	6	0.032	10.8 (1.1-103)
	Midbrain atrophy	1	2	0.582	2.6 (0.2-31.3)

	Pons involvement	0	4	0.035	14 (0.7-284)
	Medulla involvement	0	4	0.035	14 (0.7-284)
	Symmetrical cerebral involvement	4	7	0.273	2.9 (0.7-12.8)
	Thalamus involvement	1	4	0.156	6 (0.6-60.4)
	Optic nerve involvement	0	3	0.086	1.2 (0.97-1.6)
Laboratory findings	Abnormal BUN	12	2	0.005*	0.08 (0.01-0.5)
	Abnormal serum lactate	16	12	0.639	0.5 (0.07-3.5)
	Abnormal serum creatinine	5	0	0.049	0.08 (0.004-1.57)
	Abnormal serum alanine	10	14	0.083	8.4 (0.9-81)

SLE: stroke-like episode; CPEO: chronic progressive external ophthalmoplegia; CSLL: cortical stroke-like lesion; BUN: blood urea nitrogen

* p-value<0.01

† According to the first brain MRI with cortical lesion

Gastrointestinal disorders include constipation, dysmotility, gastroesophageal reflux disease, gastroparesis, and irritable bowel syndrome

§ Other endocrinopathies include hypothyroidism, delayed puberty, and irregular menstruation