

Decoding mitochondrial heterogeneity in single muscle fibres by Imaging Mass Cytometry

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Supplementary Table 1: Patient information. Information on patients detailing gender, age at biopsy, clinical information and genetic defect.

Subjects	Gender	Age (years)	Clinical information	Genetic defect	Previous reports
Pathogenic variants in nuclear-encoded proteins affecting complex I assembly					
P01	M	Adult	Exercise intolerance, unable to perform sustained aerobic exercise normal resting lactate, normal CK	<i>TMEM126B</i> (NG_053120.1) Homozygous c.635G>T, p.(Gly212Val) variant	Ahmed <i>et al.</i> (2017) Patient 14
P02	M	Adult	Exercise intolerance, muscle cramps, elevated serum lactate	<i>ACAD9</i> (NG_017064.1) Compound heterozygous c.1150G>A, p.(Val384Met) and c.1168G>A, p.(Ala390Thr) variant	Ahmed <i>et al.</i> (2017) Patient 15
Single, large-scale mtDNA deletions					
P03	F	29	CPEO and bilateral ptosis	Deletion size 4372bp Breakpoints: 8929-13301 mtDNA deletion level: 53% Deleted genes: part of <i>ATP6</i> , <i>MTCO3</i> , <i>MT-TG</i> , <i>ND3</i> , <i>MT-TR</i> , <i>ND4L</i> , <i>ND4</i> , <i>MT-TH</i> , <i>MT-S2</i> , <i>MT-L2</i> and part of <i>ND5</i>	Rocha <i>et al.</i> (2018) Patient 4
P04	F	39	CPEO, diplopia	Deletion size 7498bp Breakpoints: 7130-14628 mtDNA deletion level: 28% Deleted genes: part of <i>MTCO1</i> , <i>MT-S1</i> , <i>MT-TD</i> , <i>MTCO2</i> , <i>MT-TK</i> , <i>ATP8</i> , <i>ATP6</i> , <i>MTCO3</i> , <i>MT-TG</i> , <i>ND3</i> , <i>MT-TR</i> , <i>ND4L</i> , <i>ND4</i> , <i>MT-TH</i> , <i>MT-S2</i> , <i>MT-LS</i> , <i>ND5</i> , <i>ND6</i> , <i>MT-TE</i> and part of <i>CYTb</i>	Rocha <i>et al.</i> (2018) Patient 19
Pathogenic variants in mitochondrially-encoded tRNA^{Leu(UUR)} (<i>MT-TL1</i>)					

P05	F	25	Exercise intolerance, ptosis	m.3243A>G <i>MT-TL1</i> variant	Rocha <i>et al.</i> (2015) Patient 12
P06	F	47	Modest exercise intolerance	m.3243A>G <i>MT-TL1</i> variant	Rocha <i>et al.</i> (2015) Patient 15
P07	M	53	CPEO	m.3243A>G <i>MT-TL1</i> variant	Rocha <i>et al.</i> (2015) Patient 16
Point mutations in other mitochondrially-encoded tRNAs					
P08	M	33	Mitochondrial myopathy	m.10010T>C <i>MT-TG</i> variant	Rocha <i>et al.</i> (2015) Patient 19
P09	F	35	Mild muscle weakness	m.14709T>C <i>MT-TE</i> variant	Rocha <i>et al.</i> (2015) Patient 20
P10	M	63	Exercise intolerance, prominent exertional dyspnea	m.5543T>C <i>MT-TW</i> variant	Rocha <i>et al.</i> (2015) Patient 21
Healthy controls					
C01	M	20	Taken during anterior cruciate ligament surgery	n.a	
C02	M	24	Taken during anterior cruciate ligament surgery	n.a	
C03	F	23	Taken during anterior cruciate ligament surgery	n.a	

CK; Creatine kinase, CPEO; Chronic progressive external ophthalmoplegia, n.a., not applicable

Supplementary Table 2: List of primary and secondary antibodies. Table includes details of antibodies such as host species, isotype, dilution used and supplier.

Antibodies	Host and isotype	Dilution	Company
Primary antibodies			
Laminin (membrane marker)	Rabbit	1:50	Sigma-Aldrich (L9393)
Dystrophin (membrane marker)	Mouse	1:50	EMD Millipore (Mab 1645)
NDUFB8 (CI)	Mouse IgG1	1:50 (IMC) 1:100 (IF)	Abcam (110242)
GRIM19 (CI)	Mouse IgG2b	1:50 (IMC) 1:100 (IF)	Abcam (110240)
SDHA (CII)	Mouse IgG1	1:50 (IMC) 1:100 (IF)	Abcam (14715)
UqcCRC2 (C III)	Mouse IgG1	1:50 (IMC) 1:100 (IF)	Abcam (14745)
MTCO1 (CIV)	Mouse IgG2a	1:50 (IMC) 1:100 (IF)	Abcam (14705)
COX4+4L2 (CIV)	Mouse IgG2a	1:50 (IMC) 1:100 (IF)	Abcam (110261)
OSCP CV)	Mouse IgG1	1:50 (IMC) 1:100 (IF)	Abcam (110276)
VDAC1 (mass marker)	Mouse IgG2b	1:50 (IMC) 1:100 (IF)	Abcam (14734)
Secondary antibodies			
Anti-rabbit Alexa Fluor 405nm	Goat	1:100	Life Technologies (A31556)
Anti-IgG2a Alexa Fluor 488nm	Goat	1:200	Life Technologies (A21131)
Anti-IgG2b Alexa Fluor 546nm	Goat	1:200	Life Technologies (A21143)
Anti-IgG1 biotin	Goat	1:200	Life Technologies (A10519)
Streptavidin Alexa Fluor 647nm	Goat	1:100	Life Technologies (S32357)
Anti-IgG1 Alexa Fluor 647nm	Goat	1:200	Life Technologies (AA21240)
Anti-mouse IgG Alexa Fluor 488	Goat	1:200	Life Technologies (A11001)

Supplementary Table 3: Antibodies and their respective metal conjugates.

Antibody	Metal conjugate
Dystrophin	176Yb
UqCRC2	174Yb
MTCO1	172Yb
COX4+4L2	168Er
SDHA	153Eu
VDAC1	166Er
GRIM19	164Dy
OSCP	161Dy
NDUFB8	160Gd

Supplementary Table 4: Antibody combinations for comparison of Imaging mass cytometry and immunofluorescences. Combinations of antibodies for quadruple immunofluorescence.

Combination 1	Combination 2	Combination 3	Combination 4	Combination 5
Laminin	Laminin	Laminin	Laminin	Laminin
NDUFB8	NDUFB8	SDHA	OSCP	UqCRC2
MTCO1	COX4+4L2	TOMM22	MTCO1	MTCO1
VDAC1	VDAC1	GRIM19	VDAC1	VDAC1

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