Phenotypic diversity associated with the MT-TV gene m.1644G>A mutation, a matter of quantity.

Matthew Fraidakis, Claude Jardel, Stéphane Allouche, Isabelle Nelson, Karine Auré, Abdelhamid Slama, Isabelle Lemière, Jean Philippe Thenint, Jean Baptiste Hamon, Fabien Zagnoli, et al.

Table 1: Patients characteristics

Patient (Family)	**1 (F1)**	**2 (F2)**	**3 (II-3 F3)**	**4 (III-1 F3)**
Age (onset/present)	25/50	18/25	3/42	NN/7
First sign	Deafness	Suicide	Dystonia	Dev. delay
Cognitive decline	+	+	+	+
Cerebellar ataxia	+	-	-	+
Psychiatric signs	-	+	-	-
Deafness	+	-	-	-
Pyramidal Syndrome	-	-	+	-
Seizures, myoclonia	-	-	-	+
Dystonia	-	-	+	-
Muscle weakness	+	-	-	-
MRI Cerebellar A	+	-	+	+
Cortical A	-	+	-	-
Brainstem A	+	-	-	-
Striatum HS	-	+	+	-
White matter HS	-	-	-	+
EMG + NCV	Myogenic	Axonal	Axonal	NA
Lactatemia	**5.3** (45 y)	**3.7** (23 y)	1.7 (39 y)	1.6 (3 y)
Lactatorrachia	NA	**High** (MRS)	**4.4** (39y)	N (MRS)
Muscle Histology	SDH++/COX-	COX-	COX-	Low COX

Age is indicated in years: first number is the patient's age at onset; second number is the patient's age at diagnosis or at death (Patient 1); + = present; - = absent; A=Atrophy; Axonal = axonal sensorimotor polyneuropathy; COX-=presence of scattered cytochrome c oxidase (COX) negative fibers; Dev. Delay Development Delay; EMG + NCV = electromyography

with measurement of Nerve Conduction Velocities; High = values above highest normal values; HS=hypersignals on brain MRI; Low COX= low histochemical cytochrome c oxidase reaction. Lactatemia and lactatorrachia: the level of lactate is indicated as mM with the age at measurement in years (y) indicated between brackets; N=normal values; NA =data non available; NMR: nuclear Magnetic Resonance.

Table 2: Spectrophotometric assays of OXPHOS in the muscle of the patients with the m.1644G>A mutation

	Patient 1 (control values)	Patient 2 (control values)	Patient 3 (control values)	Patient 4 (control values)
CI	14 (22±7)	**4*** (59±32)	**7** (42±15)	84 (60-123)
CII	**78*** (31±8)	74 (78±38)	64 (61±20)	151 (105-270)
CIII	**170*** (107±26)	224(242±132)	210 (162±67)	1401 (1007-2465)
CIV	70 (56±17)	**20*** (201±99)	**47*** (193±61)	254 (175-385) ‡ 1598 (1100-2800)
II+III	**40*** (18±7)	69 (76±46)	42 (50±16)	367 (202-525)
CS	**563*** (153±35)	195 (215±110)	172 (216±70)	100 (70-150) ‡
CI/CS	**0.02*** (0.15±0.04)	**0.02*** (0.28±0.08)	**0.04*** (0.20±0.05)	-
CII/CS	0.14 (0.21±0.06)	0.38 (0.38±0.10)	0.37 (0.29±0.07)	-
CIII/CS	**0.30*** (0.72±0.20)	1.15 (1.18±0.45)	1.22 (0.78±0.22)	-
CIV/CS	**0.12*** (0.37±0.11)	**0.10*** (1.40±0.40)	**0.27*** (0.94±0.24)	-

Spectrophotometric assays of respiratory chain activities are expressed as nanomoles per minute and mg protein. They were performed on muscle homogenates for Patients 1, 2 and 3 and on isolated muscle mitochondria for Patient 4 with the exception of the two assays marked with ‡ that were performed on muscle homogenates. Each patient has been analyzed with different assay protocols, the results have therefore to be compared to their own set of control values expressed as mean±standard deviation (Patients 1, 2 and 3) or as normal range (Patient 4). Numbers marked with * are significantly abnormal. CI=respiratory complex I

(rotenone sensitive NADH ubiquinone oxido-reductase) activity; CII= respiratory complex II (succinate ubiquinone oxido-reductase) activity; CIII= respiratory complex III (antimycin sensitive ubiquinol cytochrome c oxido-reductase) activity; CIV= respiratory complex IV (cytochrome c oxidase) activity; II+III=combined respiratory complex II and III (succinate cytochrome c oxido-reductase) activity; CS=citrate synthase, a Krebs cycle enzyme, activity.

Table 3: Combined respiratory chain defect was observed in the cultured skin fibroblasts and in cybrids with the m.1644G>A mutation

	Patient 2	Patient 3	Patient 4	Control fibroblasts	Mutant cybrids	Control cybrids
VO_2 (succ)	10.6	11.3	11.0	11.5±3.2	**6.6***	12.9±4.1
VO_2 (pyr)	3.3	4.5	4.31	7.3±2.5	**2.9***	9.7±3.8
ratio	**3.11***	**2.51***	**2.55***	1.63±0.55	**2.27***	1.50±0.63
CII	31	36	34	26±8	49	43±19
CIII	28	80	30	55±17	50	47±12
CIV	**24***	**38***	**42***	83±16	**13***	71±18
II+III	26	35	35	31±7	62	50±21
CS	68	92	72	77±19	**238***	115±28
CII/CS	0.46	0.39	0.47	0.37±0.10	0.21	0.39±0.17
CIII/CS	0.41	0.87	0.42	0.79±0.25	0.21	0.41±0.11
CIV/CS	**0.35***	**0.41***	**0.58***	1.11±0.22	**0.05***	0.63±0.19

All values are expressed as expressed as nanomoles per minute and mg protein. Mutant cybrids had 100% m.1644G>A mutation. VO_2 (succ)]: respiration with succinate that enters the respiratory chain at complex II; VO_2 (pyr): respiration with pyruvate that enters the respiratory chain at complex I; ratio: ratio between the respiration rates on these two substrates; CII= respiratory complex II (succinate ubiquinone oxido-reductase) activity; CIII= respiratory complex III (antimycin sensitive ubiquinol cytochrome c oxido-reductase) activity; CIV= respiratory complex IV (cytochrome c oxidase) activity; II+III=combined respiratory complex II and III (succinate cytochrome c oxido-reductase) activity; CS=citrate synthase, a Krebs cycle enzyme, activity; control values are expressed as

mean±standarddeviation of 52 analyses for fibroblasts and 14 for cybrids; Numbers marked with * are significantly abnormal.

Table 4: Homogeneous distribution of the m.1644G>A mutation in the patients' different tissues

	muscle	blood	Buccal C	Urinary S	Nasal C	Fibros
Patient1	92%					
Patient2	100%	100%				100%
Patient 3 (II-3)	100%		98%	98%	100%	100%
Patient 4 (III-1)	61%	63%	66%	74%		66%
Pt3 mother (I-1)		64%	54%	65%	64%	
Pt3 sister (II-1)		65%	68%	79%		
Pt3 sister (II-5)		56%	52%	47%		

Proportion of the mutation evaluated with fluorescent PCR-restriction. Buccal C=DNA extracted from buccal mucosa; Urinary S=DNA extracted from the urinary sediment; Nasal C=DNA extracted from nasal mucosa; fibros=DNA extracted from cultured skin fibroblasts.