KHONDRION | bringing energy to life

MELAS SPECTRUM DISORDERS AND SONLICROMANOL PROGRESS UPDATE

Prof. Dr. Jan Smeitink, CEO – MITOACTION webinar – March 2022

Disclosure and Cautionary Note Regarding Forward-Looking Statements

This presentation contains forward-looking statements. All statements, other than statements of historical facts, contained in this document, including statements regarding our strategy, future operations, future financial position, future revenues, projected costs, prospects, plans and objectives of management, are forward-looking statements. The words "anticipate," "believe," "estimate," "expect," "intend," "may," "plan," "predict," "project," "target," "potential," "will," "would," "could," "should," "continue," and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. Any forward-looking statements represent our views only as of today and should not be relied upon as representing our views as of any subsequent date. If underlying assumptions prove inaccurate or known or unknown risks or uncertainties materialize, actual results could vary materially from the expectations and projections of Khondrion. Risks and uncertainties include but are not limited to challenges and uncertainties inherent in product development, including the uncertainties of clinical success and the timeline for the availability of sonlicromanol [KH176]. While we may elect to update these forward-looking statements at some point in the future, we specifically disclaim any obligation to do so, even if our views change.

OUTLINE

1. THE COMPANY

2. PRIMARY MITOCHONDRIAL DISEASES

3. MELAS SPECTRUM DISORDERS
   - PHENOTYPES
   - CAUSE AND CELLULAR CONSEQUENCES

4. MITOCHONDRIAL DISEASE SMALL MOLECULE DRUG DEVELOPMENT

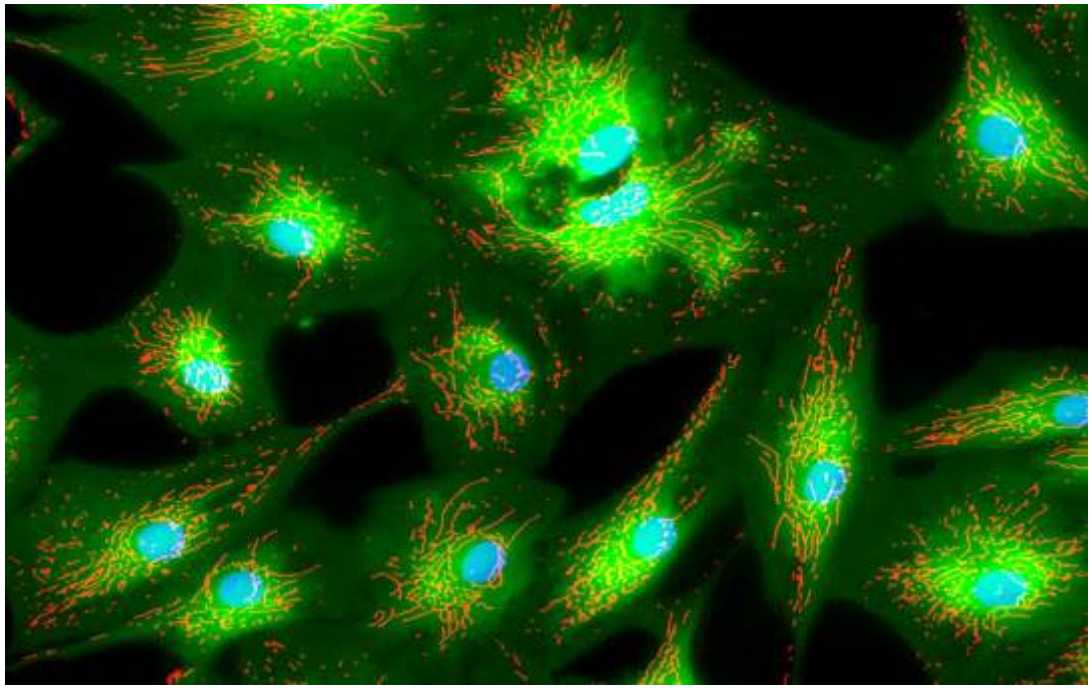
5. CLINICAL TRIALS UPDATE

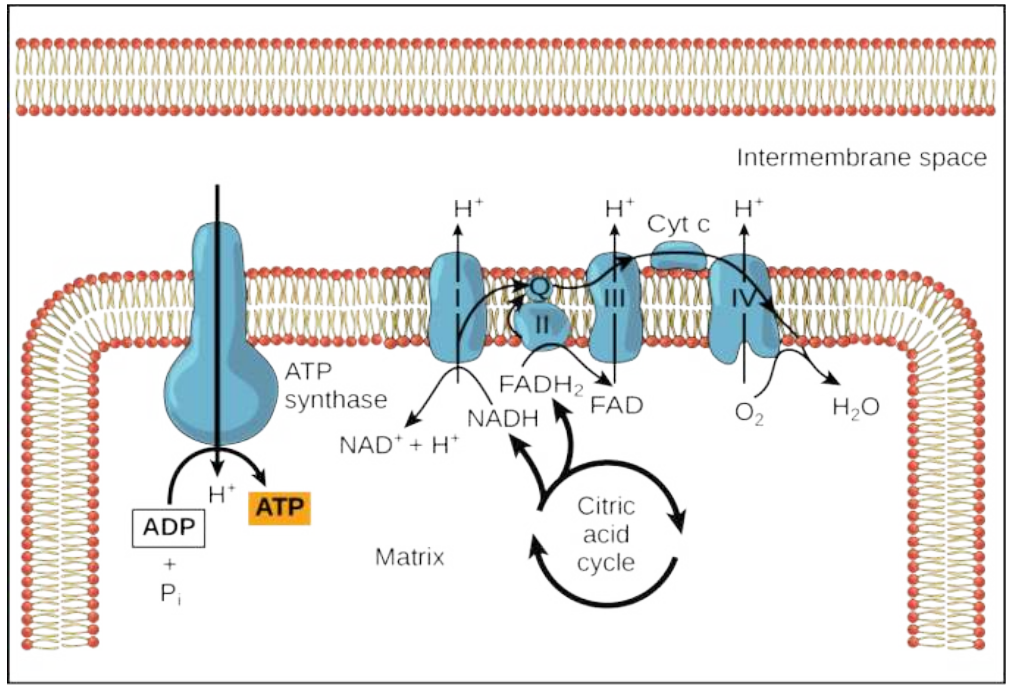
- Headquartered : Nijmegen, The Netherlands

- Founded : 2012
- Focus : Primary Mitochondrial Diseases

PRIMARY MITOCHONDRIAL DISEASES

Hampered mitochondrial OXPHOS system due to nuclear DNA or mitochondrial DNA mutations
Prevalence of 1 in 5,000 live births





Primary Mitochondrial Diseases

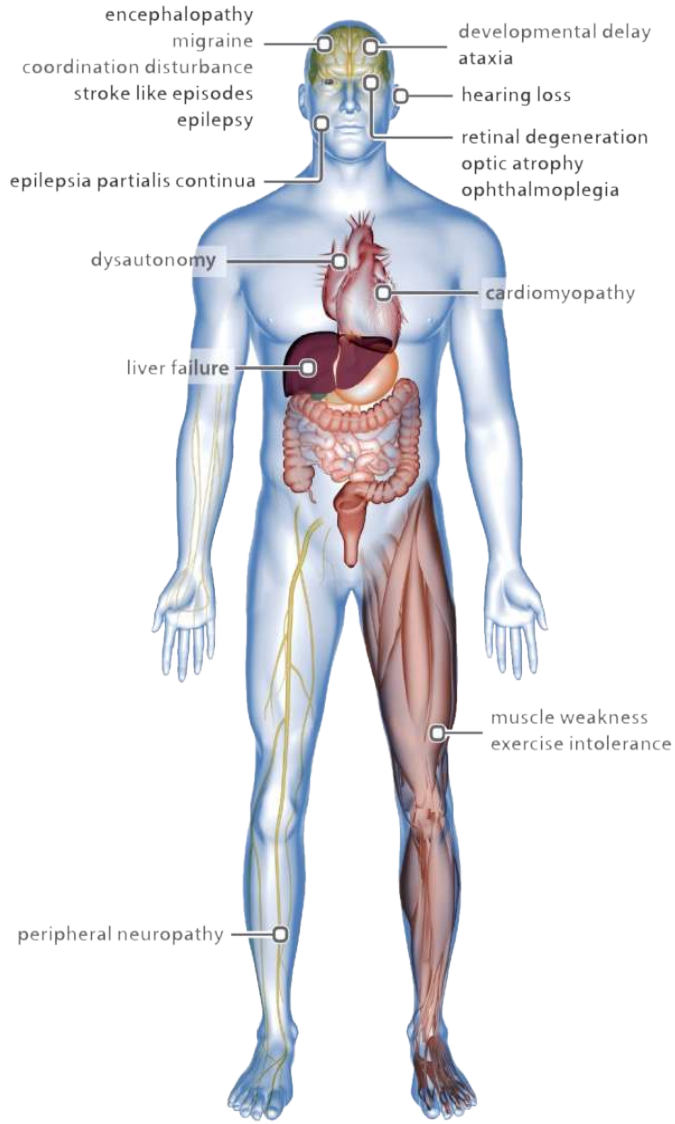
Rare, debilitating diseases

Children and Adults

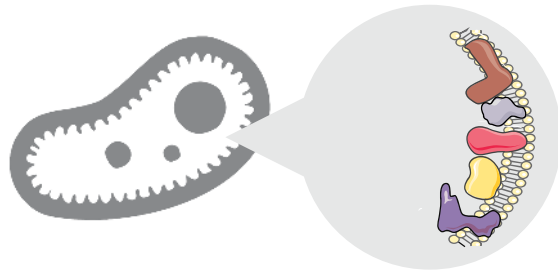
Any organ, any mode of inheritance

Great variety in clinical phenotypes

High unmet medical need



Mutations in over 200 genes known all converting to the OXPHOS system to cause Primary Mitochondrial Disease



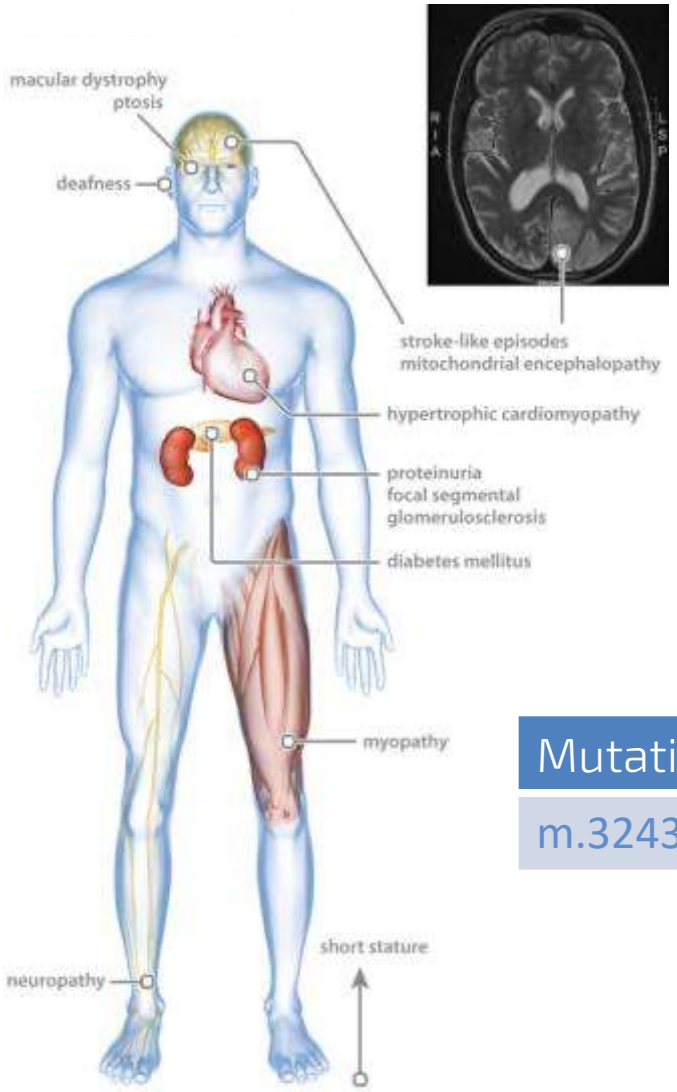
Common consequences:

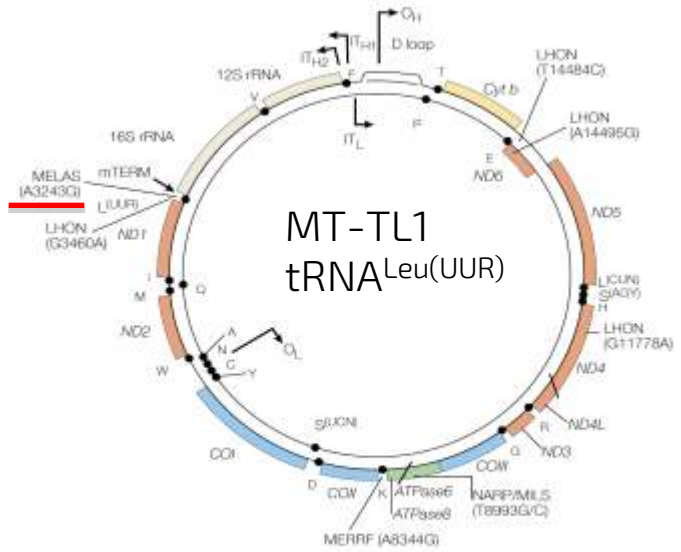
Reductive distress - disturbed redox balance

Oxidative distress – Ferroptosis due to ROS-induced lipid peroxidation

Inflammation due to increased PGE2

MELAS SPECTRUM DISORDERS





Goto et al., Nature, 1990

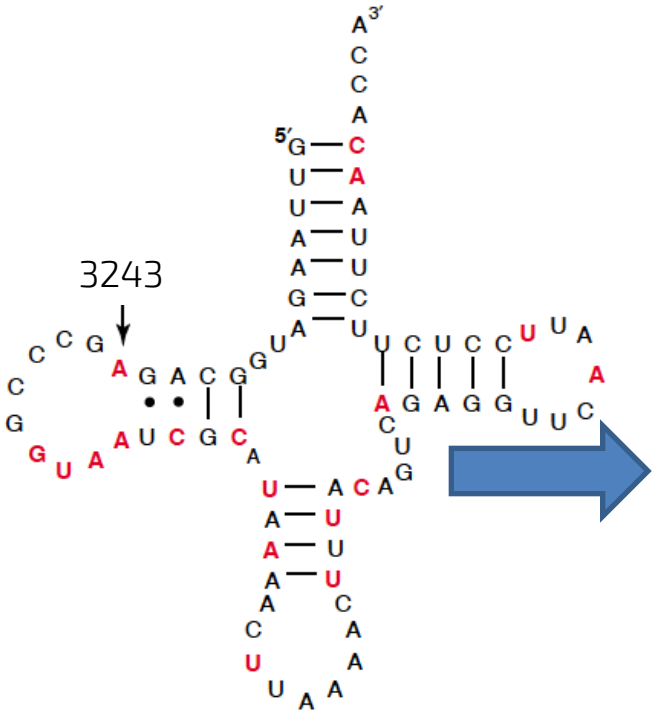
Mutation	Affected per 100,000 (CI)	"At Risk"
m.3243A>G	3.5 (2.7- 4.4)	4.4 (3.7 – 5.3)

Estimated patient number (prevalence) in EU, US and Japan: 50,000

Pavlakis et al., 1984; Verhaak et al; Orphanet J Rare Dis, 2016; Gorman et al., Nature Review Disease Primers 2016

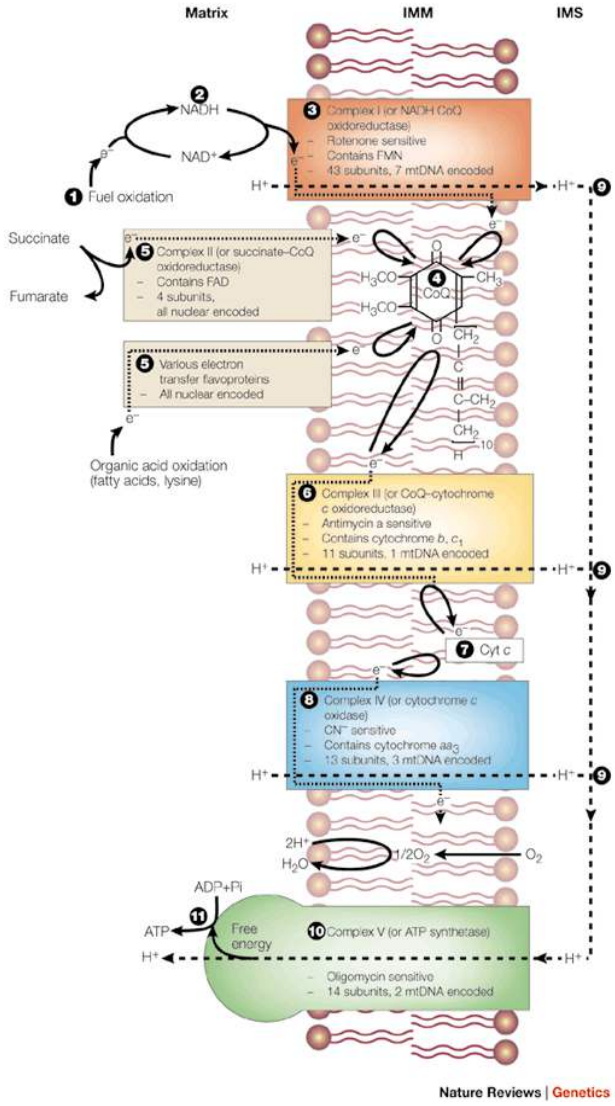
MELAS SPECTRUM DISORDERS : MT-TL1 3243A>G AND PHENOTYPES

MT-TL1 MUTATION

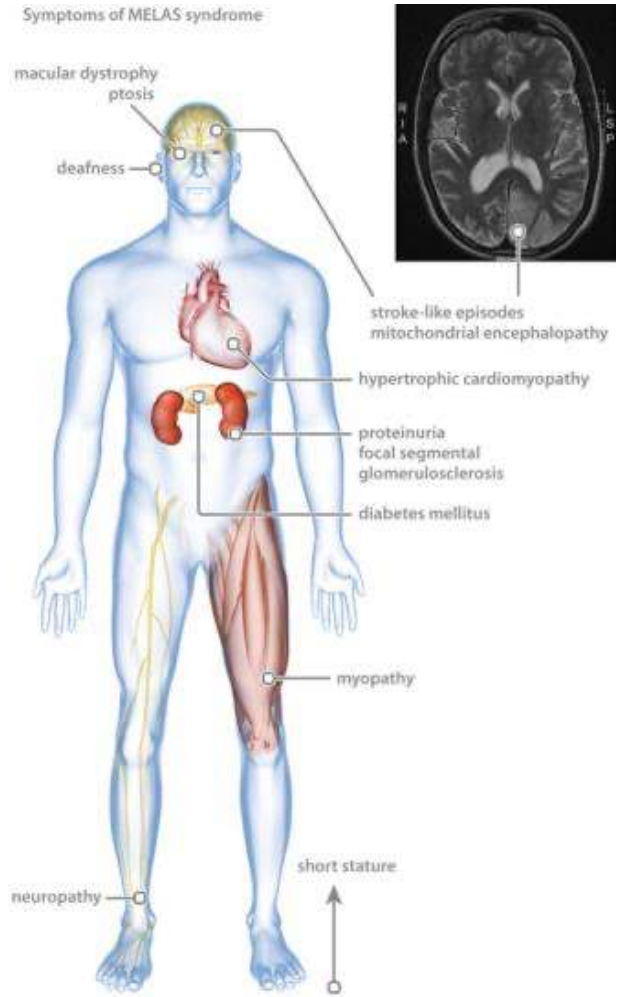


tRNA$^{Leu(UUR)}$

PROTEINS



PHENOTYPE



MELAS SPECTRUM DISORDERS: PHENOTYPES

The majority of patients with the m.3243A>G mutation do not have classical MELAS

Classical MELAS (+ strokes)

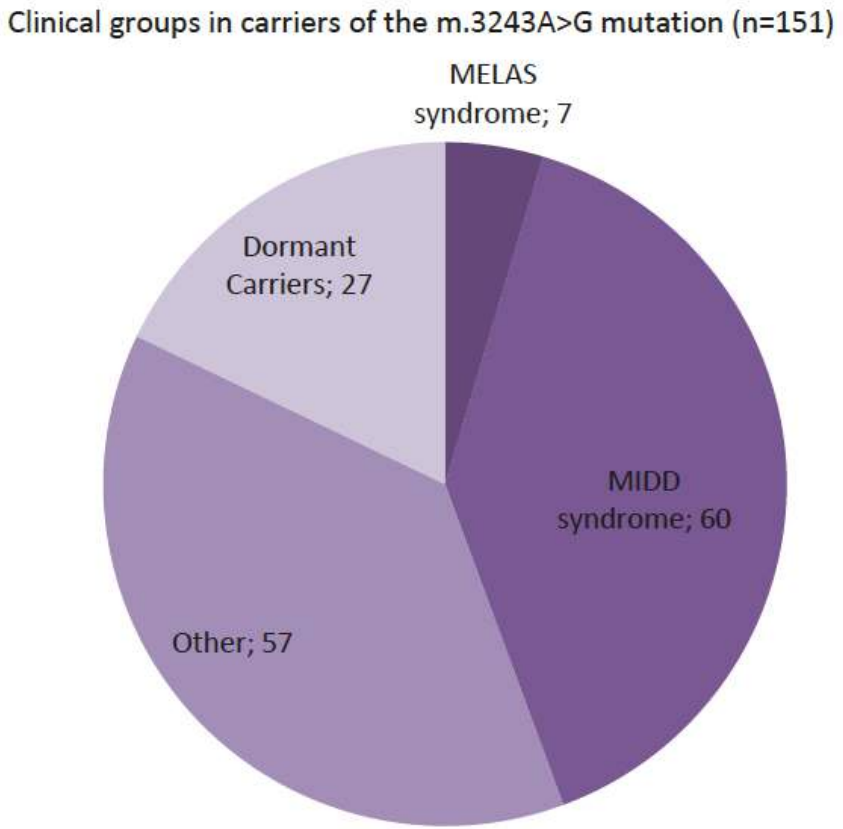
7/124 = 6%

MIDD (- strokes)

60/124 = 48%

MIXED PHENOTYPES (-strokes)

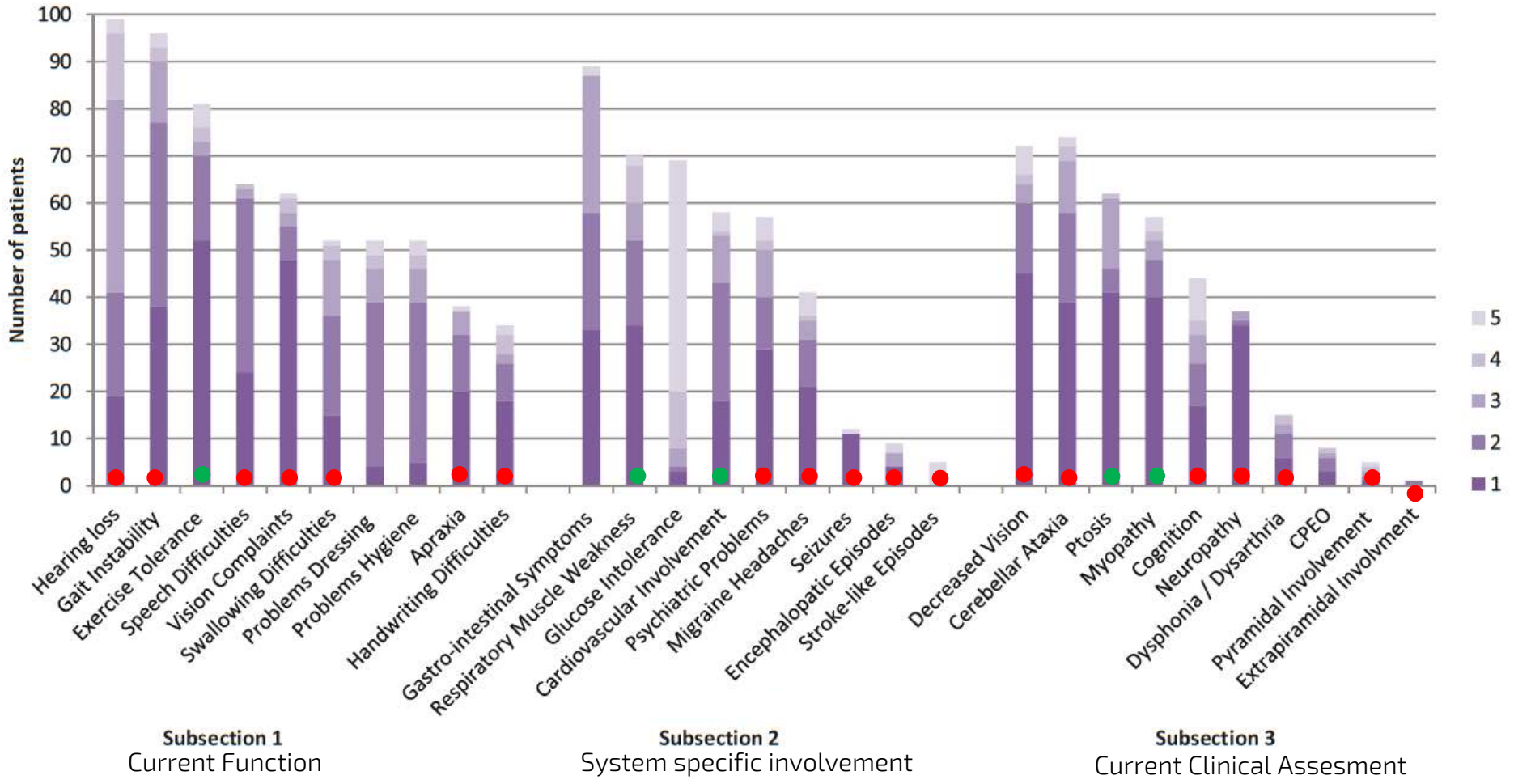
57/124 = 46%



RCMM Natural History Study

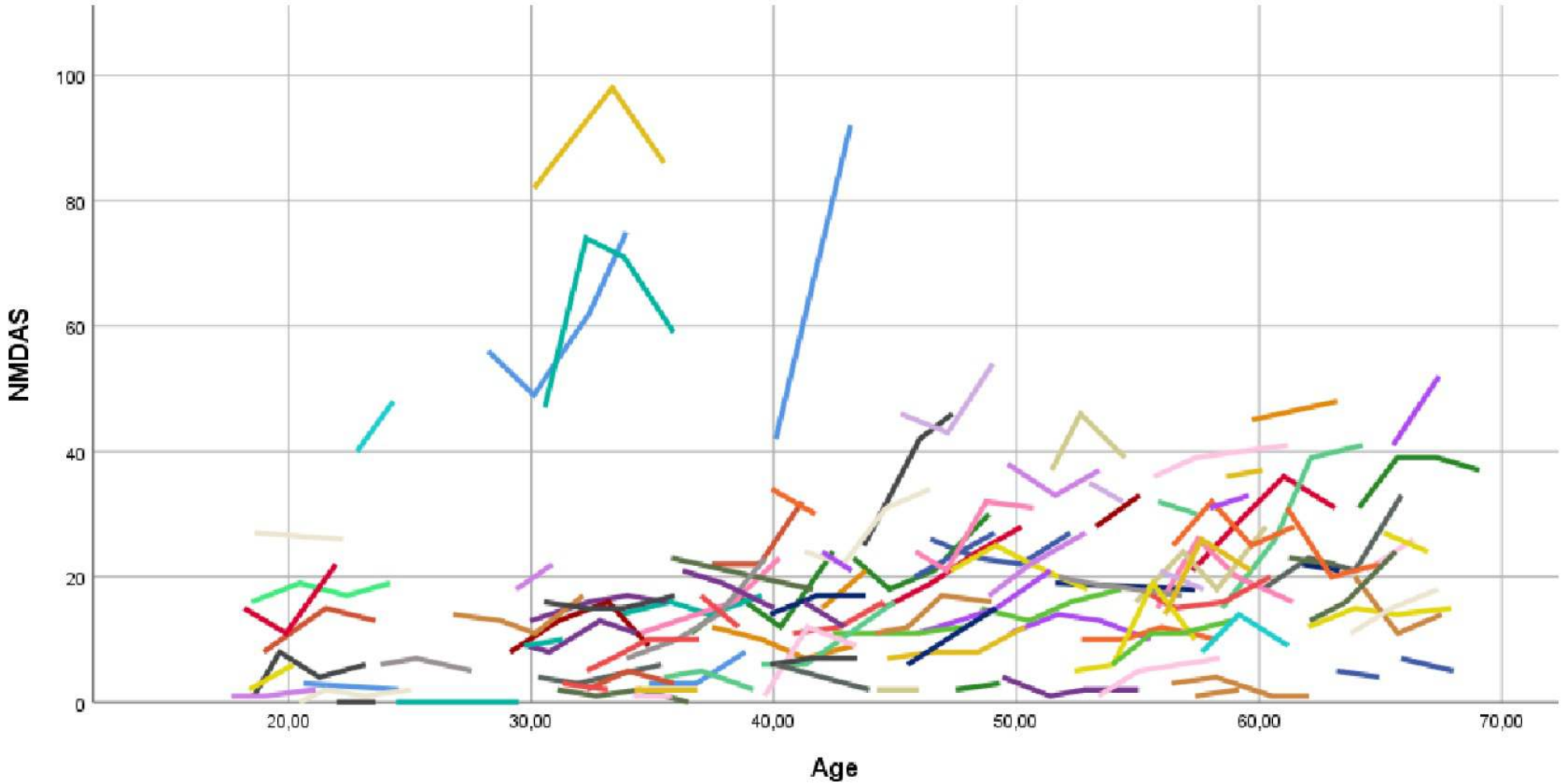
NEWCASTLE MITOCHONDRIAL DISEASE ADULT SCALE

Frequent involvement of Nervous System (•) and Muscle (•)



All scores on the NMDAS scale are shown. In order of prevalence per subsection

NMDAS NATURAL HISTORY: FOLLOW-UP DATA



The relation between Newcastle Mitochondrial Disease Adult Scale (NMDAS) and age is indicated. Age is in years.

MELAS SPECTRUM DISORDERS: DISEASE PROGRESSION

The overall progression in NMDAS score was 0.47 (95% CI 0.33 to 0.61) points per year, but there were large differences between the MELAS spectrum phenotypes:

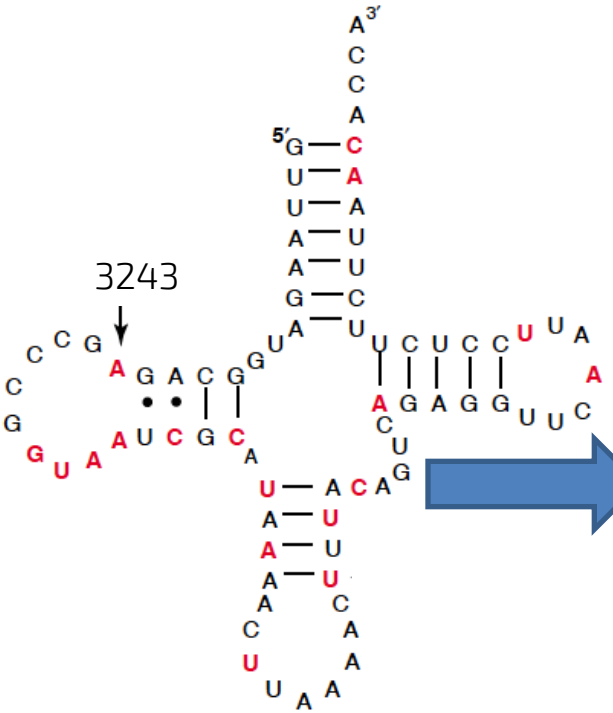
1. **Patients with classical MELAS** had a progression of **1.31** (95% CI –0.78 to 3.40) points per year. Due to the small groups of patients with MELAS (n=7), however, the CI was wide and included the null value.

2. **Patients with MIDD** showed an increase of **0.64** (95% CI 0.43 to 0.85) points per year

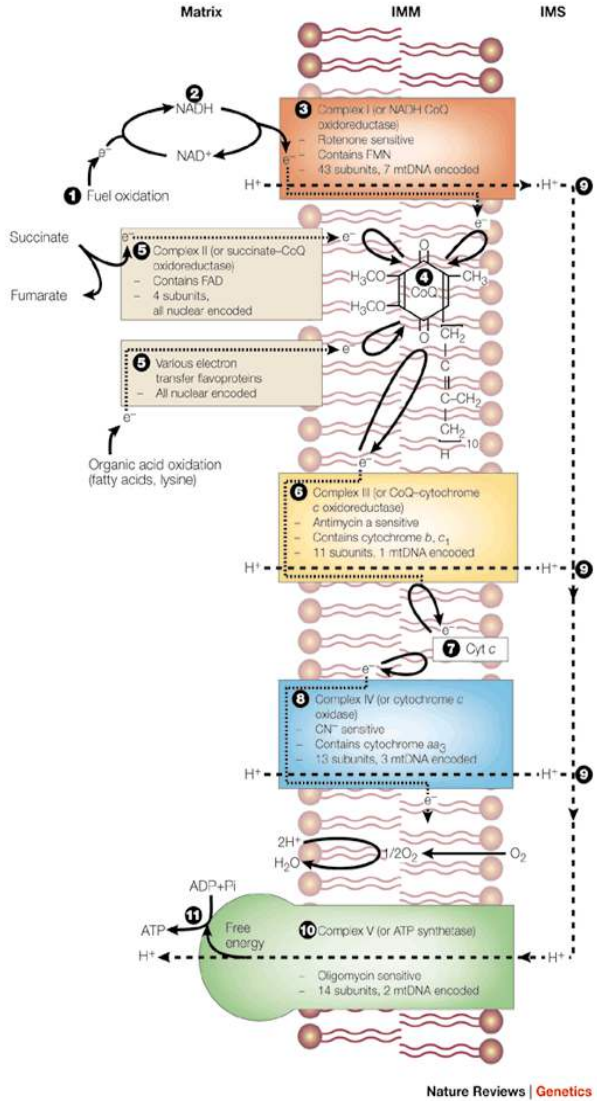
3. **The Mixed Phenotype** group showed an increase of **0.26** (95% CI 0.13 to 0.40) points per year.

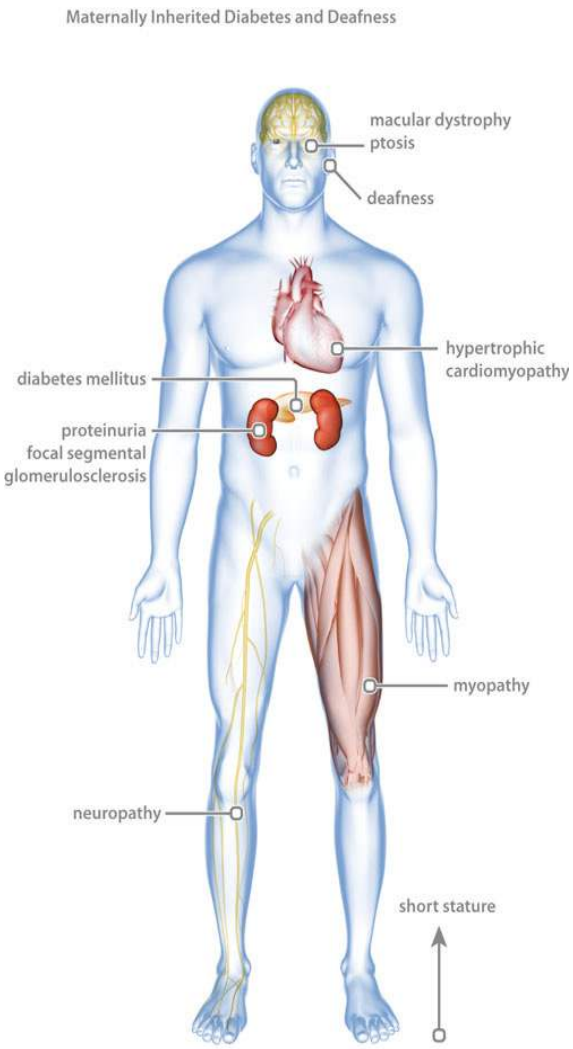
MT-TL1 3243A>G : DISEASE EXPRESSION AND SEVERITY

What factors/circumstances influence disease expression and severity?

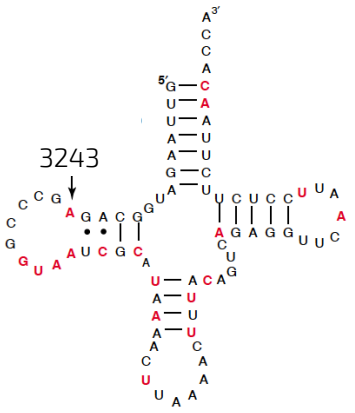


tRNA$^{Leu(UUR)}$



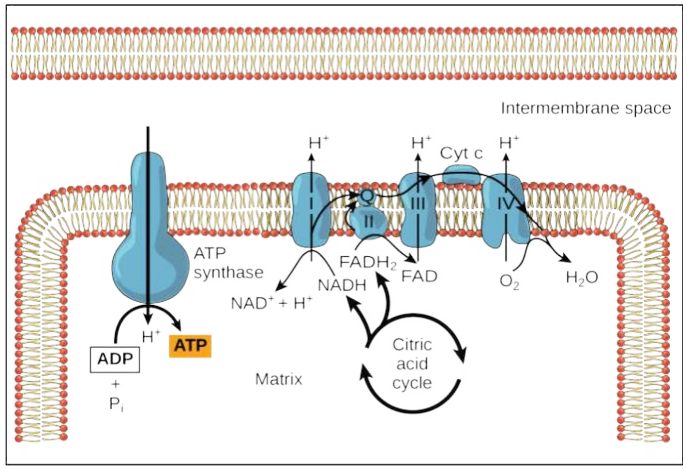


MT-TL1 3243A>G : DISEASE EXPRESSION AND SEVERITY



tRNA$^{Leu(UUR)}$

Isolated [CI] or combined OXPHOS complex [CI plus other]enzyme deficiency

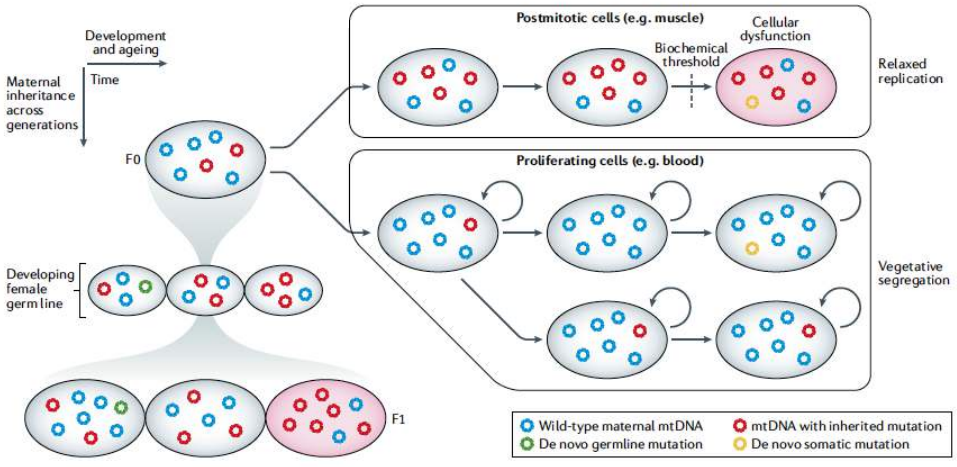


FACTORS RELATED TO EXPRESSION AND SEVERITY OF OXPHOS DEFICIENCY AND ITS CONSEQUENCES :

1. Organ and tissue seggregation and genetic bottleneck
2. Level of heteroplasmy
3. mtDNA copy number
4. Posttranslational modifications

5. Nuclear genetic determinants
6. Environmental factors
7. Age

1. ORGAN AND TISSUE SEGGREGATION AND GENETIC BOTTLENECK



Mitochondrial DNA heteroplasmy levels can change during life through:

A. Relaxed replication (continuously, cell-cycle independent) in non-dividing and dividing cells.
B. Vegetative segregation in dividing cells where mtDNAs are partioned during cell division.

In non- dividing (postmitotic) cells, relaxed replication during life can lead to an increase in the proportion of mutant mtDNA, which if it exceeds a critical threshold leads to a biochemical defect of oxidative phosphorylation.

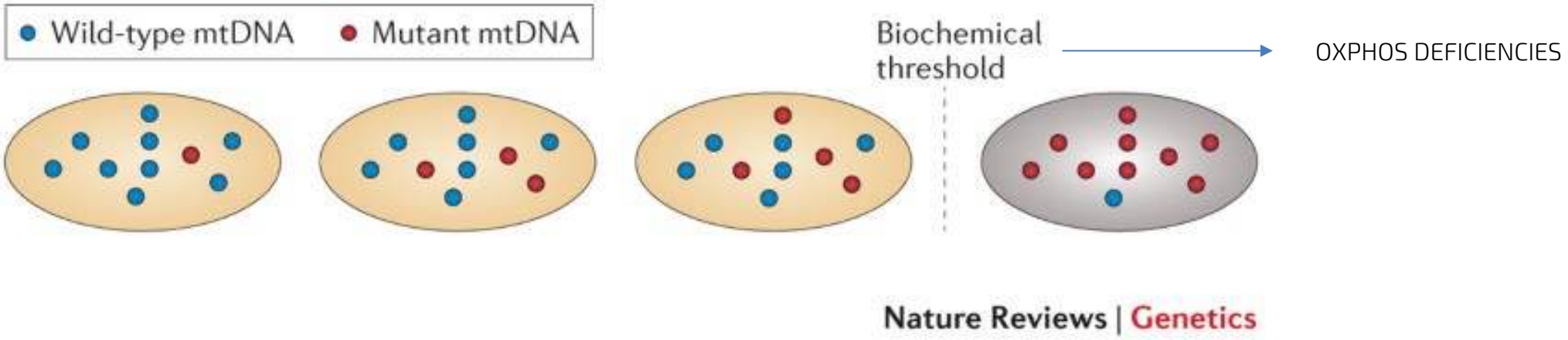
The **germline genetic bottleneck** accelerates segregation between the generations, leading to major changes in the heteroplasmy level.

Heteroplasmy can also be introduced through somatic and germline **de novo** mutations.

F0 = female patient; F1 = offspring

Stewart and Chinnery, 2021

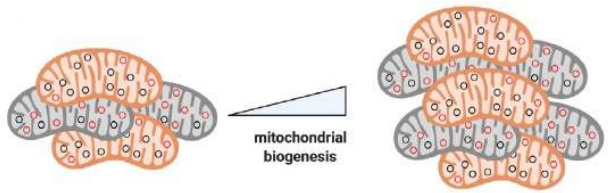
2. HETEROPLASMY: two [or more] mtDNA variants (wild-type and mutant type) exist within the same cell



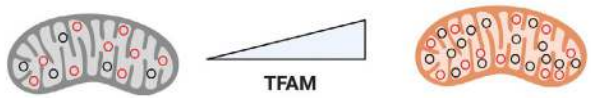
Stewart and Chinnery 2015

3. **MITOCHONDRIAL DNA COPY NUMBER:** The number of mitochondrial genomes per cell

- More mitochondria per cell



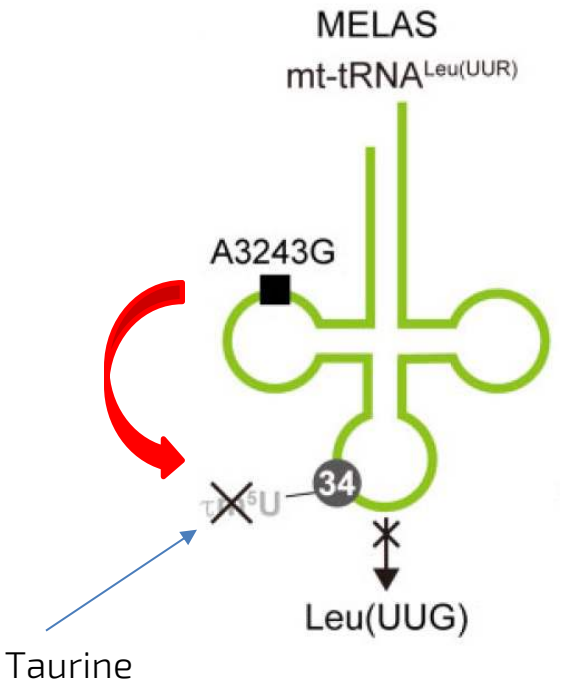
- More copies of mtDNA per mitochondrion per cell



Example: Low mtDNA copy number in skeletal muscle of m.3243A>G patients is positively associated with disease burden

John P Grady et al., EMBO Journal 2018; Filograna et al., FEBS Lett 2020

4. POSTTRANSLATIONAL MODIFICATIONS



Taurine modification is indispensable for mitochondrial translation providing the 13 subunits of the OXPHOS system

The m.3243A>G mutation leads to a disappearance of the 5-taurinemethyluridine for which taurine is the donor.

Induces impaired OXPHOS protein synthesis and/or activity

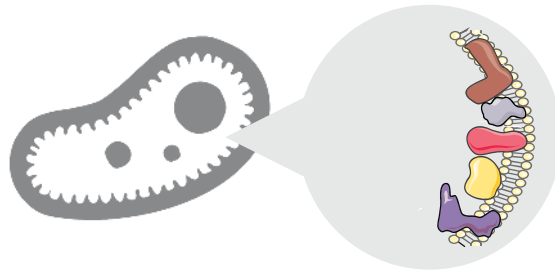
5. OTHER

a. Nuclear genetic determinants
b. Environmental factors
c. Age

Kazuhito et al., 2020

COMMON CELLULAR CONSEQUENCES OF HAMPERED OXPHOS

Mutations in MT-TL1 gene convert to the OXPHOS system to cause Primary Mitochondrial Disease



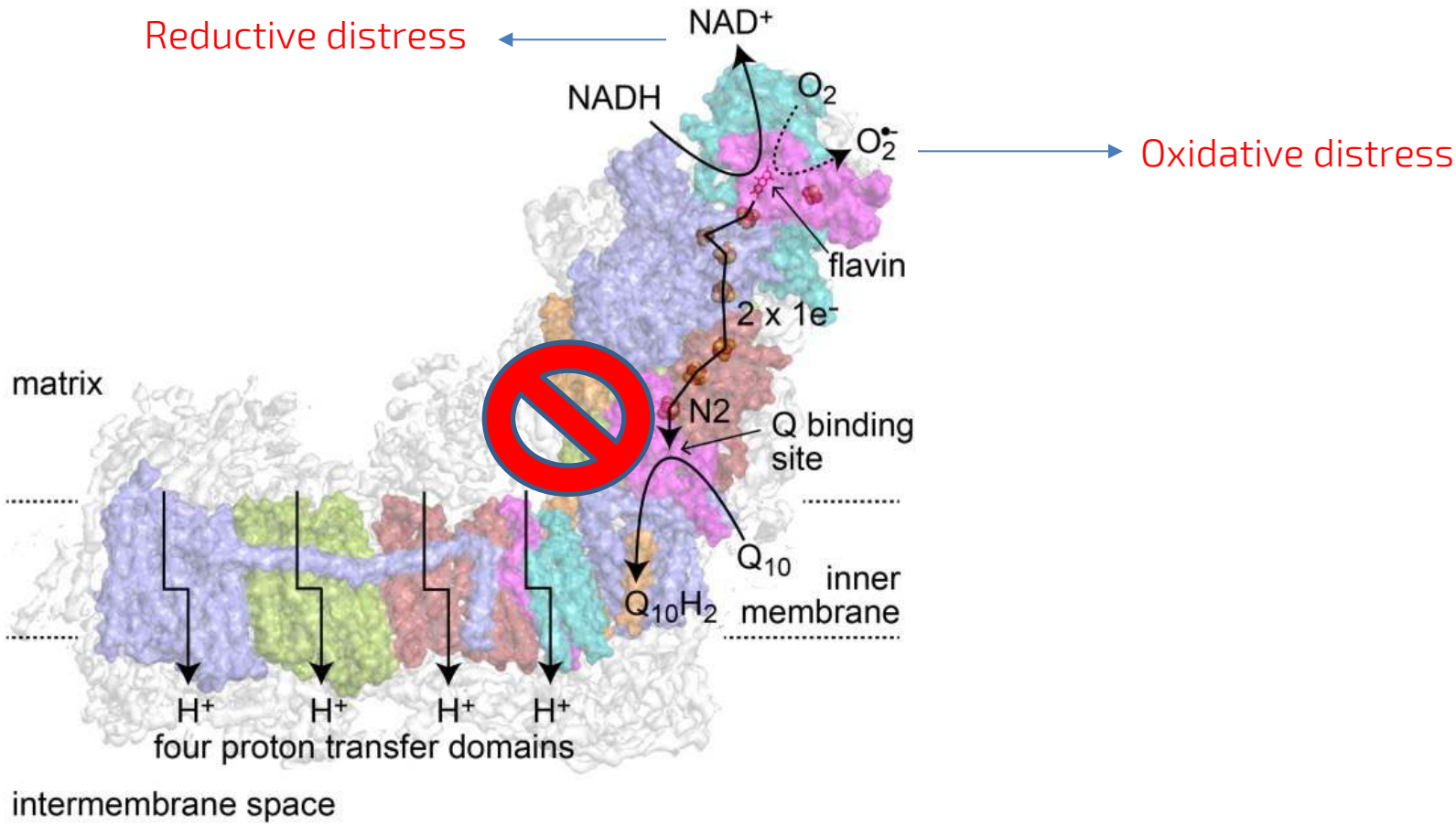
Common cellular consequences suitable for drug development:

Reductive distress - disturbed redox balance

Oxidative distress – Ferroptosis due to **ROS-induced lipid peroxidation**

Inflammation due to increased PGE2 levels

MT-TL1 3243A>G, COMPLEX I DEFICIENCY AND REDUCTIVE AND OXIDATIVE DISTRESS



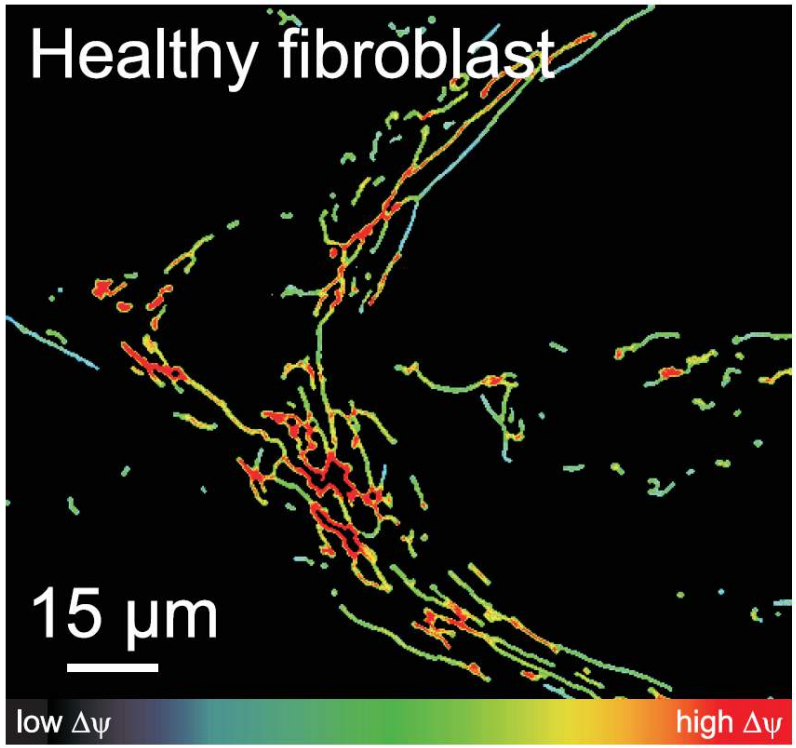
Hirst and Roessler, BBA, 2016; Koopman et al., New Eng J Med 2012 and EMBO Mol Med 2016; Patel, Trends in Pharmacology, September 2016

COMPLEX I DEFICIENCY: REDUCTIVE AND OXIDATIVE DISTRESS

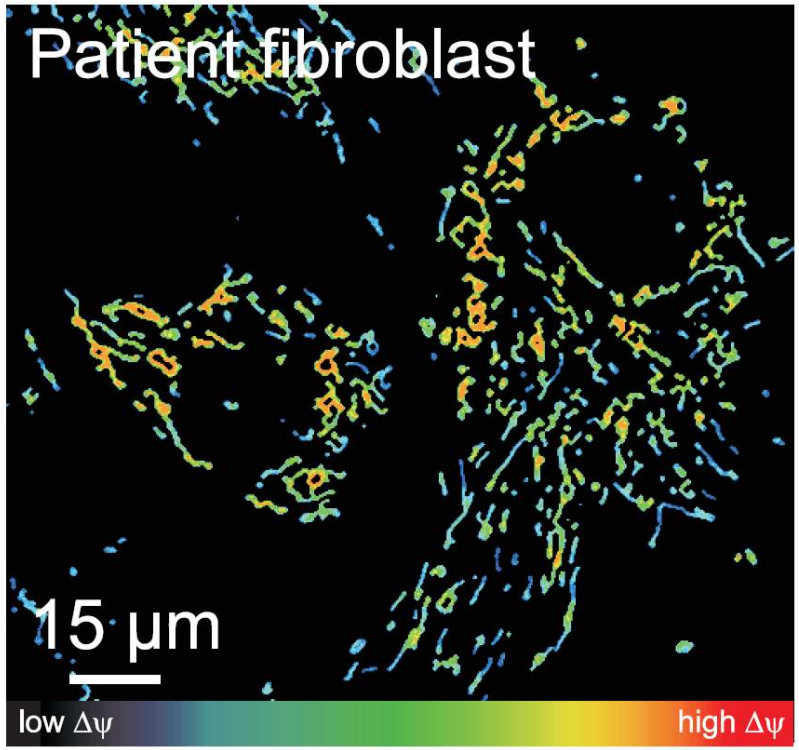
Cell line	Mutation	CI	MMP	NAD(P)H	ER_{Ca}	$[Ca]_C$ peak	$[Ca]_M$ peak	$[ATP]_M$ peak	$t_{1/2}$	Et	CM-DCF	F	Nc	F/Nc
CT1 (#5120)		113	100	100	100	100	100	100	100	100	100	100	100	100
CT2 (#5119)		105	99	86	105	104	96	101	95	102	100	99	97	102
CT3 (#5118)		103	99	109	105	100	103	110	95	98	125	105	95	111
CT4 (#4996)		n.d.	99	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	100	n.d.	n.d.	n.d.	n.d.
CT5 (MW26)		152	n.d.	n.d.	n.d.	n.d.	n.d.	97	n.d	100	n.d.	n.d.	n.d.	n.d.
CT6 (MW28)		97	n.d.	n.d.	n.d.	n.d.	n.d.	98	n.d	n.d.	n.d.	95	105	91
CT7 (MW33)		220	n.d.	n.d.	n.d.	n.d.	n.d.	101	n.d	99	n.d.	n.d.	n.d.	n.d.
P1 (#6173)		31	92	142	82	91	86	77	136	176	171	76	116	66
P2 (#4605)		40	n.d.	n.d.	n.d	93	n.d.	72	130	218	n.d.	84	149	56
P3 (#5067)		36	93	191	94	101	87	78	142	264	351	85	137	62
P4 (#5170)		39	98	140	95	97	95	102	102	205	169	65	95	68
P5 (#7276)		26	n.d.	n.d.	82	91	87	60	146	191	244	68	145	47
P6 (#4608)		75	87	130	96	108	97	97	110	195	289	123	103	119
P7 (#4827)		58	n.d.	n.d.	n.d.	82	n.d.	33	169	196	n.d.	96	177	54
P8 (#5260)		36	93	132	77	84	79	48	160	174	187	104	146	71
P9 (#5737)		53	95	127	87	93	96	86	126	131	202	91	88	103
P10 (#5077)		59	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	142	n.d.	98	91	108
P11 (#5175)		68	90	112	73	80	76	57	164	151	212	117	98	119
P12 (#6603)		18	88	170	85	91	89	74	134	222	275	120	167	71
P13 (#5199)		69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	99	112	88
P14 (#5866)		64	94	142	87	90	86	67	140	110	135	114	98	116
P15 (#5171)		73	91	130	96	96	99	93	112	186	242	133	107	125

Distelmaier et al., Brain 2009

OXIDATIVE DISTRESS AND MITOCHONDRIAL ARCHITECTURE



Normal network
Negative membrane potential



"Fragmented" network
Less negative membrane potential

Koopman et al., EMBO J, 2013

MITOCHONDRIAL DRUG DEVELOPMENT

REDUCTIVE AND OXIDATIVE DISTRESS →

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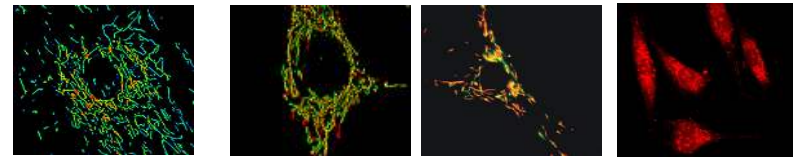
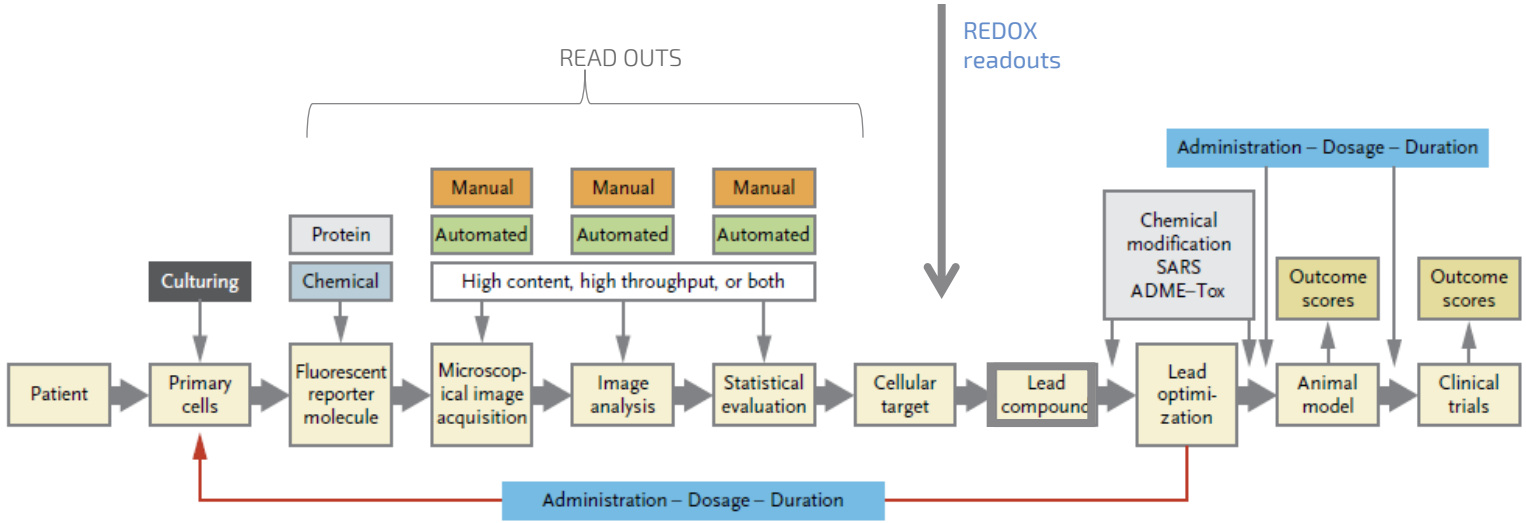
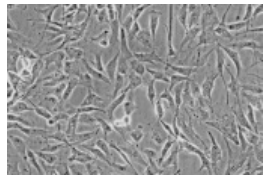


CELLULAR TARGET BASED COMPOUND OPTIMIZATION

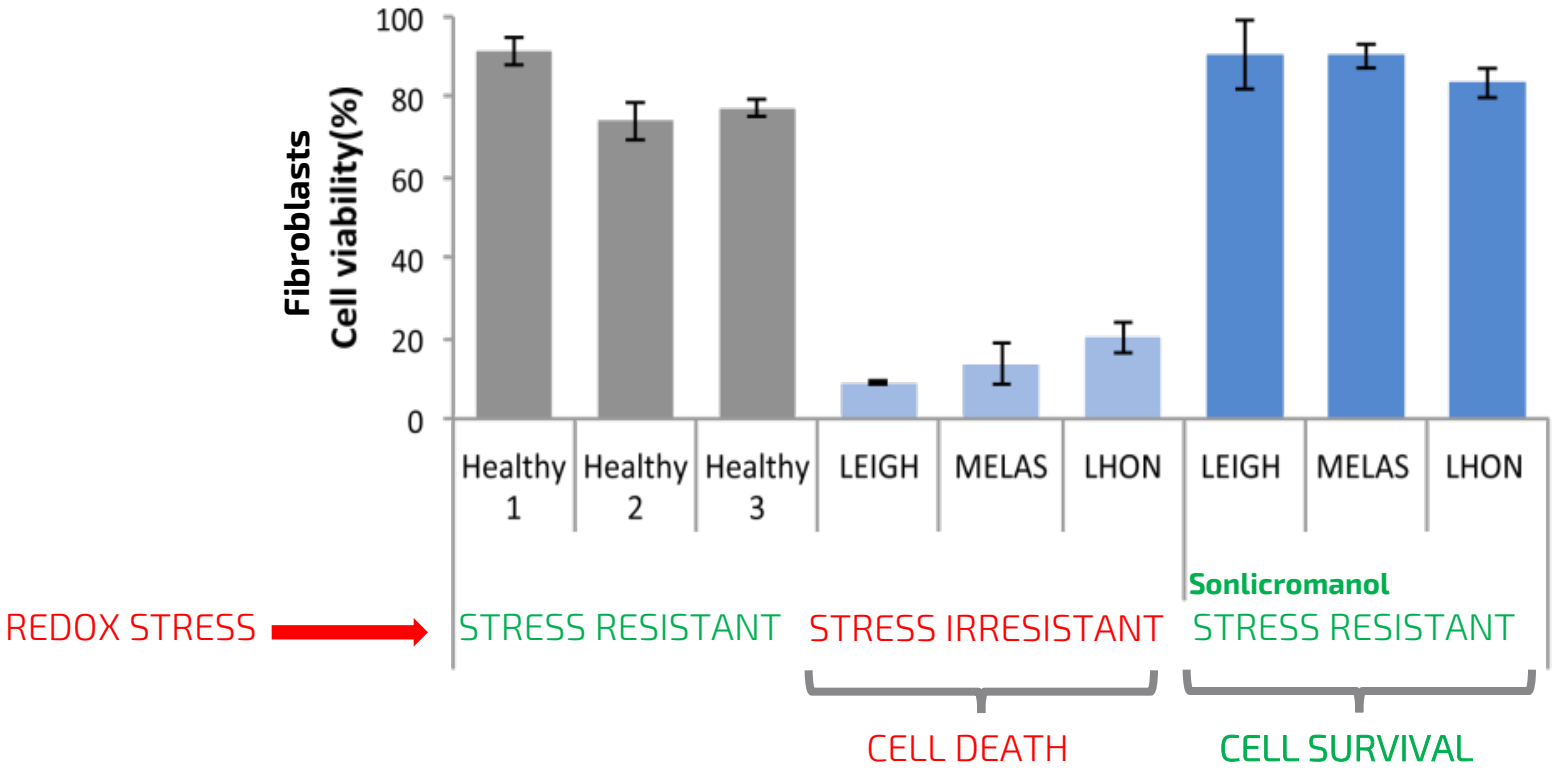




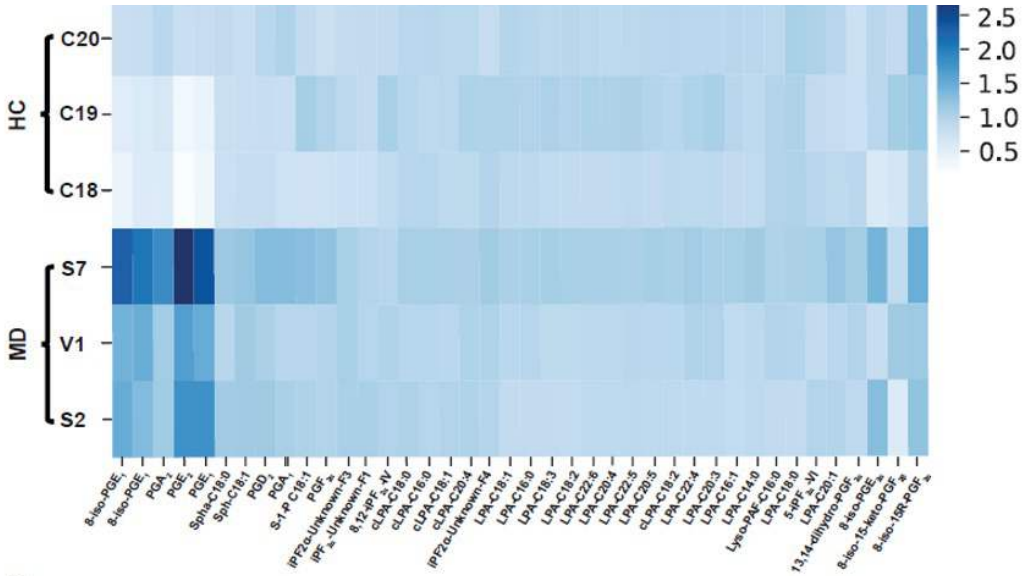
OXPHOS DEFICIENT

HIT → > 250 NCE → R/sonlicromanol

Koopman, Willems, Smeitink,NEJM, 2012; Koopman et al., EMBO Mol Med, 2016; Iannetti et al., Nature Protocols, September 2016


Fibroblasts
Cell viability(%)
100
80
60
40
20
0
Healthy 1
Healthy 2
Healthy 3
LEIGH
MELAS
LHON
LEIGH
MELAS
LHON
Sonlicromanol
REDOX STRESS
STRESS RESISTANT
STRESS IRRESISTANT
STRESS RESISTANT
CELL DEATH
CELL SURVIVAL

COMPLEX I DEFICIENT OXIDATIVE DISTRESS MARKERS NORMALIZED BY SONLICROMANOL



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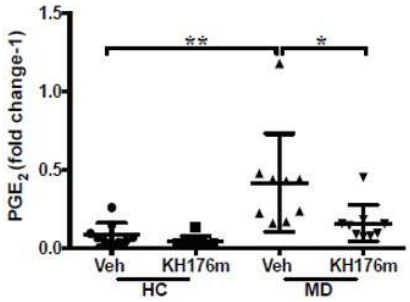

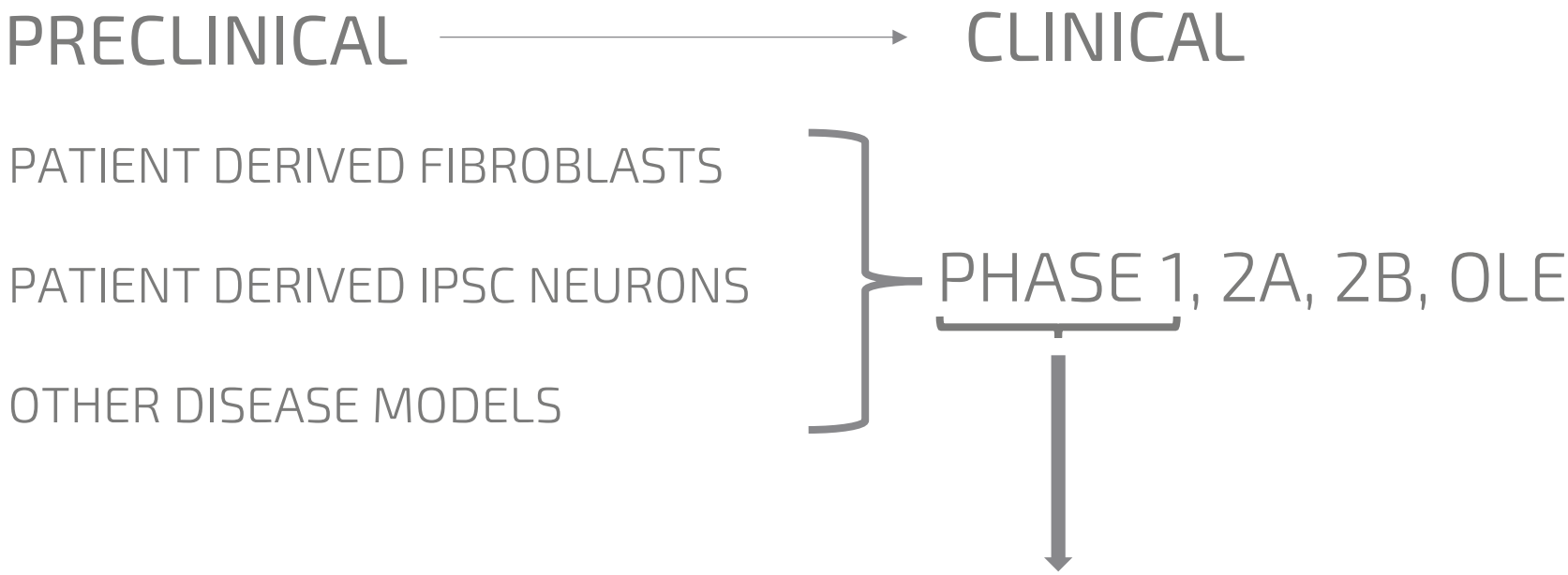

Sonlicromanol is well tolerated and has a pharmacokinetic profile supportive for a twice daily 100 mg b.i.d. oral dosing and a favorable safety profile

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Robustly designed Phase 2b study ongoing with read-out in Q3 2022

Reconfirm and expand on positive Phase 2a results; supplemented by long-term extension study data

Phase 2a – KHENERGY proof of concept trial	Phase 2b – KHENERGYZE trial	Open label extension of Phase 2b – KHENEREXT trial
• Single center • Nijmegen (NL)	• Multi-center • Nijmegen (NL), Munich (GER), Newcastle upon Tyne (UK); Copenhagen (DK)	• Multi-center • All sites participating in the KHENERGYZE trial
• Randomized, double blind, placebo-controlled • 100mg, twice daily	• Randomized, double blind, placebo-controlled • Dose-finding – 50mg and 100mg, twice daily	• Open label
• 18 adults • m.3243A>G mutation – responsible for MELAS spectrum disorders	• 27 adults • m.3243A>G mutation – responsible for MELAS spectrum disorders • Added cognition-related patient inclusion test	• Roll-over patients from Phase 2b
• 4 weeks • Outcome measures assessed after 28 days	• 4 weeks • Outcome measures assessed after 28 days	• 12 months • Outcome measures assessed every 3 months
• Sonlicromanol well tolerated and safe • Multiple parameters tested in exploratory study • Significant positive effects on several cognition-related parameters (alertness and mood)	• Confirm good safety profile and positive effects in various cognitive domains using additional, broadly validated (computerized) tests often used in CNS studies • Primary endpoint: "Visual identification test" developed by Cogstate to measure attention • In addition, measure parameters in other domains • Including hearing, migraine and fatigue	• Establish longer term safety profile • Gather additional longer-term patient data across all Phase 2b outcome measures • Assess complementary clinical outcome measures including several motor-function related parameters

- **Reconfirming Ph2a findings in larger patient pool**
- **Managing patient heterogeneity with inclusion criteria**
- **Adding cognition endpoints validated in CNS indications**
- **Adding previously unstudied outcome parameters**
- **Generating additional relevant patient data on a rolling basis in long-term extension study**

Creating valuable data package to optimize the Phase 3 study design and further support discussions with regulators

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Take home messages

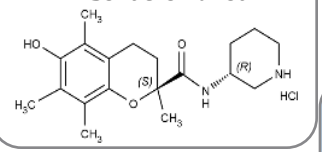
Sonlicromanol

Small molecule
- First in class
- Pipeline in a product potential

Strong intellectual property position
- Multi-layer patent protection
- Freedom to operate
- Orphan drug exclusivity

Triple mode of action
- Reductive distress modulator
- Oxidative distress modulator
- Anti-inflammatory properties

sonlicromanol



Well established production route
- Market formulation ready at start Phase 3

Safe & well tolerated
- Phase 1 in healthy volunteers
- Confirmed in Phase 2a trial and ongoing clinical studies

Excellent PK properties
- High oral bioavailability
- Ability to cross blood-brain barrier

Extensive Research Program

Phase 2a trial m.3243A>G
•Sonlicromanol showed statistically significant improvement in the cognitive domain of attention) and improvement in mood parameters

•Based on MELAS spectrum patient testimonials cognitive decline seriously affects their quality of daily life. The most affected cognitive domains being perceptual, motor function, executive function and complex attention

Phase 2b trial m.3243A>G
•A multi-center dose-finding Phase 2b study with the cognitive domain of attention as primary outcome measure and other cognitive domains as secondary outcome measures is nearing completion

Phase 2b continued 1-year open label extension study m.3243A>G
•First patients included in the one-year open label extension study to explore maintenance of effects seen, to evaluate longer-term safety and to study the effects on parameters requiring longer-term intervention (ongoing)

Phase 1 and 2 Children with PMD
•Adult equivalent doses in age ranges 5-17 years determined. First patients randomized in the phase 2 part of the clinical trial

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Thank You

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