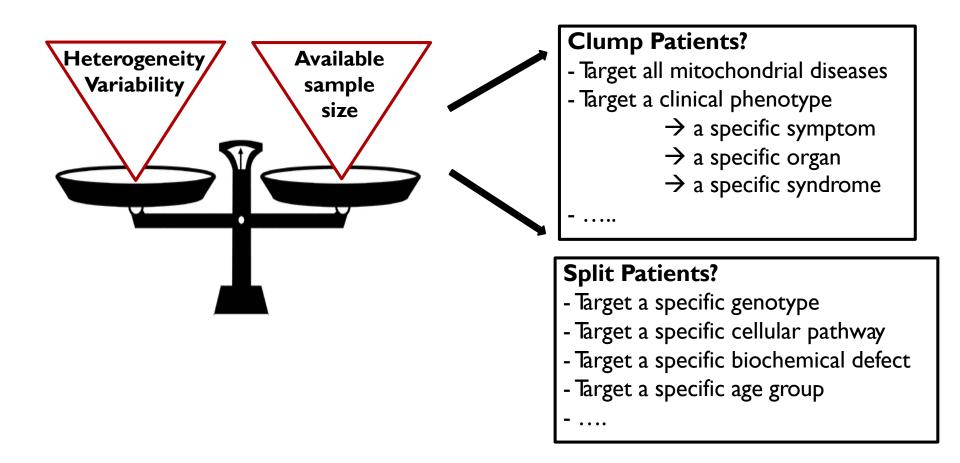


#### To clump or to split? That is the question.

## Cases

- 8 mo female with jaundice and liver failure
- 3 yo male with developmental regression and seizures
- I 5 yo female with migraines, severe constipation, fainting
- > 24 yo male with speech and balance problems
- 30 yo female with enlarged heart and strokes
- 55 yo male with weakness, muscle pain, hearing loss
- 71 yo female with dementia, diabetes, jerking limbs



#### **Clump Patients?**

-Target all mitochondrial diseases (Master protocol)

#### What is a mitochondrial disease?

- $\rightarrow$  Primary mitochondrial disease
- $\rightarrow$  Secondary mitochondrial dysfunction

## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

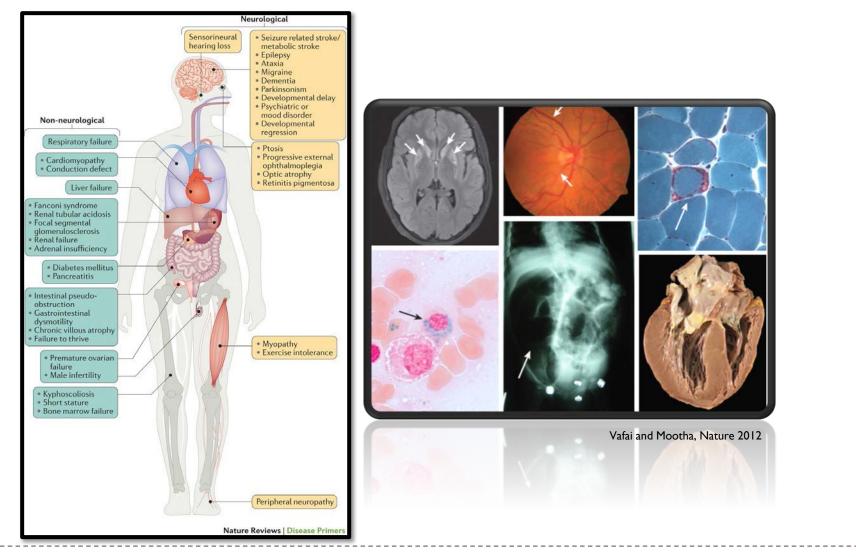
- Affect all ages from birth to late-adulthood
- Range from monosymptomatic to multi-systemic diseases
- Hundreds of presentations

- -Target all mitochondrial diseases (Master protocol)
  - → small numbers of patients with any given MD might preclude meaningful subgroup analyses
  - → differences in outcomes between patients with different MD types could be missed

- Target all mitochondrial diseases
- Target a clinical phenotype

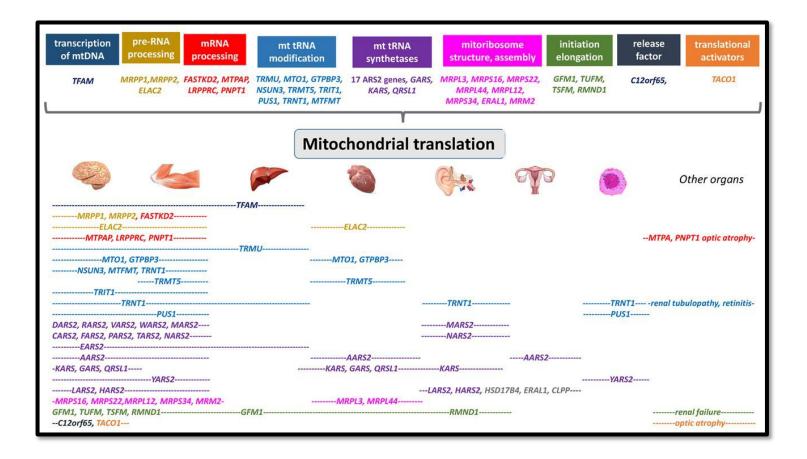
- Target all mitochondrial diseases
- Target a clinical phenotype
  → a specific symptom

## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:



- Target all mitochondrial diseases
- Target a clinical phenotype
  - $\rightarrow$  a specific symptom
  - $\rightarrow$  a specific organ

## The different phenotypes



- Target all mitochondrial diseases
- Target a clinical phenotype
  - $\rightarrow$  a specific symptom
  - $\rightarrow$  a specific organ
  - $\rightarrow$  a specific syndrome

#### The depletion syndrome

Lactic acidosis	
Failure to thrive	
Hypotonia	
Muscle weakness	
Ataxia	
Polyneuropathy	
Liver impairment	
Epilepsy	
Migraine-like headache	
Developmental delay / cognitive impairment	
Psychiatric symptoms	
Gastrointestinal symptoms	
	Neonatal – Childhood – Juvenile – Adulthood

## The different phenotypes

KSS MERRE MELAS	)
NARP LHON	
Leigh CPEO	

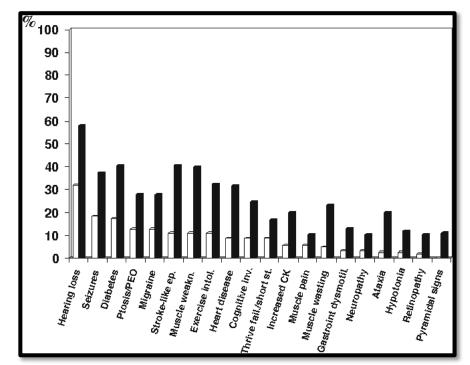
D

Tissue or Area	Symptom or Sign	Kearns–Sayre Syndrome	Myoclonus Epilepsy with Ragged-Red Fibers	Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes	Neuropathy, Ataxia, Retinitis Pigmentosa	Maternall Inherited Leigh Syndrom
	1000 to	Synaronic	1 Ibers	Subre like Episodes	riginentosa	Syndronn
Central nervous system	Seizures	- <b>-</b> -	+	*	+	+
	Ataxia	+	+	+	+	±
	Myoclonus	-	+	±		-
	Psychomotor retardation	-	-	(m)	-	+
	Psychomotor regression	+	±	+		8240
	Hemiparesis or hemianopia	-	-	+		-
	Cortical blindness	-	-	+	-	-
	Migraine-like headache	-	-	+	-	-
	Dystonia	-	-	+	- <u>-</u>	+
Peripheral ner- vous system	Peripheral neuropathy	±	*	*	+	-
Muscle	Weakness or exercise intolerance	+	+	+	+	+
	Ophthalmoplegia	+	-	557		-
	Ptosis	+	-	100		-
Eye	Pigmentary retinopathy	+	-	-	+	±
Con Th	Optic atrophy	-	-	-	±	±
Blood	Sideroblastic anemia	±	-	1.7	-	-
Endocrine	Diabetes mellitus	±	-	±	-	-
	Short stature	+	+	+		-
	Hypoparathyroidism	±	-	15	-	-
Heart	Conduction block	+		±	-	
	Cardiomyopathy	±	-	±	-	±
Gastrointestine	Exocrine pancreatic dysfunction	±	-	-		-
Ear, nose, throat	Sensorineural hearing loss	377.0	+	+	±	3752
Kidney	Fanconi's syndrome	±	-	±	-	-
Laboratory results	Lactic acidosis	+	+		-	±
	Ragged-red fibers on muscle biopsy	+	+	+	-	120
Inheritance	Maternal	843	+	+	+	+
	Sporadic	+	-	2	-	-

NEJM 2010. 354(10):1096-7

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## The different phenotypes



	Onset	Last evaluation
	$23.4 \pm 15.6$ years <sup>a</sup> (%)	$36.0 \pm 20.7$ years (%)
	• • •	• • •
Hearing loss	39 (31.0)	73 (57.9)
Generalized seizures	23 (18.3)	47 (37.3)
Diabetes	22 (17.5)	51 (40.5)
Ptosis/ophthalmoparesis	16 (12.7)	35 (27.8)
Migraine	16 (12.7)	35 (27.8)
Stroke-like episodes	14 (11.1)	51 (40.5)
Muscle weakness	14 (11.1)	50 (39.7)
Exercise intolerance	14 (11.1)	41 (32.5)
Heart disease	11 (8.7)	40 (31.7)
Cognitive involvement	11 (8.7)	31 (24.6)
Failure to thrive/short st.	11 (8.7)	21 (16.7)
Increased CK	7 (5.6)	25 (19.8)
Muscle pain	7 (5.6)	13 (10.3)
Muscle wasting	6 (4.8)	29 (23.0)
Vomiting	5 (4.0)	7 (5.6)
Gastrointestinal dysmotil.	4 (3.2)	16 (12.7)
Neuropathy	4 (3.2)	13 (10.3)
Ataxia	3 (2.4)	25 (19.8)
Hypotonia	3 (2.4)	15 (11.9)
Retinopathy	3 (1.8)	13 (10.3)
Myoclonus	3 (2.4)	8 (6.3)
Hypothyroidism	2 (1.6)	5 (4.0)
Psychiatric involvement	1 (0.8)	8 (6.3)
Optic neuropathy	1 (0.8)	6 (4.8)
Hypogonadism	1 (0.8)	5 (4.0)
Pyramidal signs	_	14 (11.1)
Respiratory impairment	_	6 (4.8)
Status epilepticus	_	5 (4.0)

#### **Split Patients?**

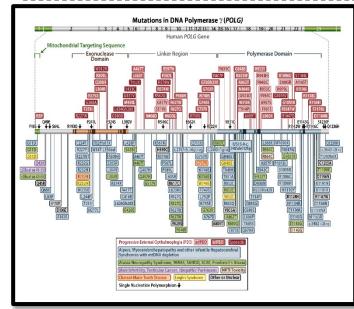
-Target a specific genotype

## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

• Caused by mutations in nDNA, mtDNA or both

#### nDNA (~350 genes)

			Pri	mary role	e specifi	c to OXPHO	S bioger	nesis			
	OXPHOS Subunits					Electron Ca	arriers	mtDNA Homeostasis	mt-tRNA Biogenesis/ Aminoacylation		
	CI	CII	CIII	CIV	CV	CoQ	Cyt. c	DNA2*	MT-TA	GTPBP3 AARS	
MT-ND1 MT-ND2 MT-ND3 MT-ND4 MT-ND4L MT-ND5 MT-ND6	NDUFB3 NDUFB9 NDUFB10 NDUFB11 NDUFS1 NDUFS2 NDUFS3		MT-CYB CYC1 UQCRB UQCRC2 UQCRQ	MT-CO1 MT-CO2 COX4I1 COX4I2 COX5A COX6A1 COX6B1	MT-ATP6 MT-ATP8 ATP5A1 ATP5E	COQ2	CYCS HCCS	MGME1 POLG POLG2 RNASEH1 <sup>®</sup> TFAM TWNK	MT-TC MT-TD MT-TE MT-TF MT-TG MT-TH MT-TI	MTFMT CARS MTO1 DARS NSUN3 EARS PUS1° FARS2 QRSL1 GARS TRIT1° HARS TRMT5 IARS2	
NDUFA1 NDUFA2	NDUFS4 NDUFS6			COX7B COX8A		COQ9 PDSS1		eotide ools	MT-TK MT-TL1	TRMU KARS	
NDUFA9 NDUFA10 NDUFA11	NDUFS7 NDUFS8 NDUFV1			NDUFA4		PDSS2	ABAT <sup>®</sup> DGUOK	SUCLA2 <sup>®</sup> SUCLG1 <sup>®</sup>	MT-TL2 MT-TM MT-TN	MARS NARS PARS	
NDUFA12 NDUFA13	NDUFV2						MPV17 RRM2B <sup>a</sup> SAMHD1 <sup>a</sup>	TK2 TYMP"	MT-TP MT-TQ	RARS: SARS:	
	OXP	HOS Asse	mbly Facto	rs		)	Granne i		MT-TR MT-TS1	TARS2 VARS2	
CI	CII	CIII	C	V	CV	mtRNA Exp		Mitoribosome	MT-TS2 MT-TT	WARS YARS	
ACAD9 FOXRED1 NDUFAF1 NDUFAF3 NDUFAF3 NDUFAF4 NDUFAF6 NUBPL <sup>d</sup> TIMMDC1 TMEM126B	SDHAF1	BCS1L LYRM7 TTC19 UQCC2 UQCC3	COA3 COA5 COA6 COA7 COX10 COX14 COX15	SURF1	ATPAF2 TMEM70	Process ELAC2 FASTKI HSD17, LRPPR MRM2 MTPAP PNPT1 TRMT1	02 810 C	Biogenesis MT-RNR1 ERAL1 MRPL3 MRPL12 MRPL44 MRPS7 MRPS16 MRPS22 MRPS23 MRPS23	MT-TV MT-TW MT-TY	Translation C12orf65 GFM1 GFM2 RMND1 TAC01 TSFM TUFM	
					t on OXP	HOS ± othe	r cellula	r functions			
Fe-S cluster biogenesis	Co-Factor	S	tein Quality Control	Proce	Import/ essing	Mitochondrial Morphology <sup>e</sup>	Metabol Transpo	ort <sup>e</sup> Metabo	olism	Metabolism of Toxic Compound	
ABCB7 BOLA3 FDX1L FDXR FXN GLRX5 IBA57 ISCA2 ISCU LYRM4 NFS1 NFU1		lodificatio neostasis A PNPL SERA 6 TAZ	48	GFE MIP PMI TIM	M1 AJC19' ER PCA M8A M50 Sis/ gy*	CHCHD10 C19orf70 (QIL1, DNM1L GDAP1 MFF MFN2 MFF0 MFN2 MST01 OPA1 SACS SLC25A46° STAT2 TRAK1	SLC194 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC254 SLC394 MICU1	3      ALDH18A1        11      DLAT        13      DLD        14      FH        172      HAAO        19      IDH3B        124      IDH3B        125      KYNU        132      42	MDH2 MECR NADK2 PDHA1 PDHB PDHX PDHX PDHX PDH3 PDP1 PPA2	D2HGDH ECHS1 ETHE1 HIBCH L2HGDH NAXE TXN2 Unclear Function APOPT1 OPA3 CEP89 RTN4IF C190f12 SFXN4 C10BP TMEM6	



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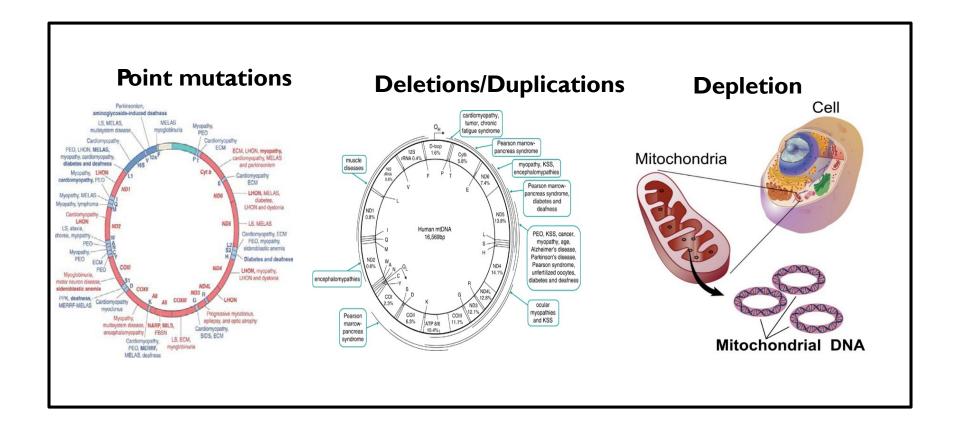
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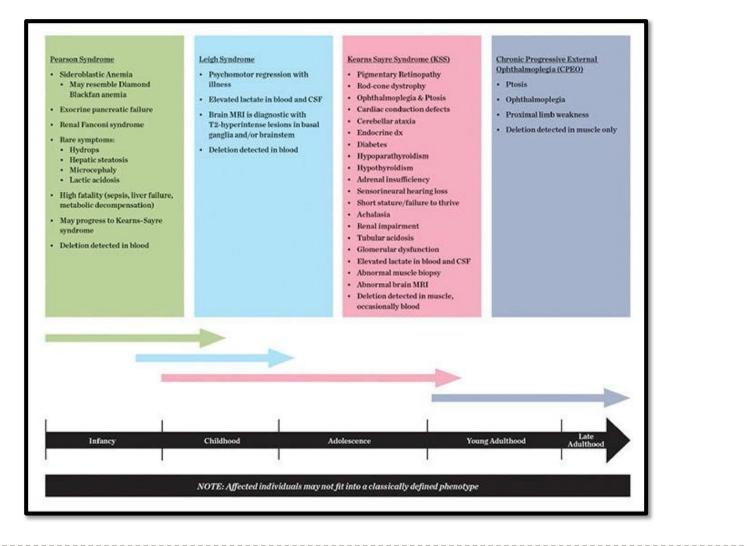
Alpers-Huttenlocher syndrome (AHS)	nDNA (POLG-related)	Intractable epilepsy, psychomotor regression and liver disease; might also include the clinical features of MCHS and MEMSA	55-57
Childhood myocerebrohepatopathy spectrum (MCHS)		Neuropathy, ataxia, hypotonia, myoclonus (spontaneous muscle contractions), choreoathetosis (the occurrence of involuntary jerky, writhing movements of muscles or muscle groups) and Parkinsonism, in addition to renal tubulopathy	
Ataxia neuropathy spectrum (ANS; previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO))		Sensory axonal neuropathy with variable sensory and cerebellar ataxia	
Myoclonic epilepsy myopathy sensory ataxia (MEMSA; previously referred to as spinocerebellar ataxia with epilepsy (SCAE))		Epilepsy. PEO, seizures, dysarthria, dementia, spasticity and myopathy	
	POLG1, which encodes α-DNA polymerase subunit γ1	Ataxia, peripheral sensory neuronopathy, Parkinsonism, premature ovarian failure, psychiatric symptoms, MELAS syndrome and epilepsy	195
	POLG2, which encodes DNA polymerase subunit γ2	Ptosis and proximal myopathy, dystrophy, cerebellar ataxia and gastrointestinal symptoms	196

Gorman et al. Nature reviews primers 2016

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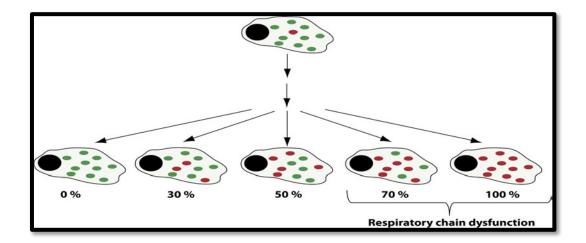
#### mtDNA (16 569 bp and contains 37 genes)



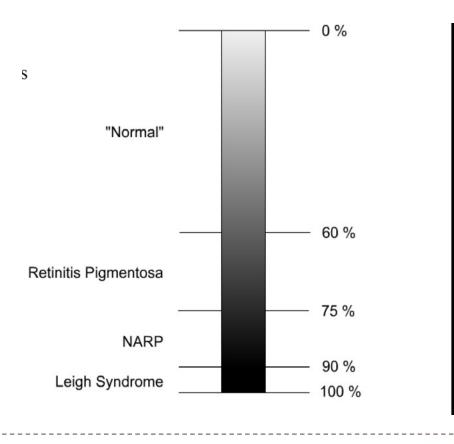


#### Mitochondrial DNA

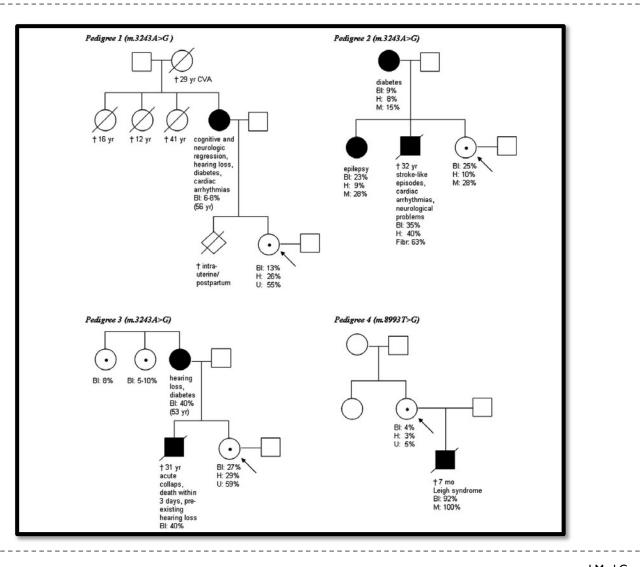
- Heteroplasmy
- Random mitotic segregation
- Threshold expression



Importance of tissue heteroplasmy: m.T8993G mutant load



Carelli V et al. Arch Neurol 2002;59:264-70

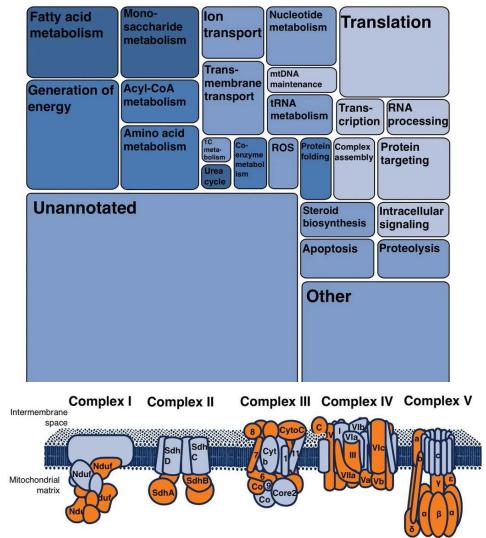


| Med Genet -2012-101172

#### **Split Patients?**

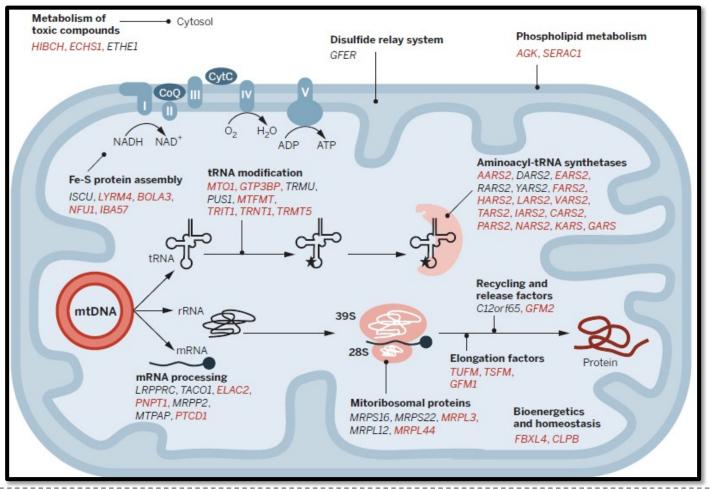
- Target a specific genotype
- Target a specific cellular pathway

# Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:



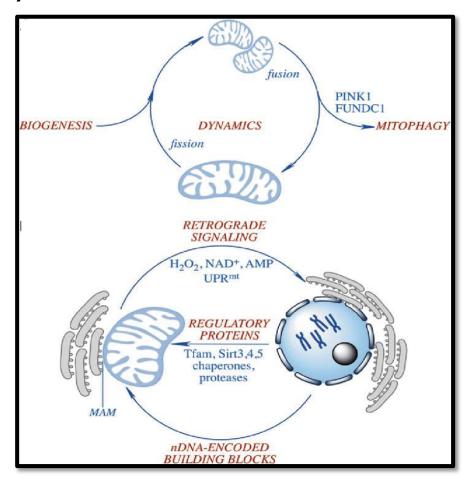
## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

#### Mitochondrial maintenance and translation



## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

Mitochondrial dynamic

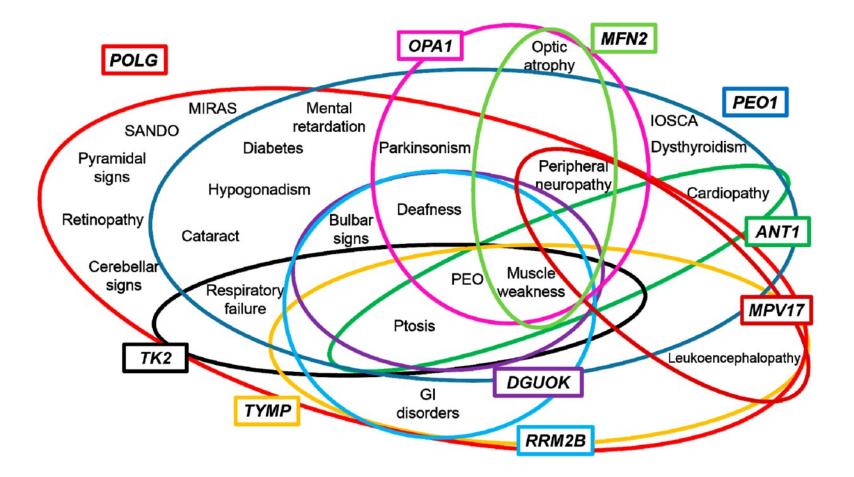


## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

#### Mitochondrial protein localization

ACAD8 ACAD9 ACADM ACADS ACADSB ACAT1 ALAS2 ALDH2 ALDH4A1 ALDH6A1 AMT ATPAF2 AUH	FH GCDH GCSH GLUD1 HADH HARS2 HIBCH HMGCS2 HMGCL HSD17B10 HSPD1 IDH2 IDH3B	PCCB PCK2 PDHA1 PDHB PDHX PDP1 POLG POLG2 PYCR1 RAR52 RMRP SARDH SARDH SAR52 SCO1	ARM52 BCL2 CPT1A DNM1L GCK GK KIF1B MAOA PINK1	AK2 DIABLO GATM GFER HTRA2 PANK2 PPOX	MTND1 MTND2 MTND3 MTND4 MTND4L MTND5 MTND6 MTC01 MTC01 MTC01 MTC02 MTC03 MTC03 MTATP6 MTATP8	ABCB7 ACADVL ADCK3 AGK ATP5E C12orf62 COX412 COX6B1 CPT2 CRAT CYCS CYP11A1 CYP11B1 CYP11B2	DHODH DNAJC19 FASTKD2 GPD2 HADHA HADHB HCCS L2HGDH MMAA MPV17 NDUFA1 NDUFA1 NDUFA2 NDUFA10	NDUFB3 NDUFB9 NDUFV2 NDUFS1 NDUFS2 NDUFS2 NDUFS4 NDUFS6 NDUFS6 NDUFS7 NDUFS8 OPA1 OPA3 PDSS1	SDHD SLC25A3 SLC25A4 SLC25A12 SLC25A13 SLC25A15 SLC25A19 SLC25A20 SLC25A22 SLC25A38 SPG7 TIMM8A UCP1 UCP2
BCKDHA BCKDHB BCS1L C&of38 C10of2 C12of65 C20of7 COA5 COX10 COX10 COX15 CPS1 D2HGDH DARS2 DBT DECR1 DGUOK DLD	ISCU IVD KARS MCCC1 MCCC2 MCEE MRPS16 MRPS22 MTFMT MTPAP MUT NAGS NDUFAF1 NDUFAF1 NDUFAF3 NDUFAF4 NUBPL	SCO2 SDHAF1 SDHAF2 SOD2 SUCLA2 SUCLG1 SURF1 TACO1 TK2 TMEM70 TK70 TKMU TSFM TTC19 TUFM UNG XPNPEP3 YARS2	9		Mitochondrial nner membrane 81	CYP24A1 CYP27A1 CYP27B1		SDHA SDHB SDHC AIFM1 AKAP10 AMACR APTX BAX BOLA3 CYB5R3 ETHE1 FXN GDAP1 HK1	UCP3 UQCRB UQCRQ HLCS LRPPRC LRRK2 MFN2 MLYCD NFU1 PARK2 PARK7 SACS SPG20 WWOX
DMGDH ETFA ETFB ETFDH	OAT OGDH OTC OXCT1 PC PCCA		Mitoch		ubmitochondrial location		AFG3L2 COQ2 COQ6 COQ9 GLDC	GLRX5 HOGA1 MMAB MMADHC PDSS2	PNKD PUS1 REEP1 STAR TMEM126A

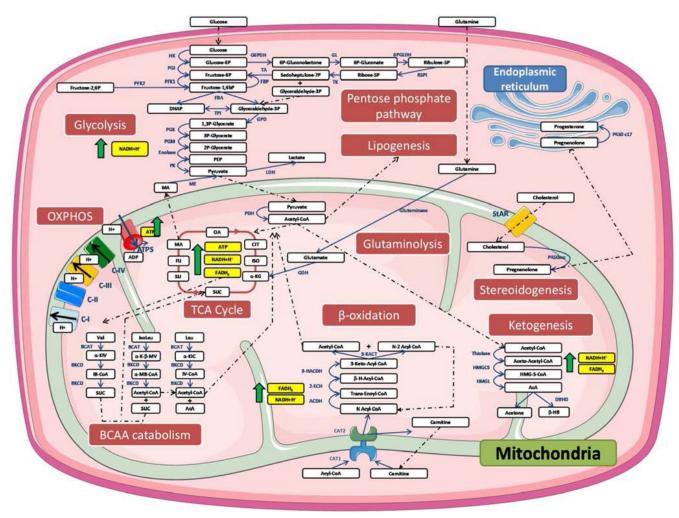
## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:



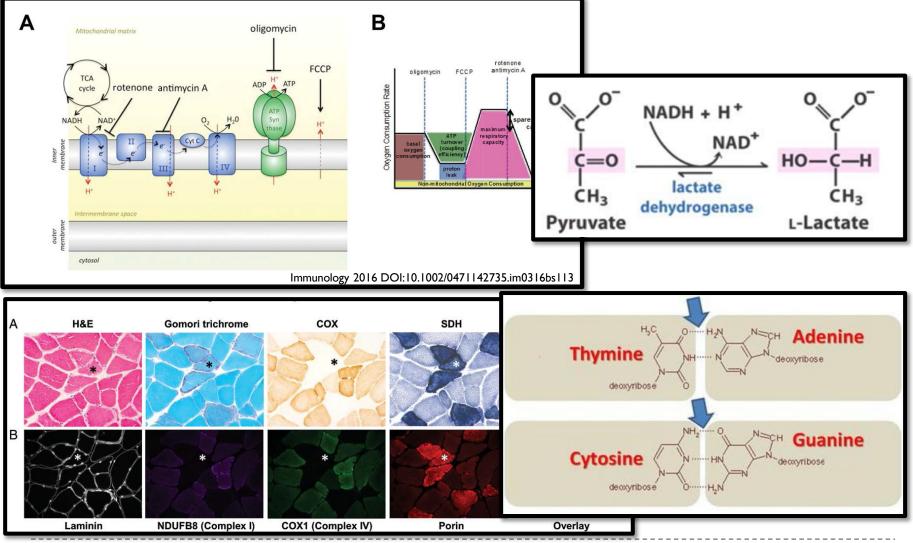
#### **Split Patients?**

- Target a specific genotype
- Target a specific cellular pathway
- Target a specific biochemical defect

## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

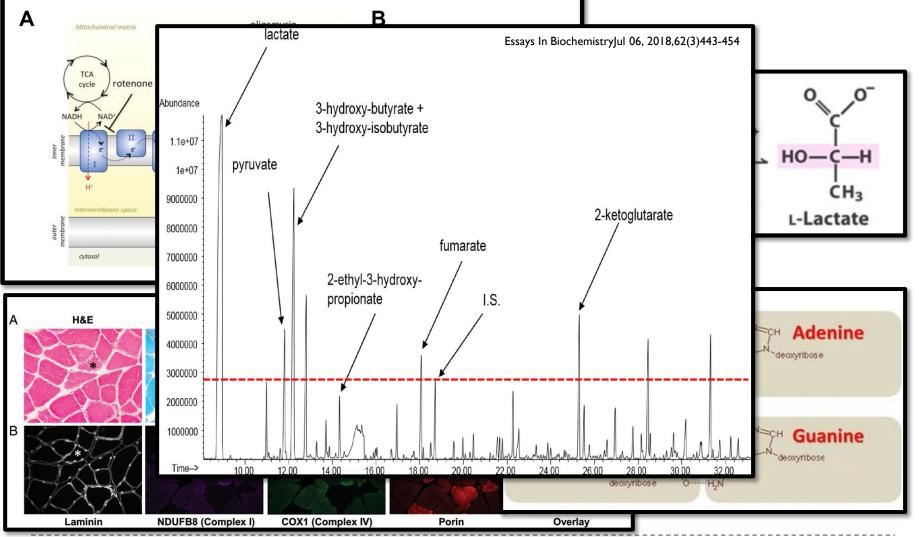


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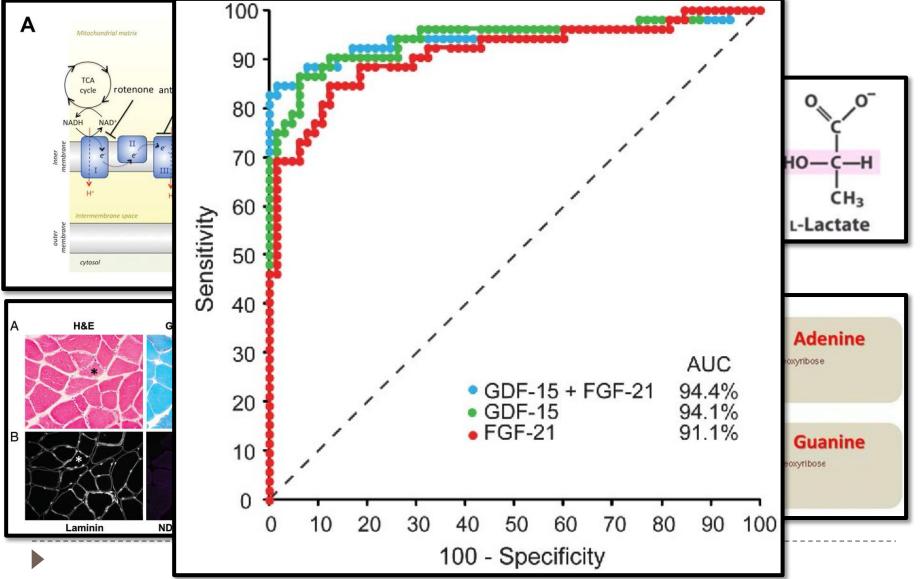
•The Journal of pathology 2017 DOI:10.1002/path.4809

# Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:



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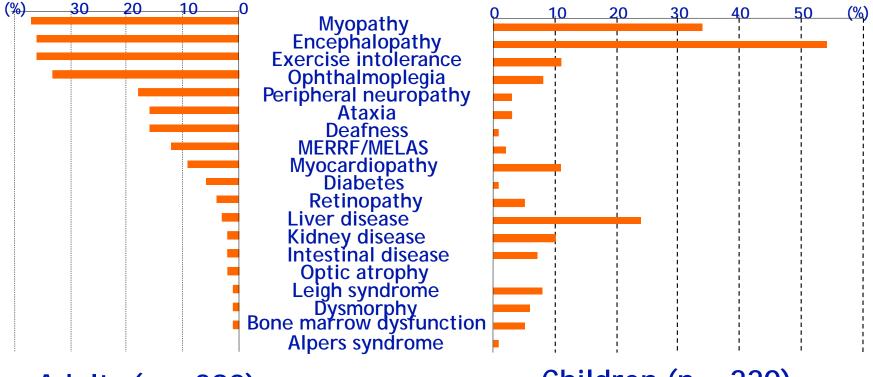


Neurology 2016 DOI:10.1212/wnl.000000000002705

#### **Split Patients?**

- Target a specific genotype
- Target a specific cellular pathway
- Target a specific biochemical defect
- Target a specific age group

## Mitochondrial disorders are one of the most complex and heterogeneous group of diseases:

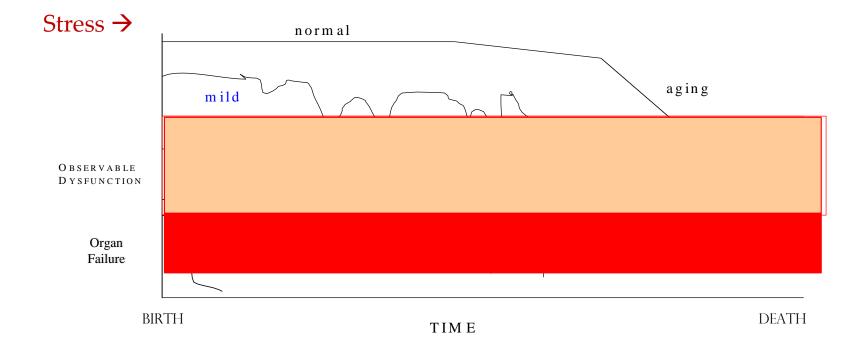


Adults (n = 390)

Children (n = 220)

### **Other considerations**

#### **Episodic, progressive Phenotype**



- Considerable heterogeneity
- No clear genotype-phenotype correlations
  - Is there consensus among clinical experts?
  - Uncertainties about the correct diagnosis in non-expert settings
  - Is there diagnostic consistency across centers?
- Clumping vs. splitting?

## How to select the right trial patient population?

One size does not fit all

- Stable or highly variable condition?
- Life limiting or symptom control?
- Short or long-term endpoints?

## How to select the right trial patient population?

#### One size does not fit all

Selection will need to be tailored by:

- Drug mechanism of action
- Therapeutic effects
- Off target effects
- Outcome measure



How to adequately power studies Overcome recruitment problems

- Availability of natural history data
- Trial design
- Patient input

