Mitochondrial transfer RNA gene mutations – proving pathogenicity and understanding transmission

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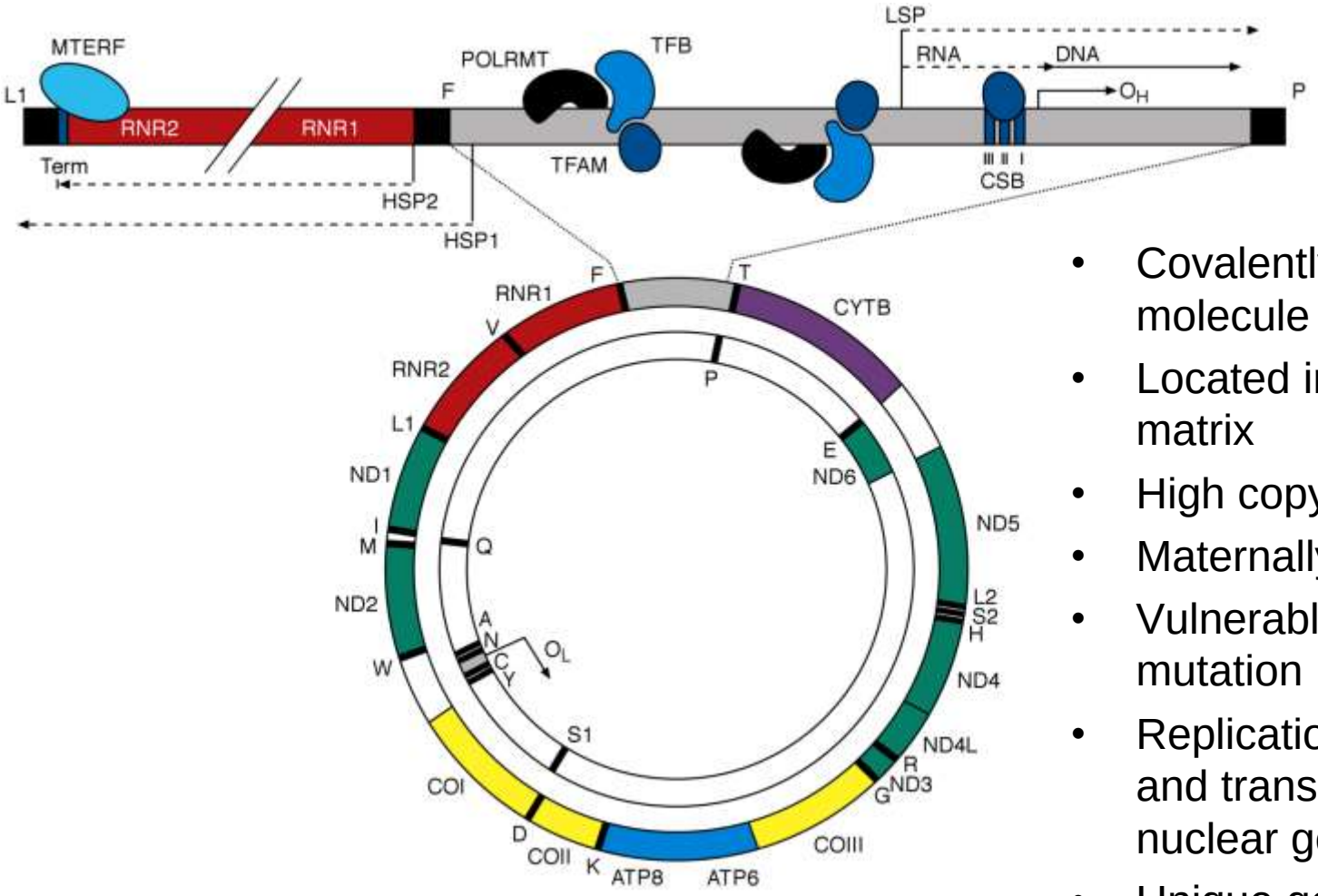
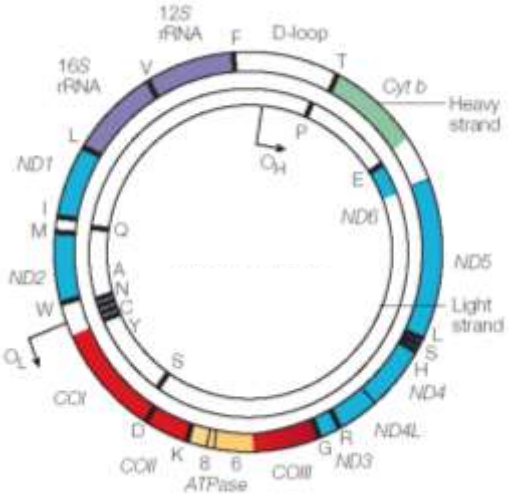
Mitochondrial DNA (mtDNA)



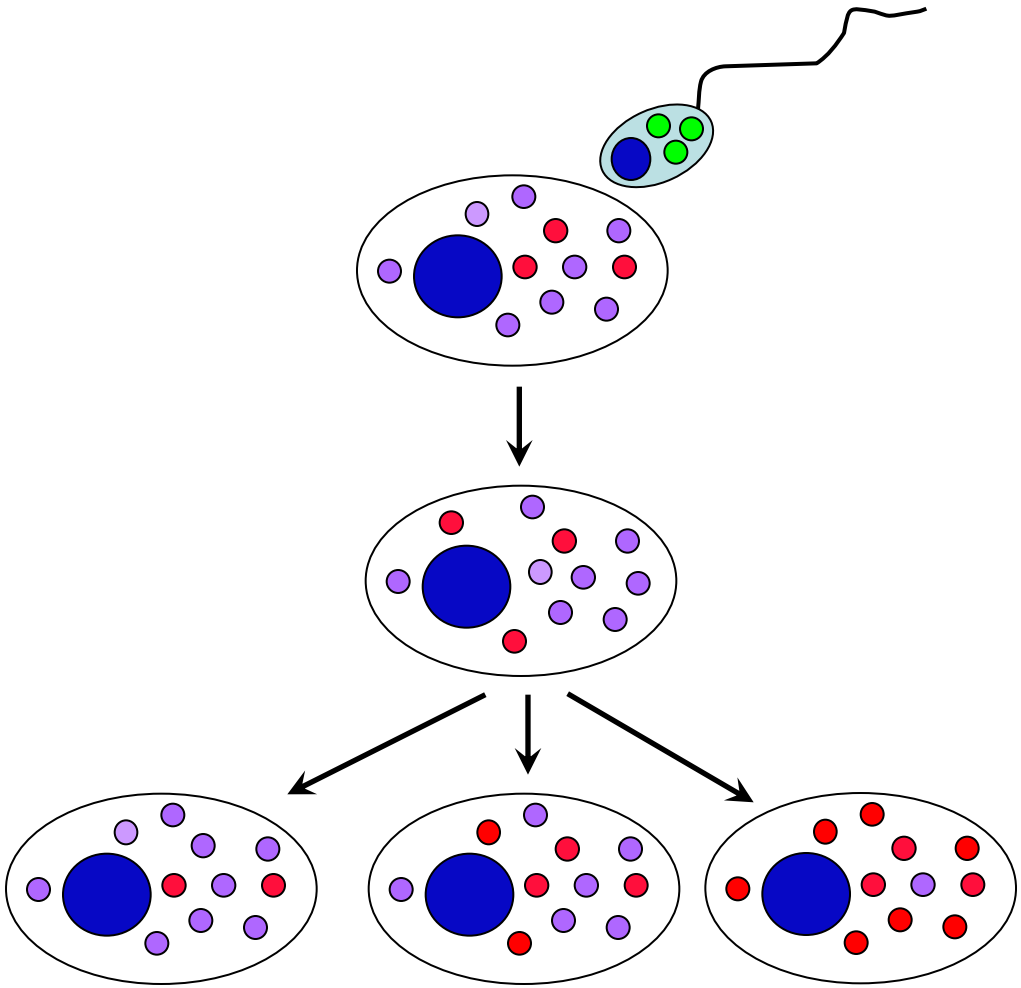
- Covalently-closed DNA molecule (16.6 kb)
- Located in mitochondrial matrix
- High copy number
- Maternally-inherited
- Vulnerable to ROS-induced mutation
- Replication, transcription and translation mediated by nuclear gene products
- Unique genetics

Tuppen et al. *Biochim Biophys Acta* 2010;**1797**:113-128

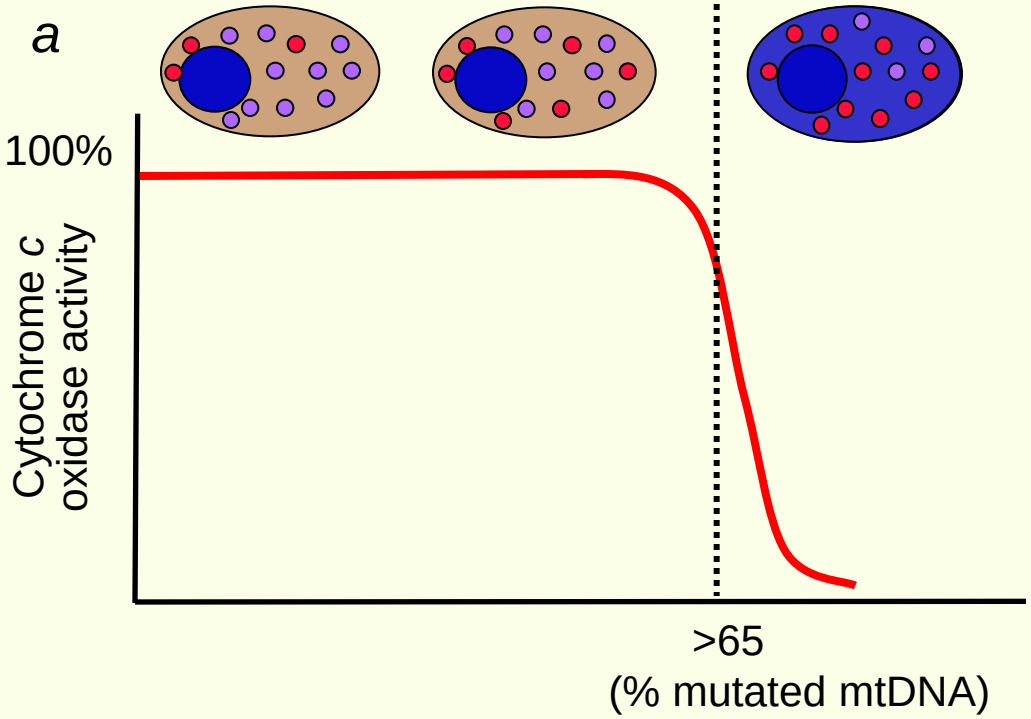


Mitochondrial DNA – genetic rules

- Maternal inheritance
- Heteroplasmy
- Threshold effect
- Mitotic Segregation



Threshold effect



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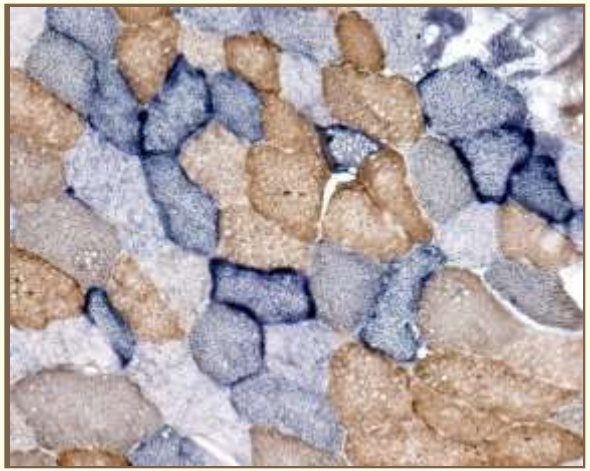
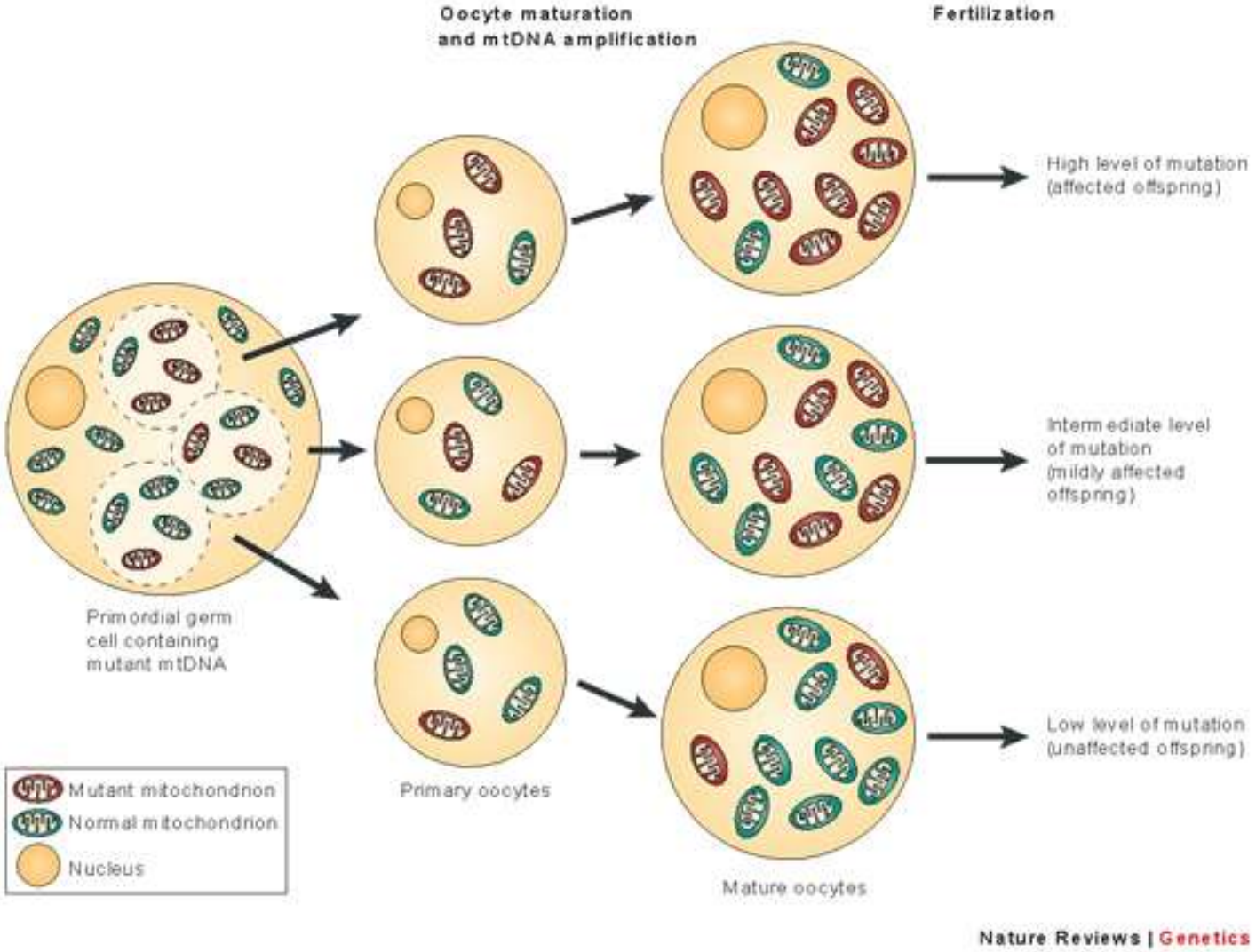




Taylor and Turnbull *Nature Rev Genet.* 2005;**6**:389-402

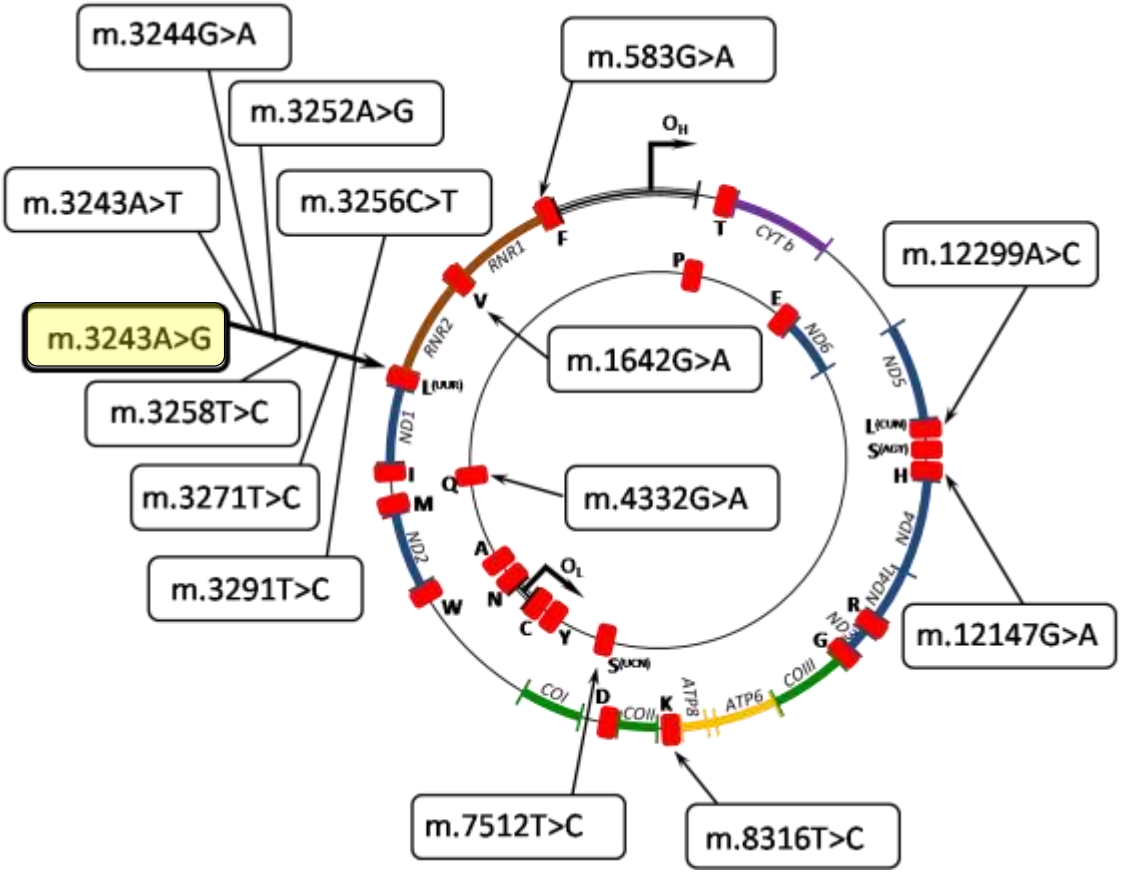
mt-tRNA Mutation and Disease

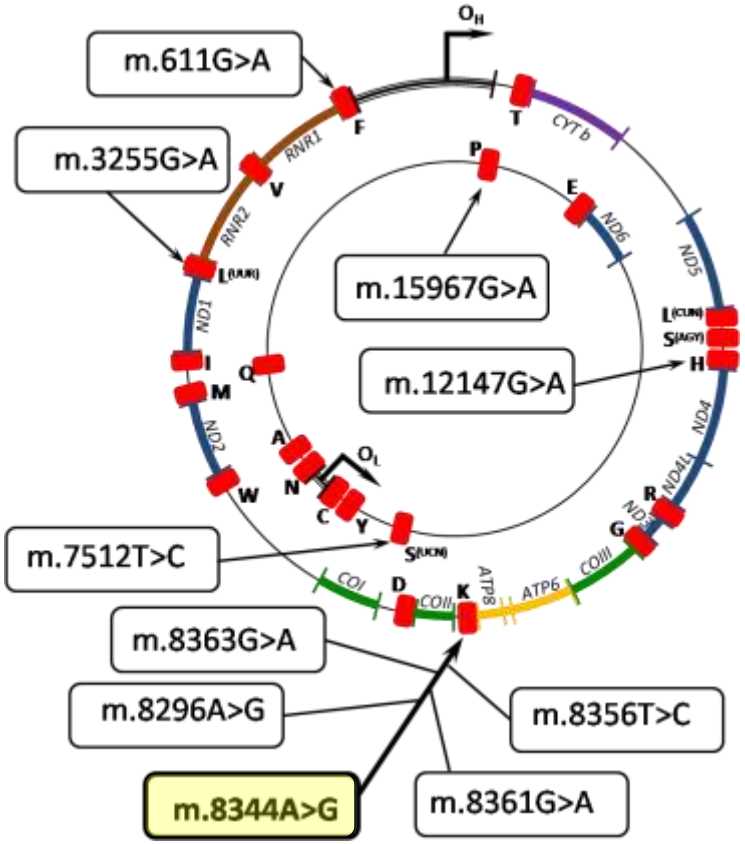
- Point mutations in mitochondrial tRNA (*MTT*) genes are responsible for the majority of mitochondrial disease presentations
- >200 pathogenic mt-tRNA mutations described
- Well-characterised heteroplasmic mt-tRNA mutations may be associated with specific clinical phenotypes

    m.3243A>G *MTTL1* mutation – MELAS (**M**itochondrial myopathy, **E**ncephalopathy, **L**actic **A**cidosis and **S**troke-like episodes)

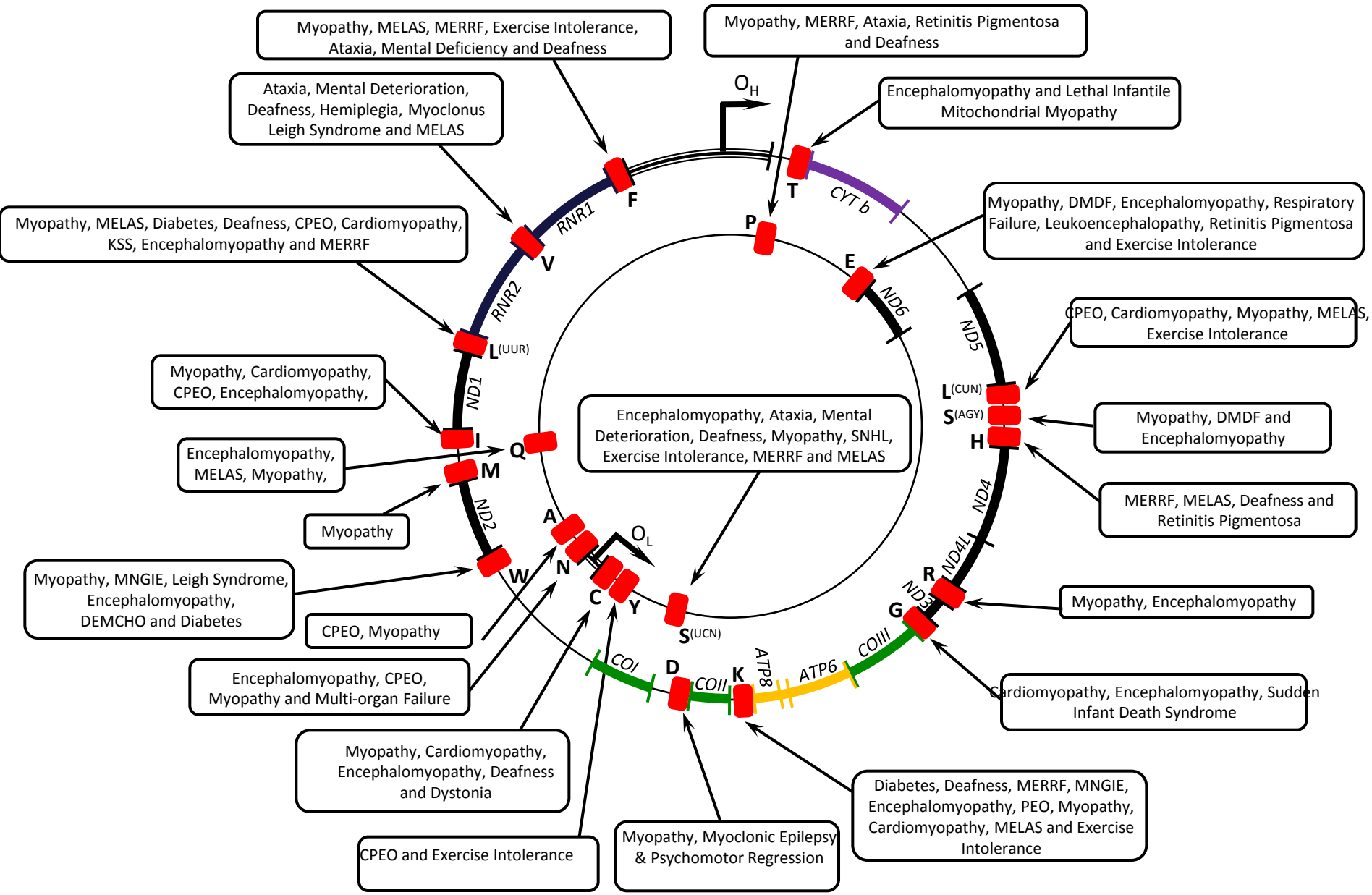
    m.8344A>G *MTTK* mutation – MERRF (**M**yoclonic **E**pilepsy with **R**agged-**R**ed **F**ibres)

MTT gene mutations associated with MELAS and MERRF phenotypes





Clinical presentations associated with *MTT* gene mutations



Characteristic clinical syndrome? (MELAS, MERRF, LHON, Pearson syndrome, Leigh Syndrome, Alpers)

YES

Test common mtDNA or *POLG* mutations in blood

Negative result, further investigations required

NO

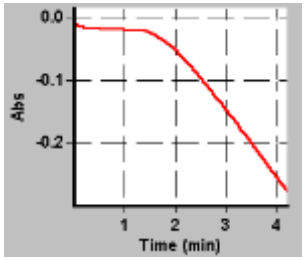
MUSCLE BIOPSY

Histochemistry

COX **COX/SDH**

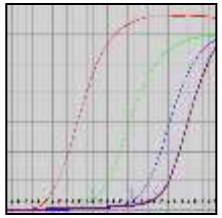
cytochrome *c* oxidase (COX)-deficient fibres (uniform decrease or mosaic pattern)

Biochemistry



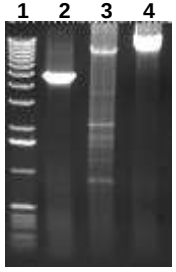
Measurement of respiratory chain complex activities and Ubiquinone (CoQ_{10})

Molecular Genetics



real-time PCR (mtDNA depletion)

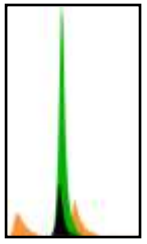
POLG, PEO1, TK2, DGUOK, RRM2B, SUCLA2, MPV17 analysis



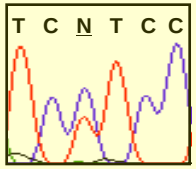
Long-range PCR (mtDNA rearrangements)

single, large-scale mtDNA deletion disorders

multiple mtDNA deletion disorders *– POLG, POLG2, PEO1, SLC25A4* analysis



Pyrosequencing, fluorescent PCR-RFLP (common mtDNA point mutations)



mtDNA sequencing (novel mtDNA mutations)

single fibre PCR and family studies

Complex I deficiency – mtDNA and nuclear-encoded genes (structural and assembly factors)
Complex II deficiency – *SDHA, SDHB, SDHC, SDHD* analysis
Complex III deficiency – *MTCYB* (mtDNA), 10 nuclear structural genes, *BCS1L*
Complex IV deficiency – mtDNA, COX assembly factors (*SURF1, SCO1, SCO2, COX10, COX15*…)
Multiple complex deficiencies – mtDNA, numerous nuclear mtDNA maintenance and translation genes
Ubiquinone deficiency - *CABC1, COQ2, COQ9, PDSS1* gene analysis

Assigning pathogenicity to novel *MTT* gene variants

Fulfilment of canonical criteria, including:

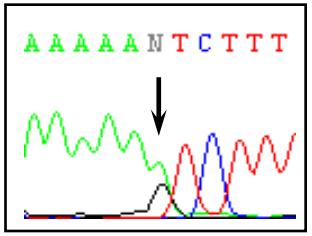
- Absence from databases of control human mtDNA sequences
- Occurrence at evolutionary conserved sites (majority of cases)
- Usually heteroplasmic, with the highest mutation load in clinically affected tissues
- Predicted to alter tRNA structure
- Associated with a biochemical/cellular defect
- Clear segregation of mutation load in COX-deficient muscle fibres

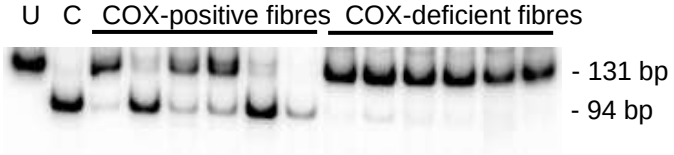
DiMauro and Schon *Am J Med Genet.* 2001;**106**:18-26

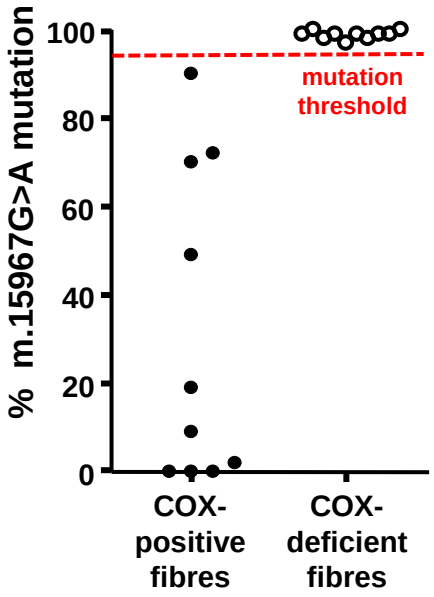
McFarland et al. *Trends Genet.* 2004;**20**:591-596

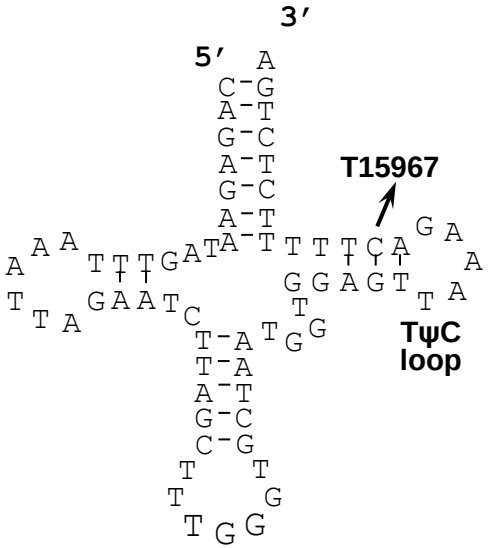
A New Mitochondrial Transfer RNA^{Pro} Gene Mutation Associated With Myoclonic Epilepsy With Ragged-Red Fibers and Other Neurological Features

Emma L. Blakely, PhD; S. Anand Trip, PhD, MRCP; Helen Swalwell, PhD; Langping He, PhD; Damian R. Wren, DM, FRCP; Philip Rich, FRCR, FRCS; Douglass M. Turnbull, MD, PhD; Salah E. Omer, FRCP; Robert W. Taylor, PhD, FRCPath









	TψC stem		TψC stem
Patient	GGAGT	TAAAG--	ATTTT
	15978		15964
Human	GGAGT	TAAAG--	ACTTT
Gorilla	GGAGT	CGAGG--	ACTTT
Chimpanzee	GAAGT	TAATT--	ACTTT
Bovine	GAGAC	TGCA---	GTTTC
Mouse	GGGGA	GTAGC--	TCCTT
Rat	GGGGA	GGTAGT-	TCCTT
Chicken	GGAGG	TTTGAGC	CCTCC
X. laevis	GGAGG	TTTGAGT	CCTTC
S. cerevisae	GTTAG	TTCGAGT	CTATC

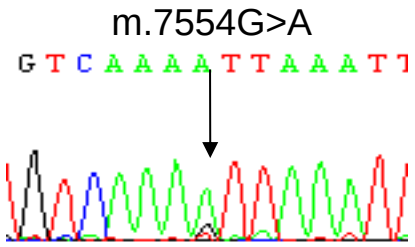
Blakely *et al*. *Arch. Neurol.* 2009;**66:**399-402.

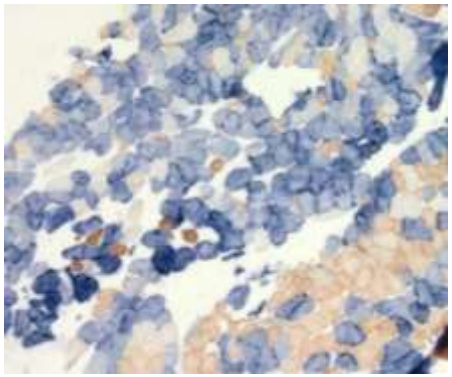
Identification of rare or novel *MTT* gene mutations in Newcastle Mitochondrial NCG Diagnostic Laboratory since April 2007

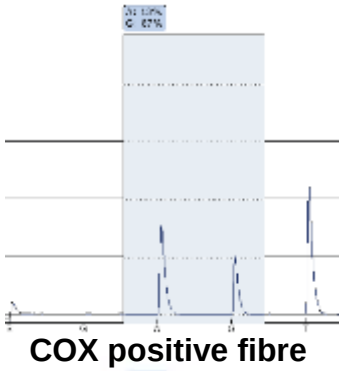
Mutation	Gene	Sporadic or Transmitted	Single Fibre studies	Novel or previously published
m.586G>A	tRNA-Phe	Sporadic	Yes	Novel
m.618T>G	tRNA-Phe	Sporadic	Yes	Novel
m.3243A>T	tRNA-Leu(UUR)	Transmitted	Yes	Published
m.3291T>C	tRNA-Leu(UUR)	?	Yes	Published
m.5543T>C	tRNA-Trp	Transmitted	No – previously done	Published
m.7451A>T	tRNA-Ser(UCN)	?	Yes	Novel
m.7554G>A	tRNA-Asp	?	Yes	Novel
m.8304G>A	tRNA-Lys	Transmitted	Not possible	Novel
m.8313G>A	tRNA-Lys	Sporadic	Yes	Published
m.9997T>G	tRNA-Gly	Transmitted	Homoplasmic	Published
m.10010T>C	tRNA-Gly	?	No – previously done	Published
m.12258C>A	tRNA-Ser(AGY)	Transmitted	No – previously done	Published
m.12261T>C	tRNA-Ser(AGY)	Transmitted	Yes	Novel
m.12264C>T	tRNA-Ser(AGY)	Transmitted	Yes	Novel
m.12283G>A	tRNA-Leu(CUN)	Sporadic	Yes	Novel
m.12317T>C	tRNA-Leu(CUN)	?	Yes	Novel
m.14723T>C	tRNA-Glu	Sporadic	Yes	Novel
m.15967G>A	tRNA-Pro	Sporadic	Yes	Novel
m.16023G>A	tRNA-Pro	?	Yes	Novel

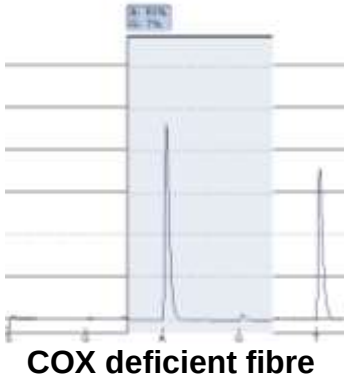
Case History – Patient TJ

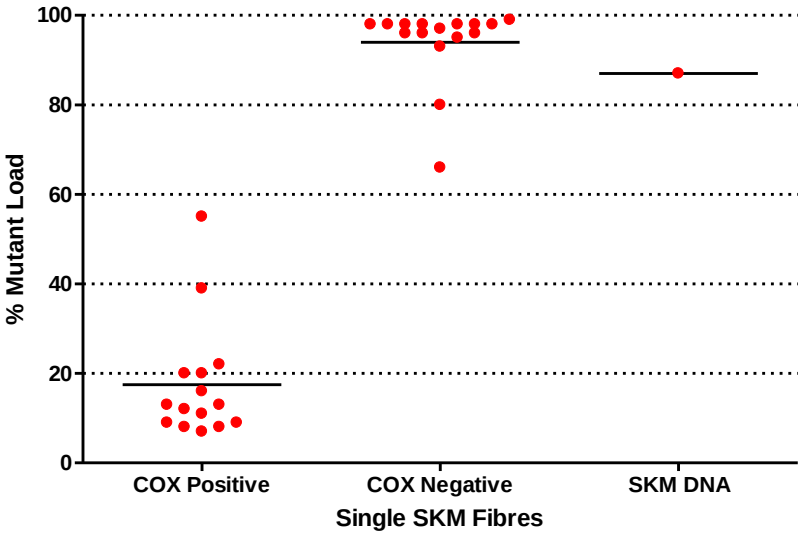
- Patient presented with delayed milestones, nystagmus, ataxia, myopathy, abnormal ECG, raised serum lactate and many COX-ve, ragged-red fibres on muscle biopsy
- He has a sister with possible MS
- Negative for mtDNA rearrangements, common *POLG1,* m.3243A>G and m.8344A>G mutations (Oxford)
- mtDNA sequencing revealed a novel m.7554G>A mt-tRNA^{Asp} (*MTTD*) variant





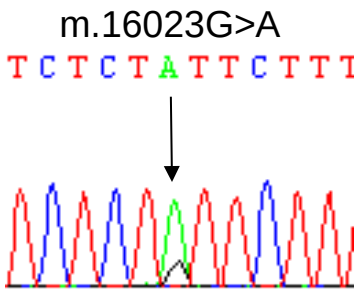


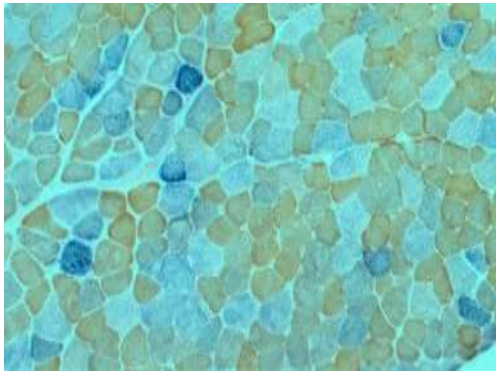


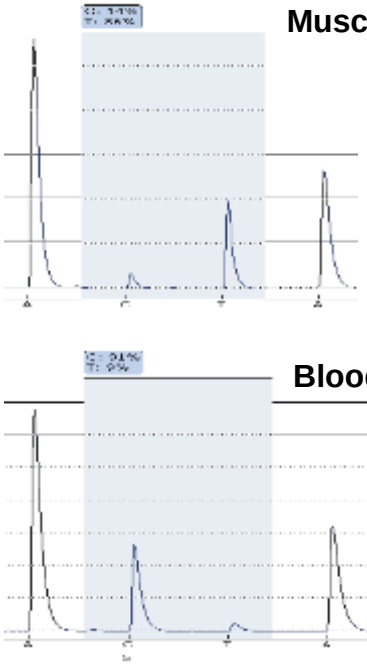


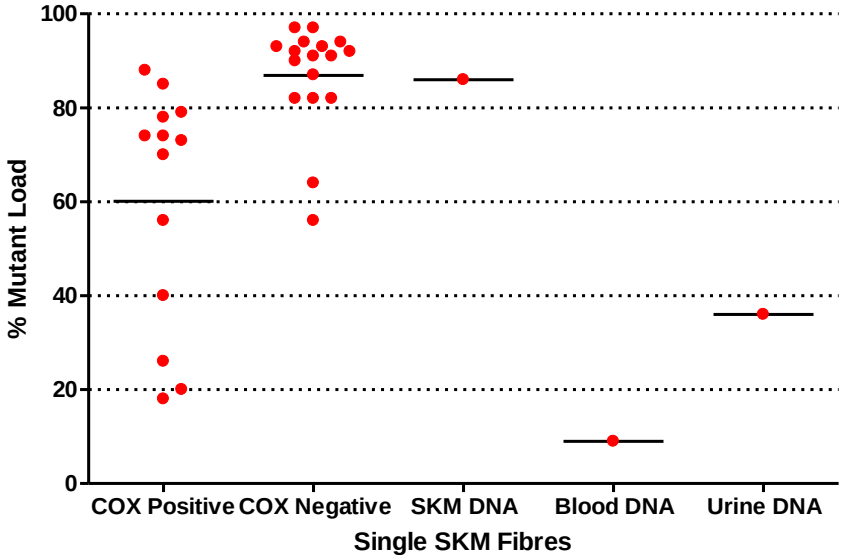
Case History – Patient AM

- Patient presented with deafness and retinopathy
- Muscle biopsy showed mild myopathic changes, ~60% COX-deficient fibres and occasional RRFs
- Brain MRI revealed marked white matter vascular changes
- Family history of migraine and deafness
- m.3243A>G and mtDNA rearrangements excluded (Newcastle)
- mtDNA sequencing revealed a novel m.16023G>A tRNA^{Pro} (*MTTP*) variant









Transmission of mt-tRNA (*MTT*) gene mutations

- mt-tRNA mutations can be sporadic or maternally-inherited
- Difficult to provide accurate genetic counseling for risk of transmission which is mutation specific and further complicated by the mitochondrial bottleneck
- Which pathogenic mt-tRNA mutations are inherited?
- Why are some inherited and others not?
- Can we accurately determine whether a mt-tRNA mutation will be transmitted?

HUMAN MUTATION Mutation in Brief 30:E984-E992 (2009) Online

MUTATION IN BRIEF

HUMAN MUTATION

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Pathogenic Mitochondrial tRNA Mutations – Which Mutations Are Inherited and Why?

Joanna L. Elson[1], Helen Swalwell[1], Emma L. Blakely, Robert McFarland, Robert W. Taylor,* and Doug M. Turnbull

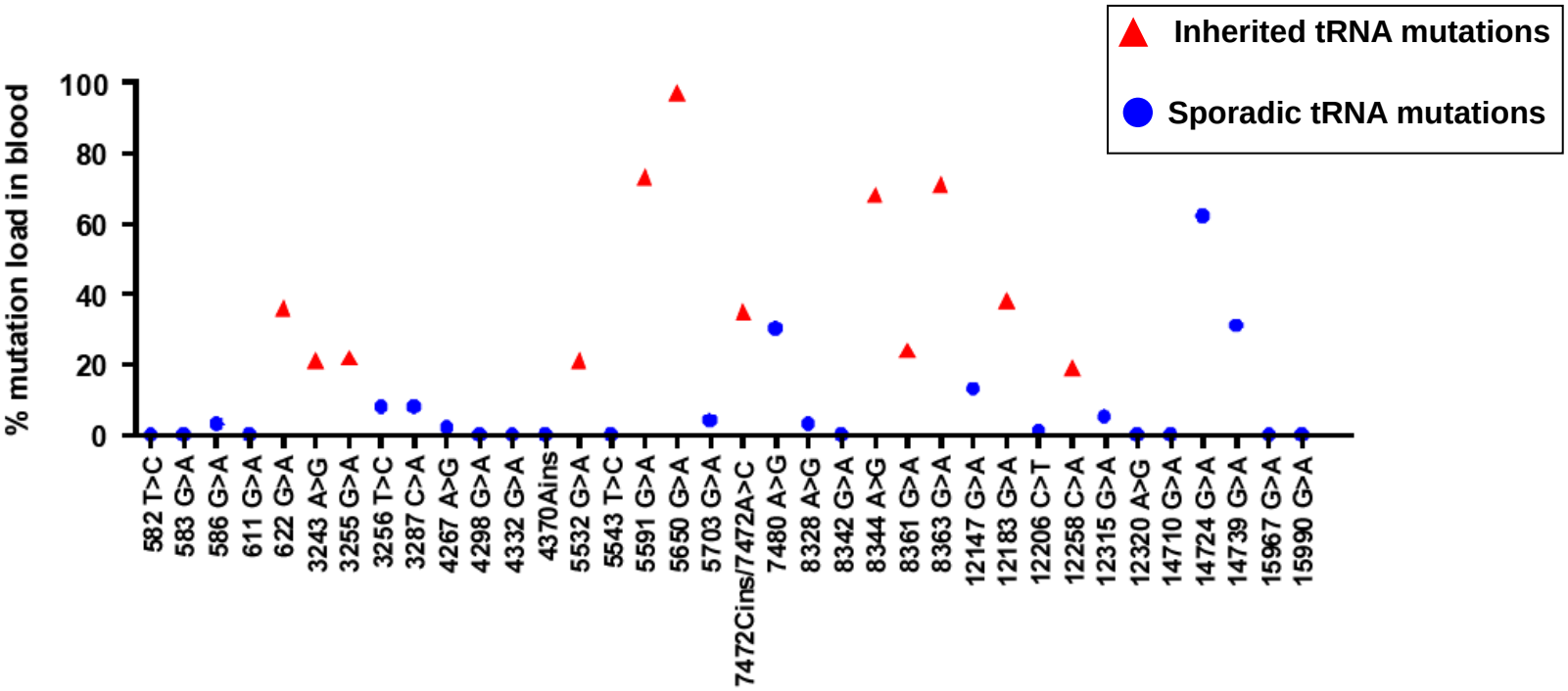
- Studied 36 well-characterised pathogenic mt-tRNA mutations
- 12 maternally-inherited, 24 were sporadic mutations

Determined the likelihood of transmission is not related to:

- the patient's clinical features
- the mutation load in muscle
- the evolutionary conservation of the mutation site
- the threshold mutation level for COX deficiency

Elson et al. *Human Mutation* 2009; **30**:E984-992

The level of mutation in blood was significantly lower in sporadic mt-tRNA mutations when compared to inherited mt-tRNA mutations (P = 0.0003)



These data suggest that those mt-tRNA mutations which exert a major (*biochemical*) phenotype in rapidly dividing cells and are selected against, are unlikely to be inherited

Elson et al. *Human Mutation* 2009; **30**:E984-992

Summary

- Pathogenic mt-tRNA mutations are common and are associated with marked clinical heterogeneity
- Novel mt-tRNA gene mutations are still being identified
- Proving pathogenicity of novel mt-tRNA mutations is complex but necessary
- mt-tRNA mutations may be maternally transmitted or sporadic and determining inheritance is key for counseling families
- Recent data suggest those exhibiting a strong genotype in dividing cells are unlikely to be inherited, therefore determining the level of mutation in several mitotic tissues, including blood, is essential

Acknowledgments

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Angela Baker

http://www.mitochondrialncg.nhs.uk

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wellcome trust

Muscular Dystrophy Campaign

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust