

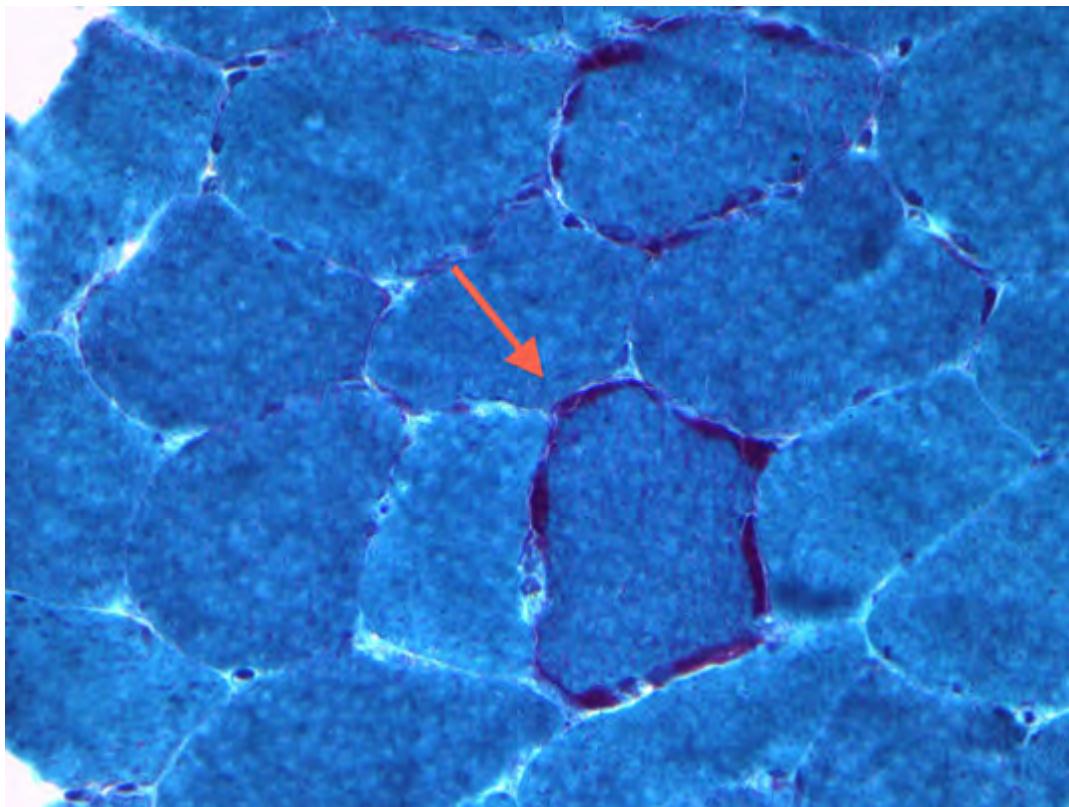
New phenotypes in mitochondrial myopathies

**Sydney Neurophysiology Workshop,
Park Hyatt, November 15th, 2014**

Objectives

- Illustrate the phenotypic heterogeneity of mitochondrial disease
- Illustrate the genetic heterogeneity of mitochondrial disease
- Demonstrate the impact of NGS on the diagnosis of mitochondrial disease

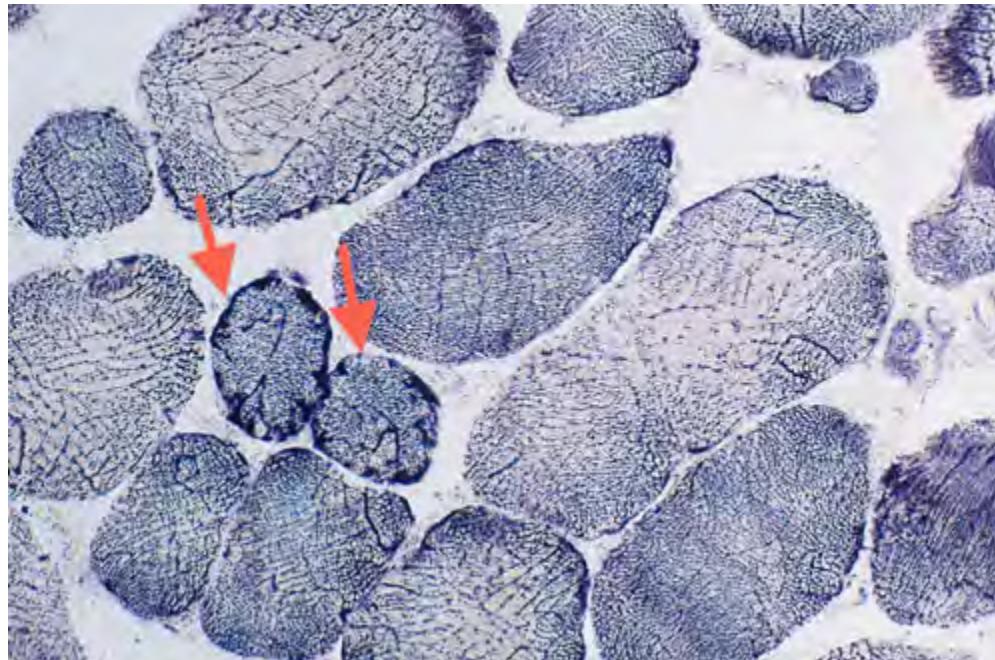
Pathological hallmark of mitochondrial disease



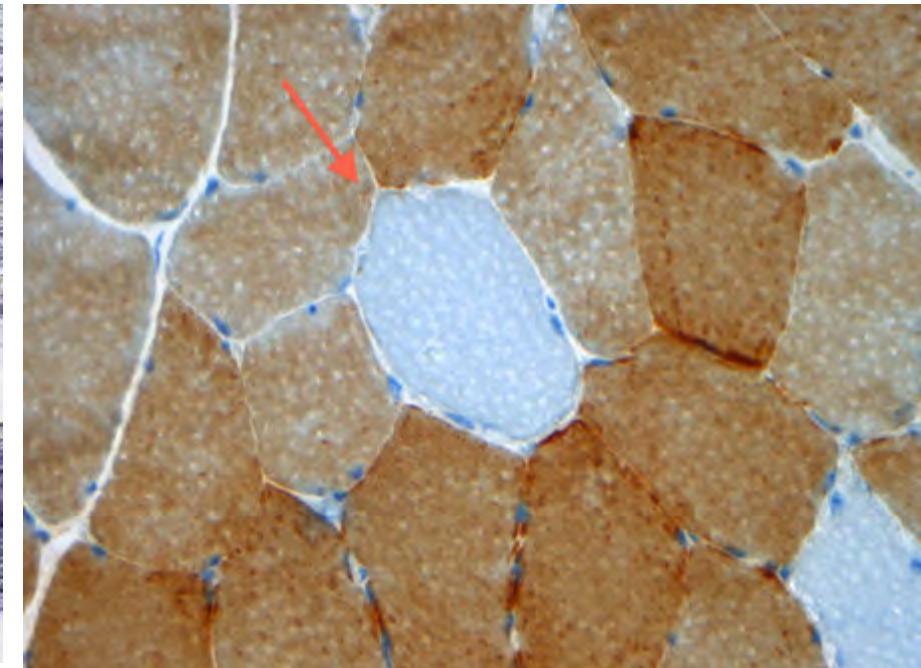
Modified Gomori trichrome stain: “**ragged-red fibres**”

(Engel and Cunningham, 1963)

Pathological hallmark of mitochondrial disease

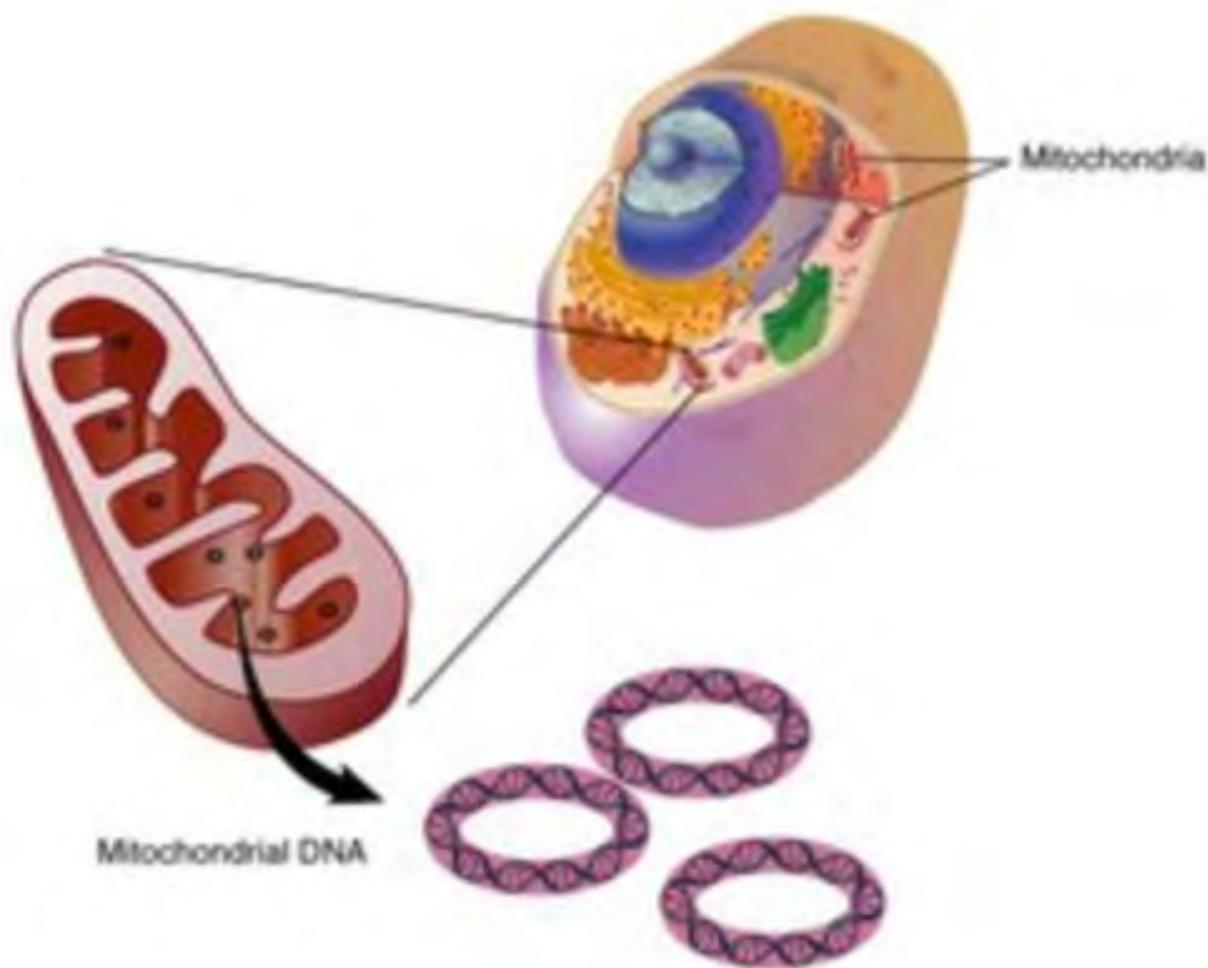


SDH stain:
“ragged-blue fibres”



SDH/COX stain:
“ragged-blue fibres”

Mitochondrial DNA



Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies

I. J. Holt, A. E. Harding & J. A. Morgan-Hughes

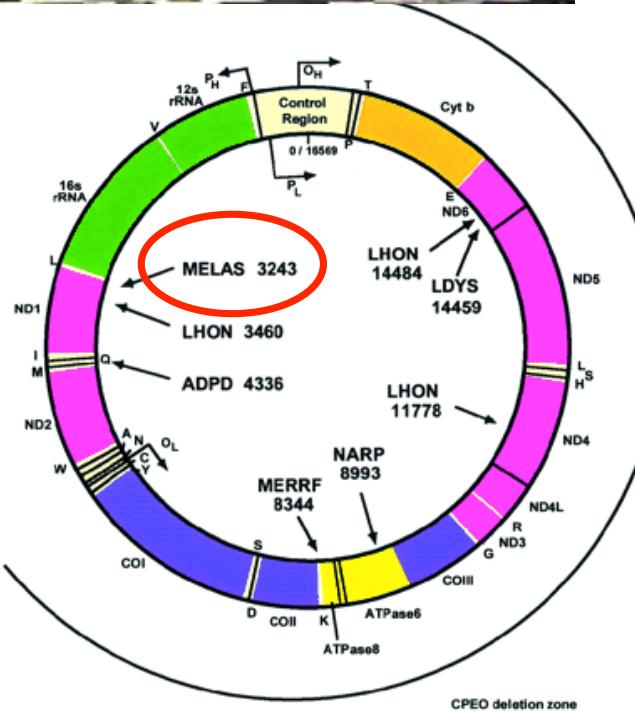
Department of Clinical Neurology, Institute of Neurology,
Queen Square, London WC1N 3BG, UK

(Holt et al., Nature 1988)

MELAS



MERRF



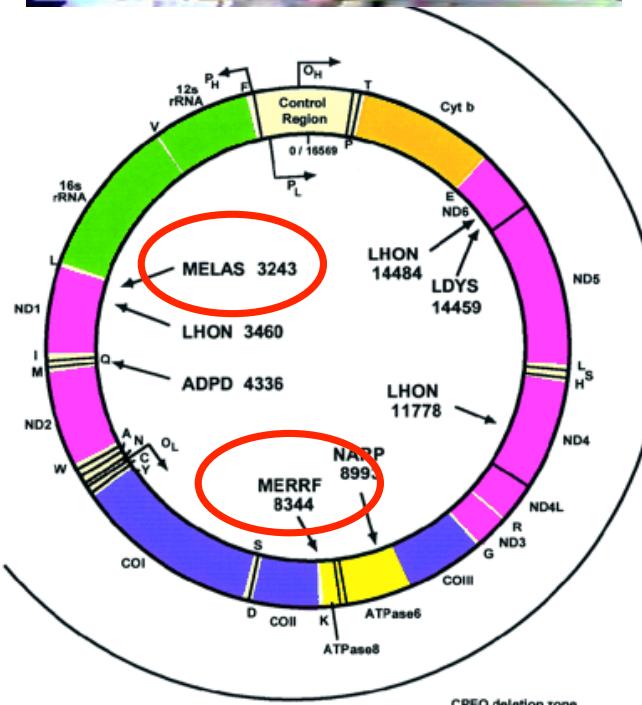
KSS/CPEO



MELAS



MERRF



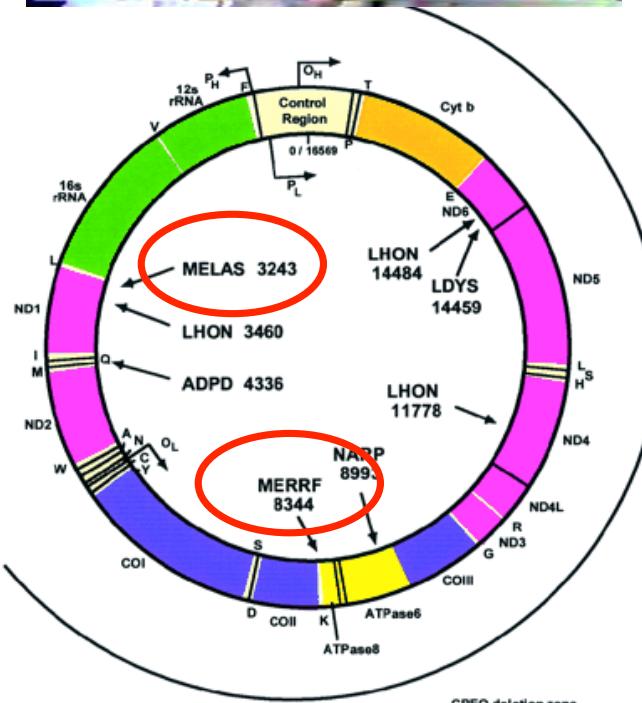
KSS/CPEO



MELAS



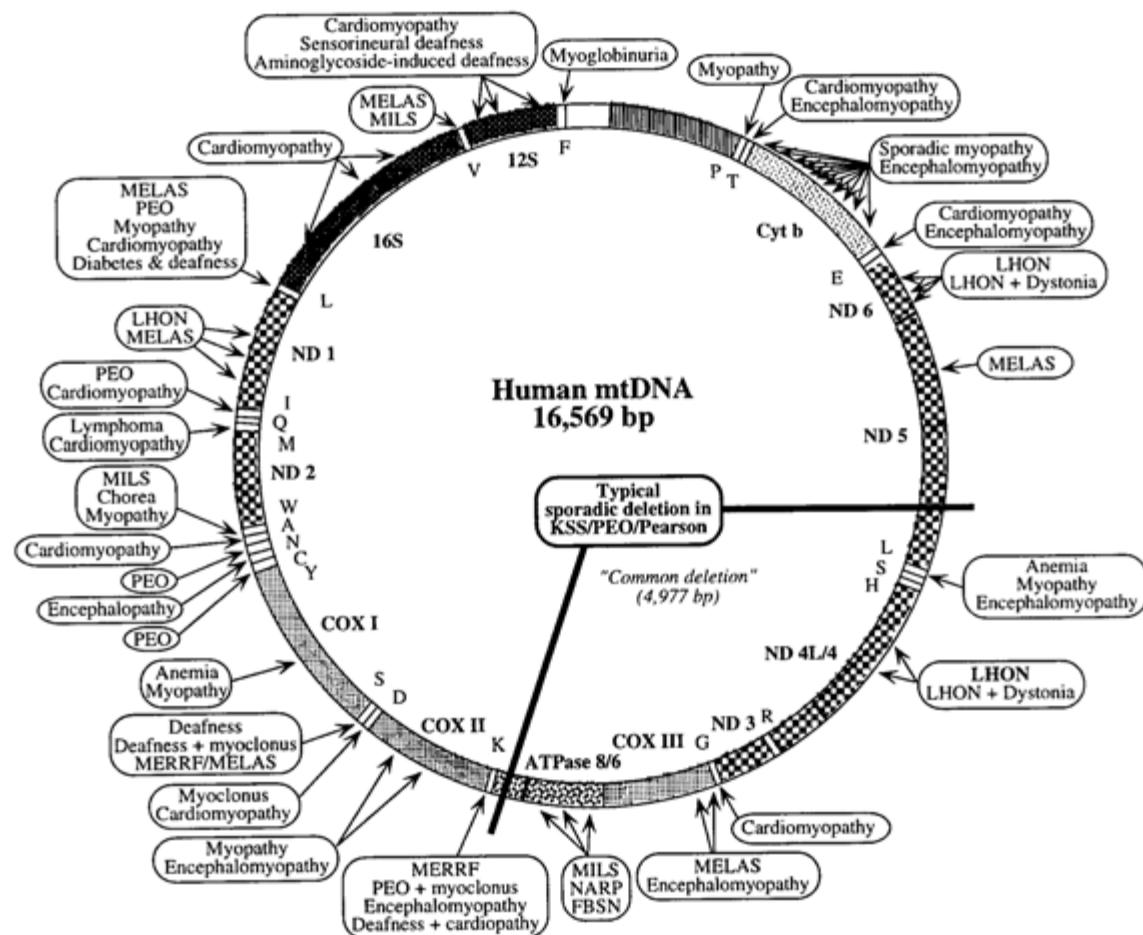
MERRF



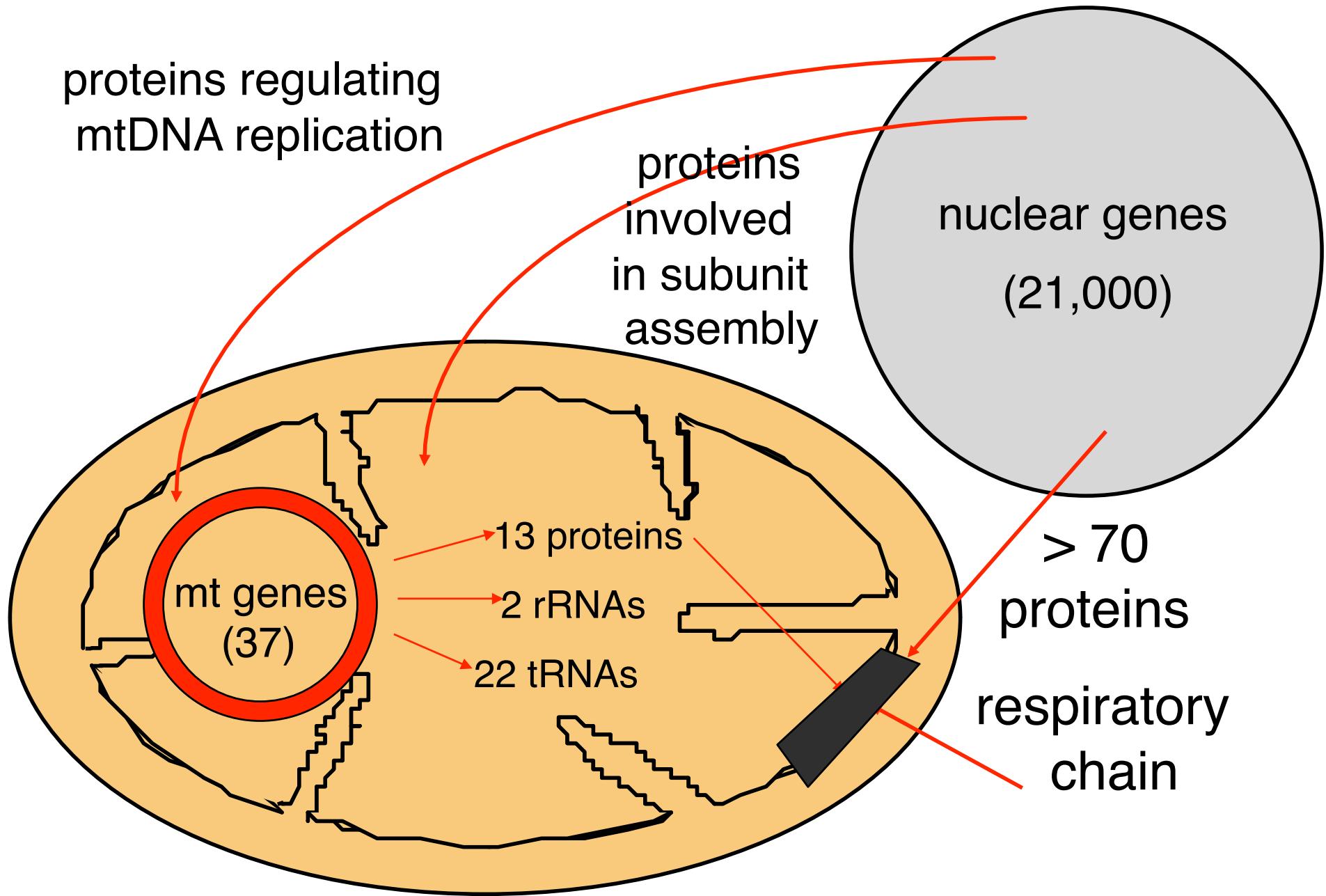
KSS/CPEO



mtDNA mutations cause MD

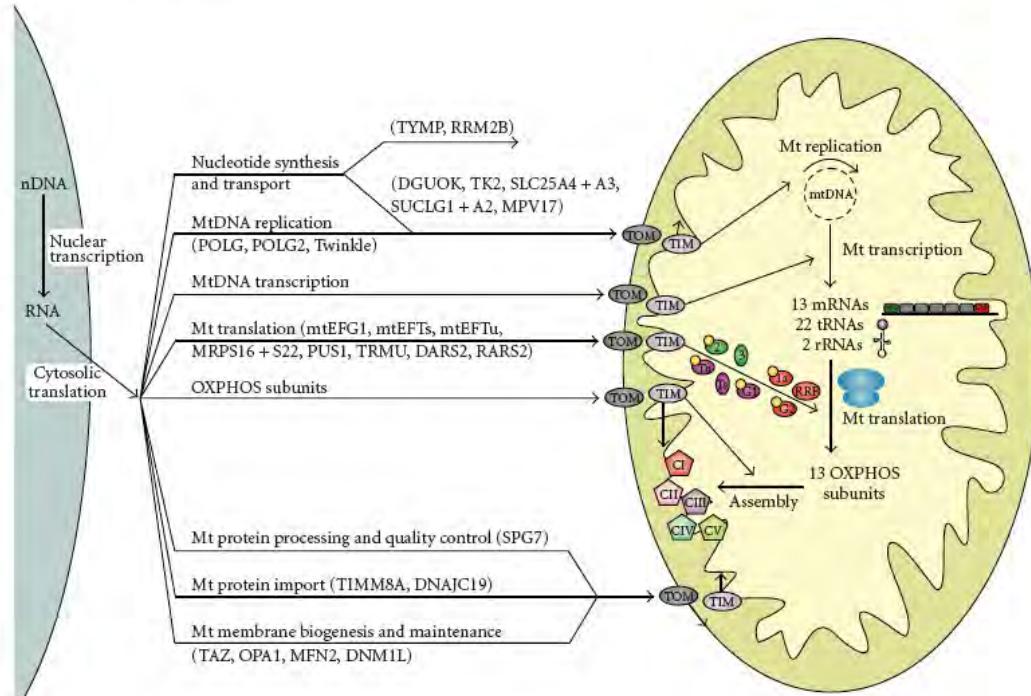


Courtesy from Eric Schon

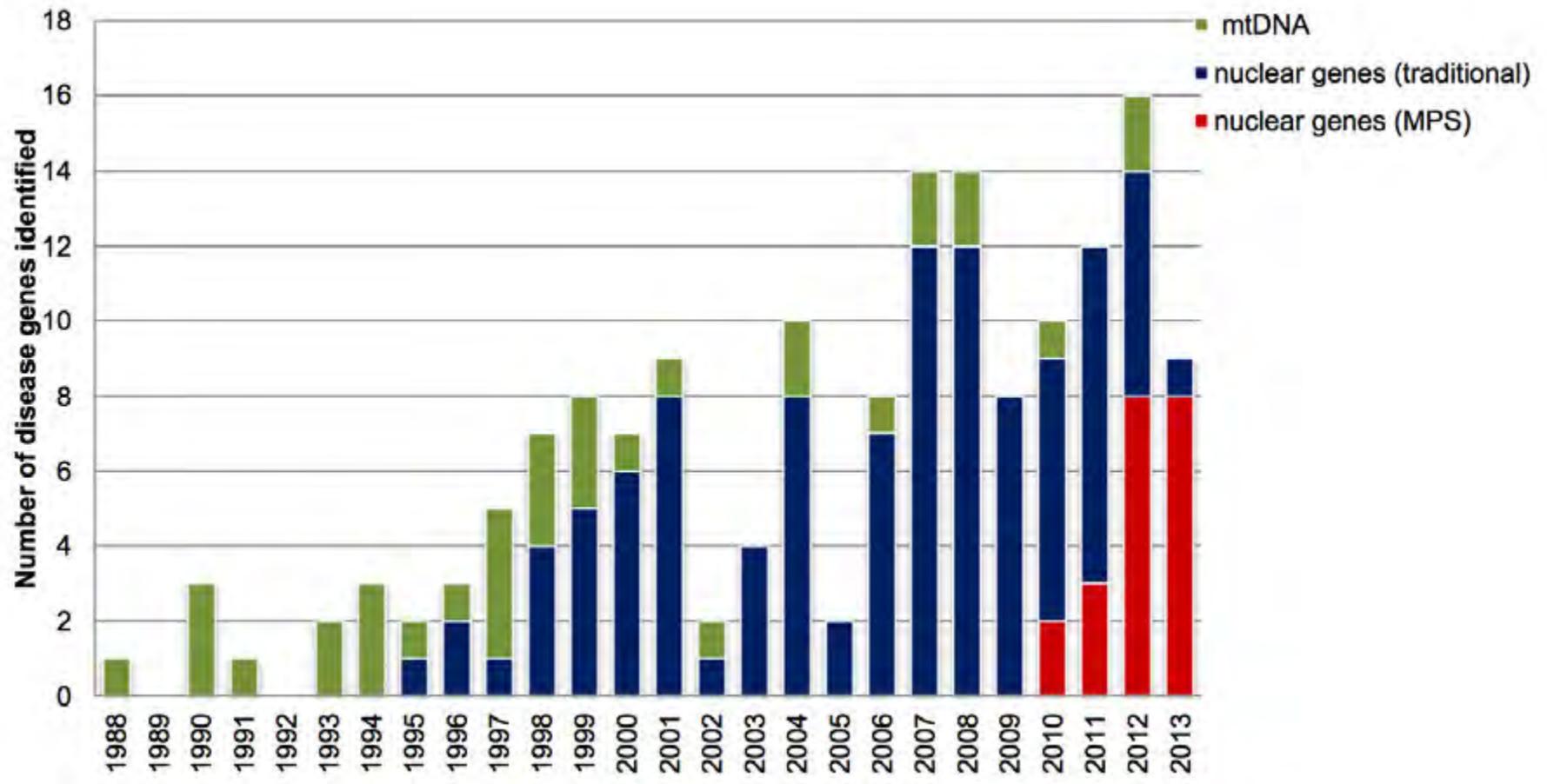


Nuclear-mitochondrial interactions

Journal of Biomedicine and Biotechnology

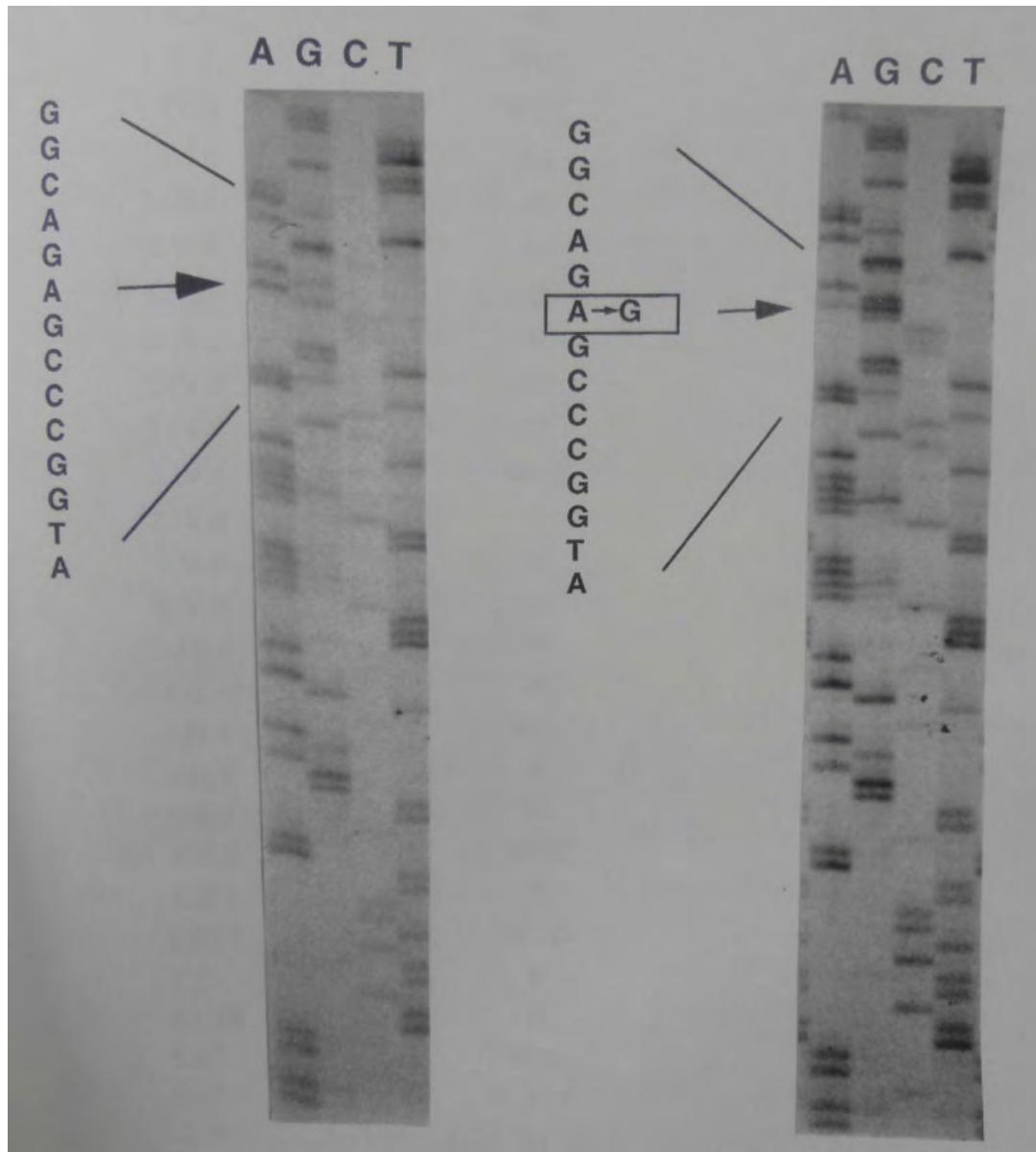


Genes associated with mitochondrial disease

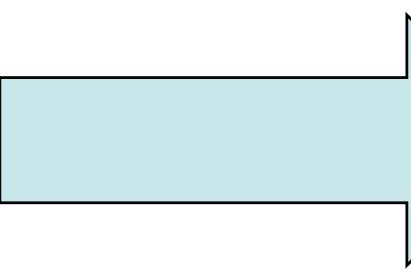


NGS: >100 nuclear and >100 mitochondrial genes
associated with mitochondrial disease

Sanger sequencing



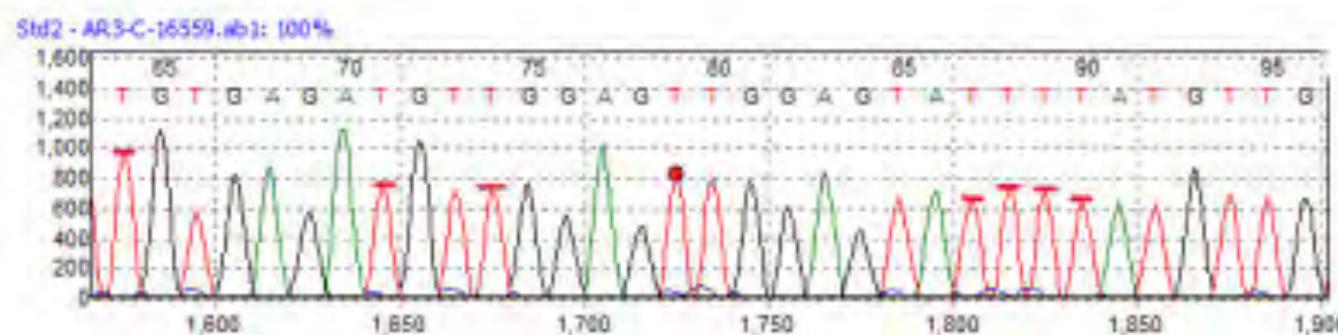
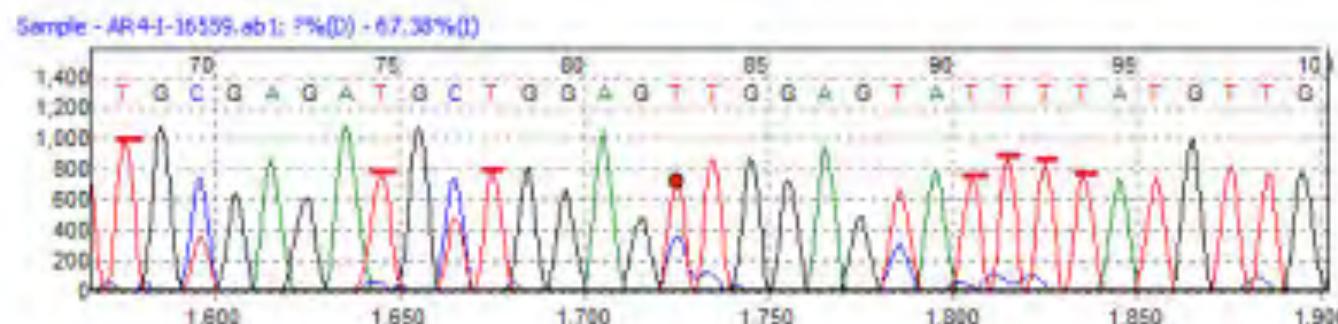
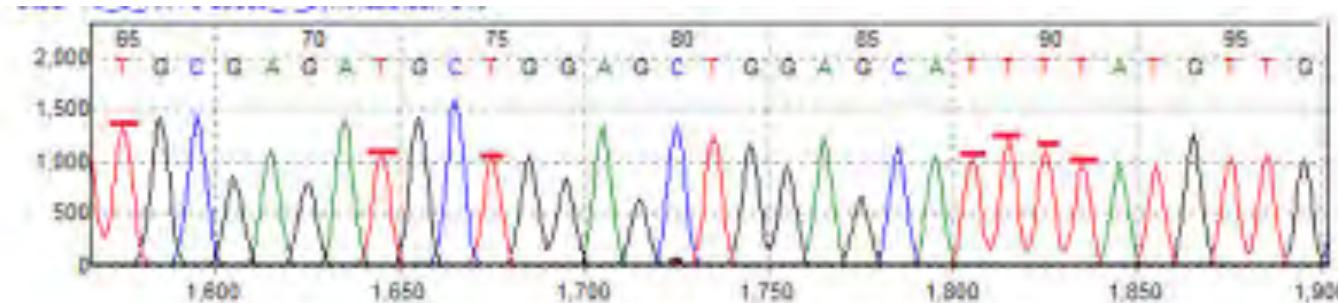
(Sue 1996)



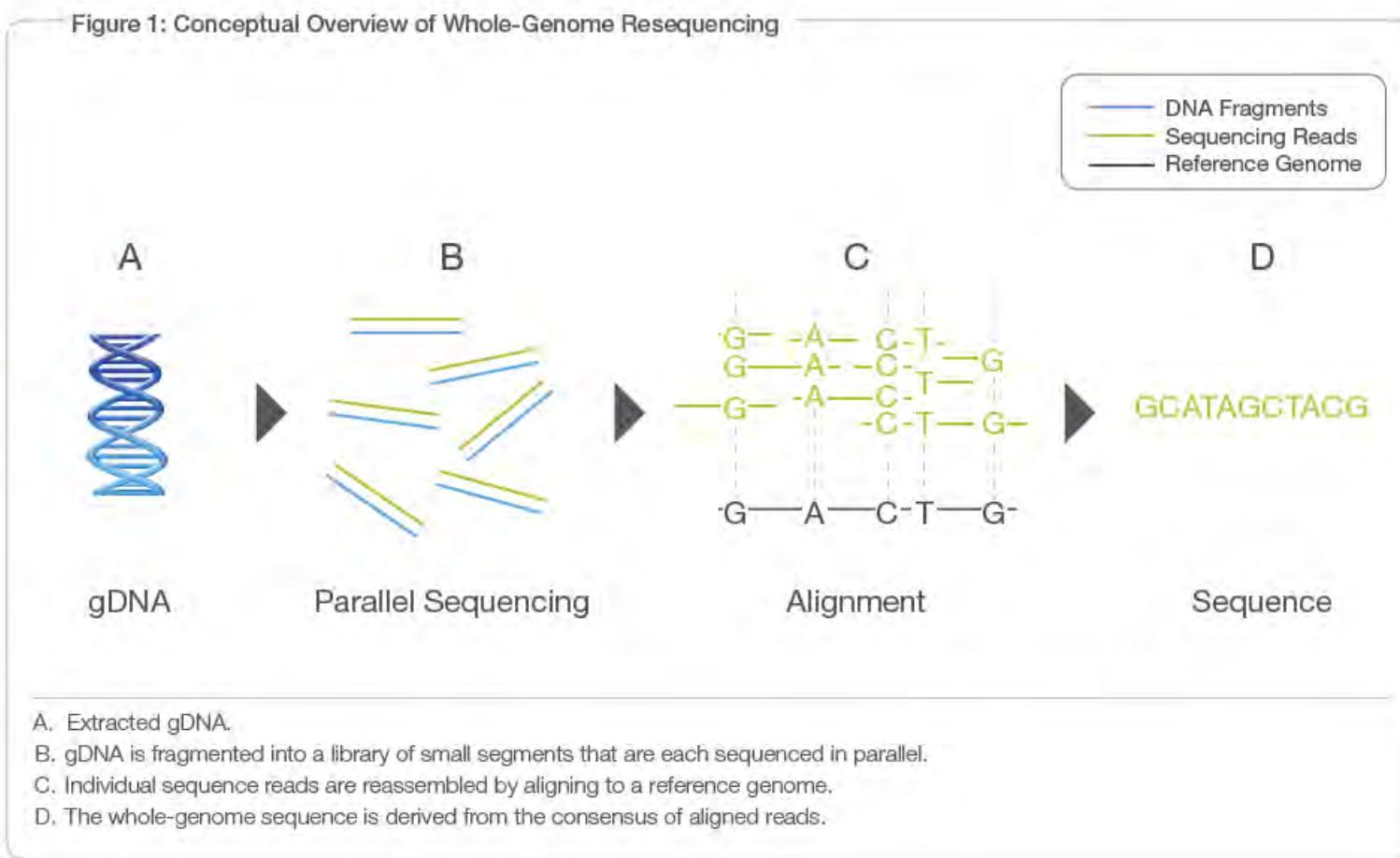
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1561 cgtaacatgg taagtgtact ggaaagtgcg cttggacgaa ccagagtgt aacttaacaca
1621 aagcacccaa cttacactt ggagatttc acttaacttg accgctctga gctaaaccta
1681 gccccaaacc cactccaccc tactaccaga caacccitac caaaccattt acccaaataa
1741 agtataggcg atagaaattt aaacccgtcg caatagatat agtaccgaa gggaaagatg
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1861 ttaacttagaa ataactttgc aaggagagcc aaagctaaga ccccccgaac cagacgagct
1921 acctaagaac agctaaaaga gcacacccgt ctatgttagca aaatagtggg aagattata
1981 ggttagaggcg acaaaccatc cgagccgtt gatactgtt tgccaagat agaatcttag
2041 ttcaacttta aatttgcctt cagaaccctt taaatccct tggtaattt actgttagt
2101 caaagaggaa cagcttttgc gacacttagga aaaaacccgt tagagagagt aaaaattt
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m.3243A>G

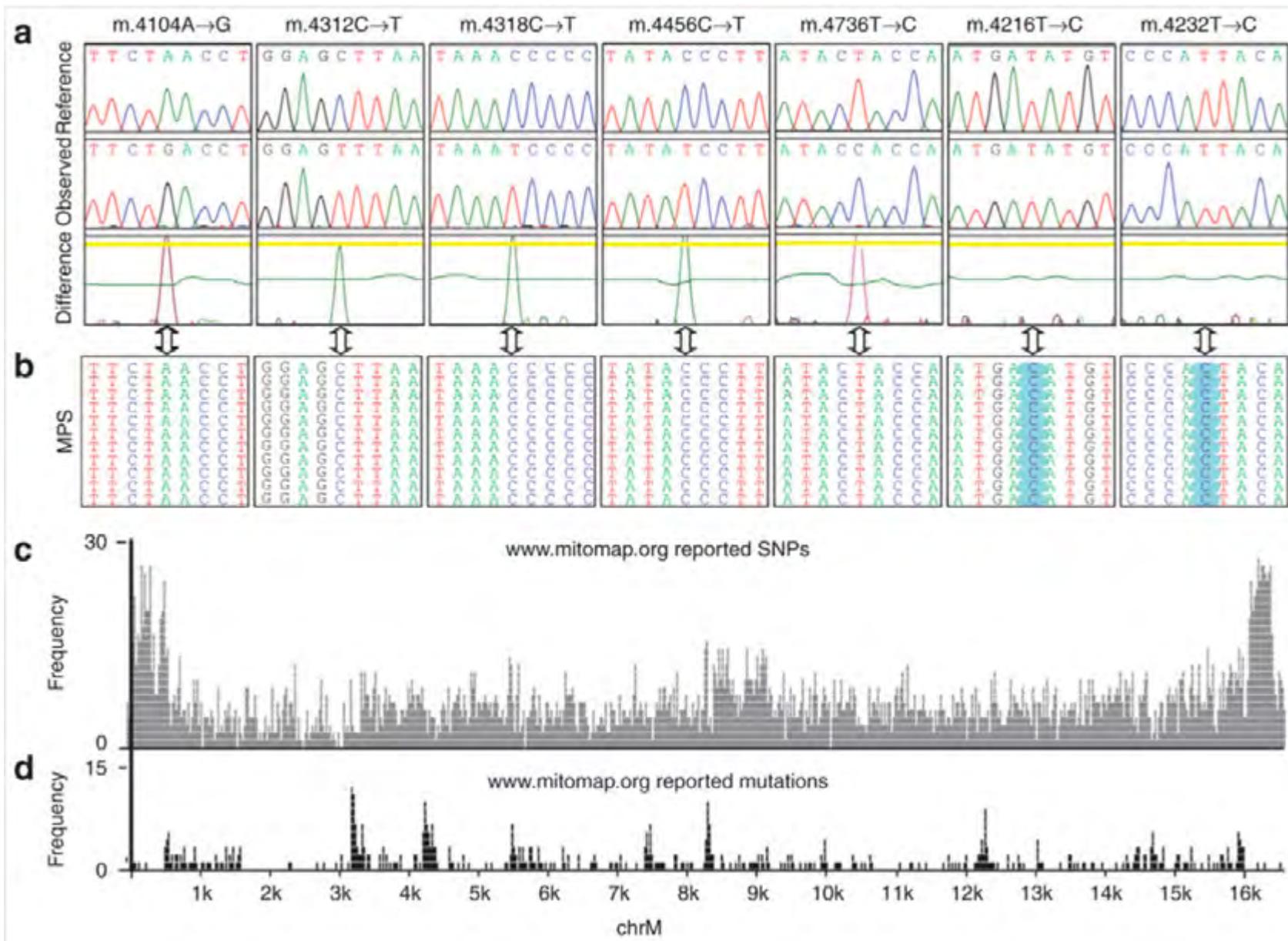
Sanger sequencing



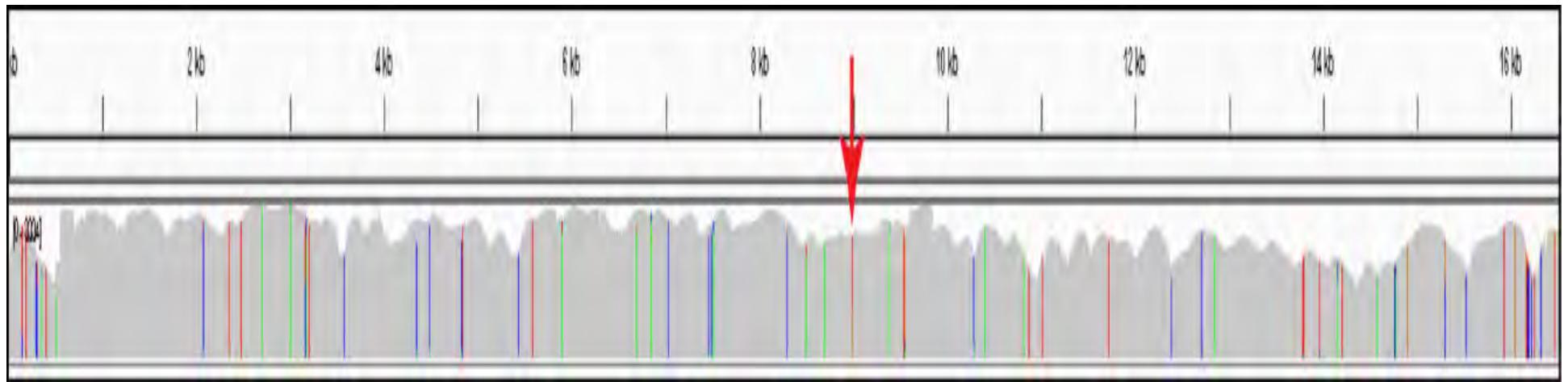
Next generation sequencing



Next generation sequencing



Next generation sequencing



Analysis of mt DNA

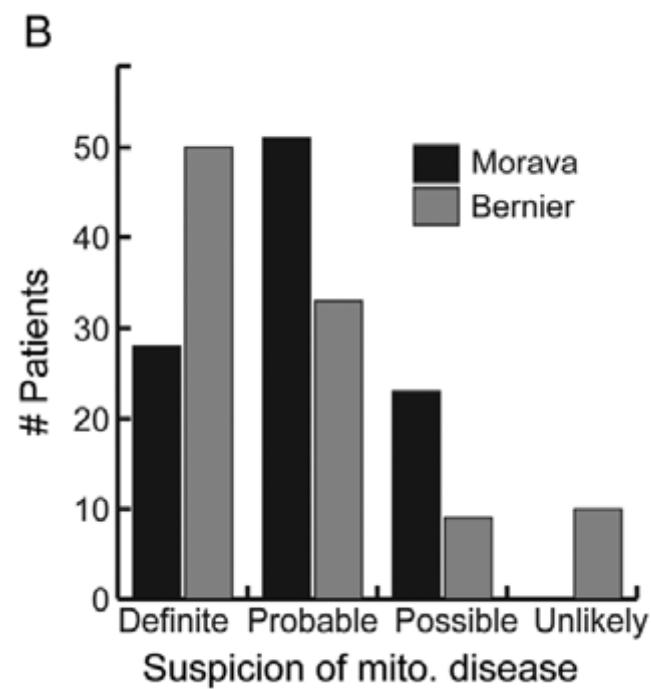
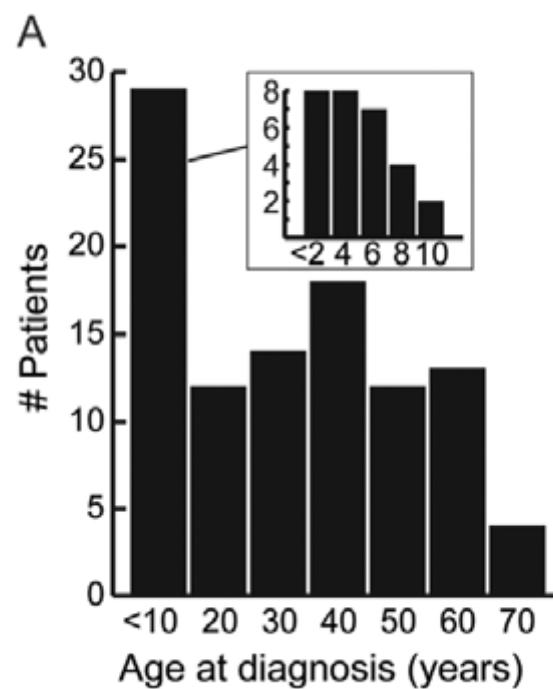
Next generation sequencing

- Target exome sequencing
- Whole exome sequencing
- Whole genome sequencing

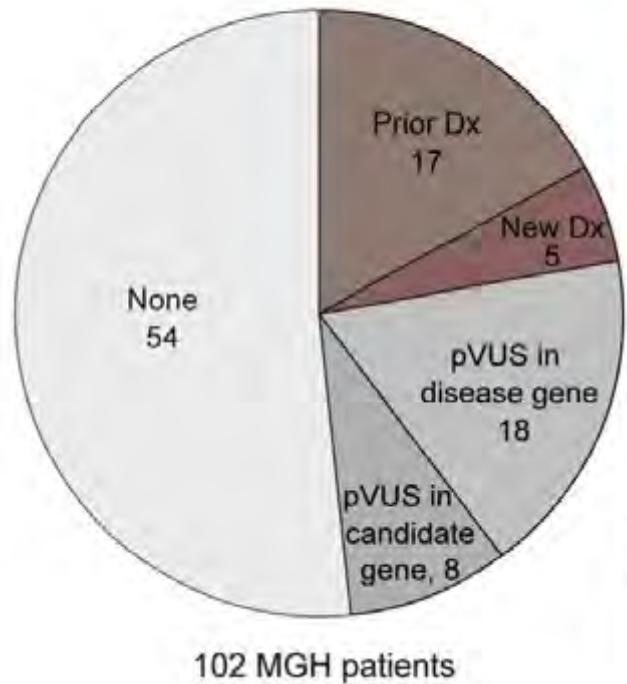
Next generation sequencing

- **Target exome sequencing**
 - Sequence a subset of genes known to cause disease

Targeted exome sequencing of suspected mitochondrial disorders



(Lieber et al., Neurology 2013)



17 patients with prior molecular Dx recovered by MitoExome sequencing

12 mtDNA MELAS(6)^a, MERRF(3)^b, NARP(2)^c, 12kb deletion^d
 4 recessive mito. disease HADHA^e, POLG(3)^{f,g}
 1 X-linked mito. disease PDHA1

5 patients with new molecular Dx in known disease gene

1 dominant mito. disease *POLG*
1 recessive mito. disease *NDUFV1*
3 recessive DDx genes *DPYD*^{0.1}, *KARS*¹, *WFS1*¹

18 patients with pVUS in known disease gene

7 mtDNA	COX1, COX2, ND1, ND4, RNR1(2), TRNL1, [COX2]
4 dominant mito. disease	POLG(3), POLG2
3 X-linked mito. disease	AIFM1, HCCS, NDUFA1 ^b
4 recessive DDX genes	DNA1 ^b , DPYD ^b , SECISBP2 ^b , WFS1 ^b , [ETFB, MAN2B1 ^b]

8 patients with pVUS in gene not previously linked to mito. disease

2 hemizygous X-linked	<i>APEX2</i> , <i>MAOA</i> , <i>[ACSL4, ASMTL]</i>
6 autosomal recessive	<i>ACACA</i> ¹ , <i>ACACB</i> ¹ , <i>AK8</i> ¹ , <i>ATP5A1</i> ^c , <i>ECH1</i> ¹ , <i>THG1L</i> ^e , <i>[ABCB1</i> ¹¹ , <i>ANGEL2</i> ¹ , <i>CERK</i> ^e , <i>HAO2</i> ^e , <i>NDUFAB1</i> ^e , <i>RNASEH1</i> ¹

(Lieber et al., Neurology 2013)

HUMAN GENETICS

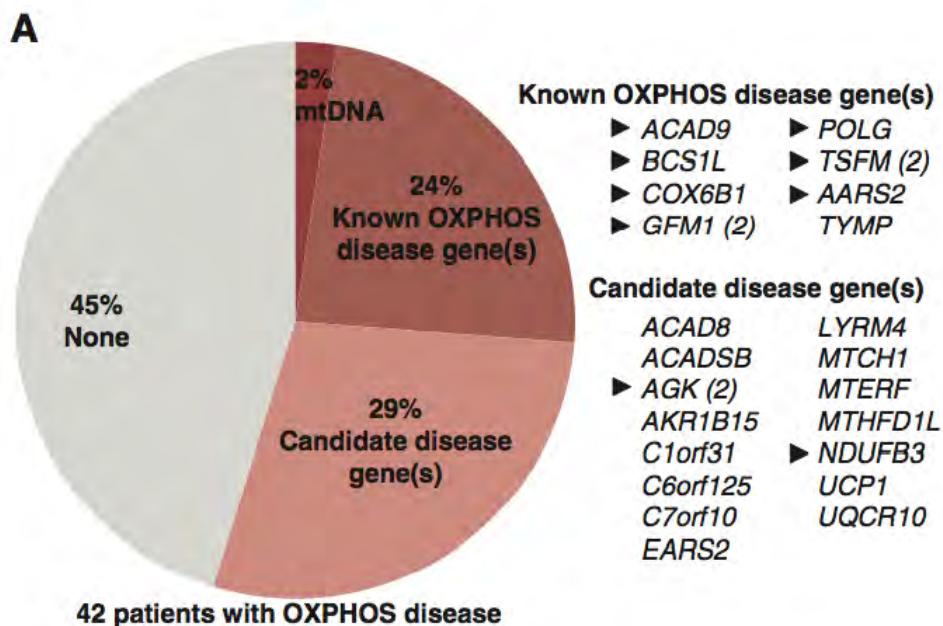
Molecular Diagnosis of Infantile Mitochondrial Disease with Targeted Next-Generation Sequencing

Sarah E. Calvo,^{1,2,3*} Alison G. Compton,^{4*} Steven G. Hershman,^{1,2,3} Sze Chern Lim,^{4,5}
Daniel S. Lieber,^{1,2,3} Elena J. Tucker,^{4,5} Adrienne Laskowski,⁴ Caterina Garone,^{6,7} Shangtao Liu,¹
David B. Jaffe,³ John Christodoulou,^{8,9} Janice M. Fletcher,^{10,11} Damien L. Bruno,^{4,12}
Jack Goldblatt,¹³ Salvatore DiMauro,⁶ David R. Thorburn,^{4,5,12†} Vamsi K. Mootha^{1,2,3†}

(Calvo et al., Neurology 2013)

Molecular Diagnosis of Infantile Mitochondrial Disease with Targeted Next-Generation Sequencing

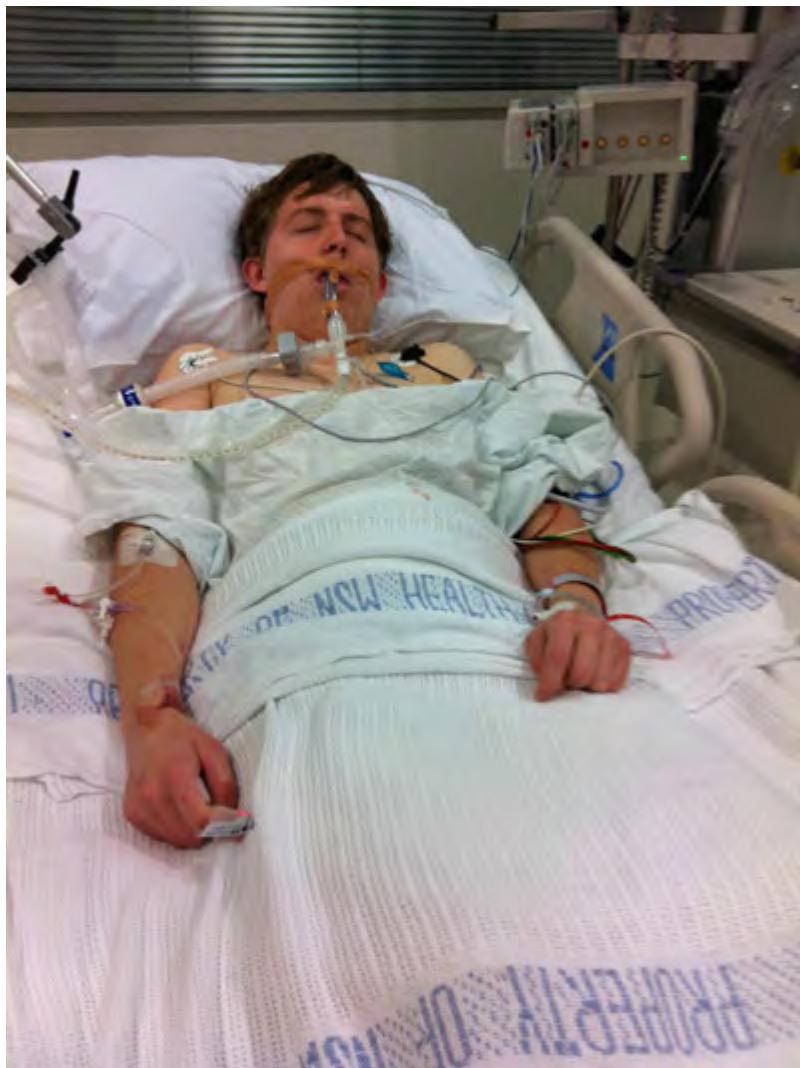
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(Calvo et al., Neurology 2013)



17 yo male presenting with diplopia and ptosis.



6 months later developed bilateral visual loss,
dysphagia and respiratory failure

Se:401
Im:204

[H]

[A]

[F]

HR 3D FLAIR Sag



Se:401
Im:213

[H]

[A]

[F]

HR 3D FLAIR Sag



Se:402
Im:5

[AF]

[R]

[PH]

AX 3D FLAIR

Se:402
Im:8

[AF]

[R]

[PH]

AX 3D FLAIR



Se:402
Im:16

[AF]

[R]



[PH]

AX 3D FLAIR

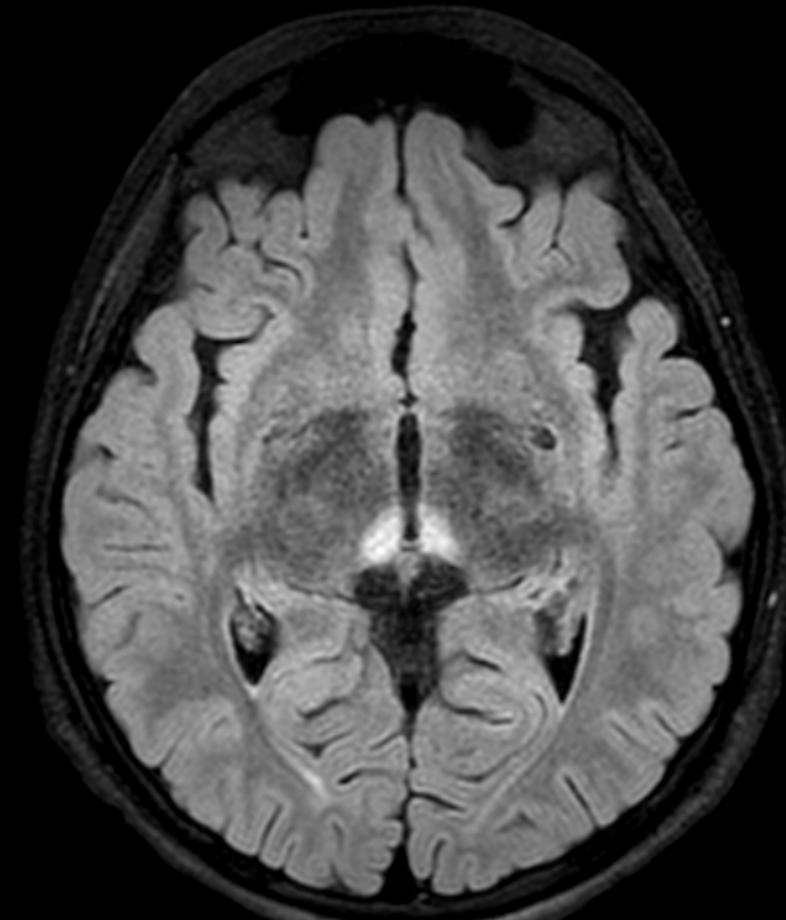
Se:402
Im:23

[AF]

[R]

[PH]

AX 3D FLAIR



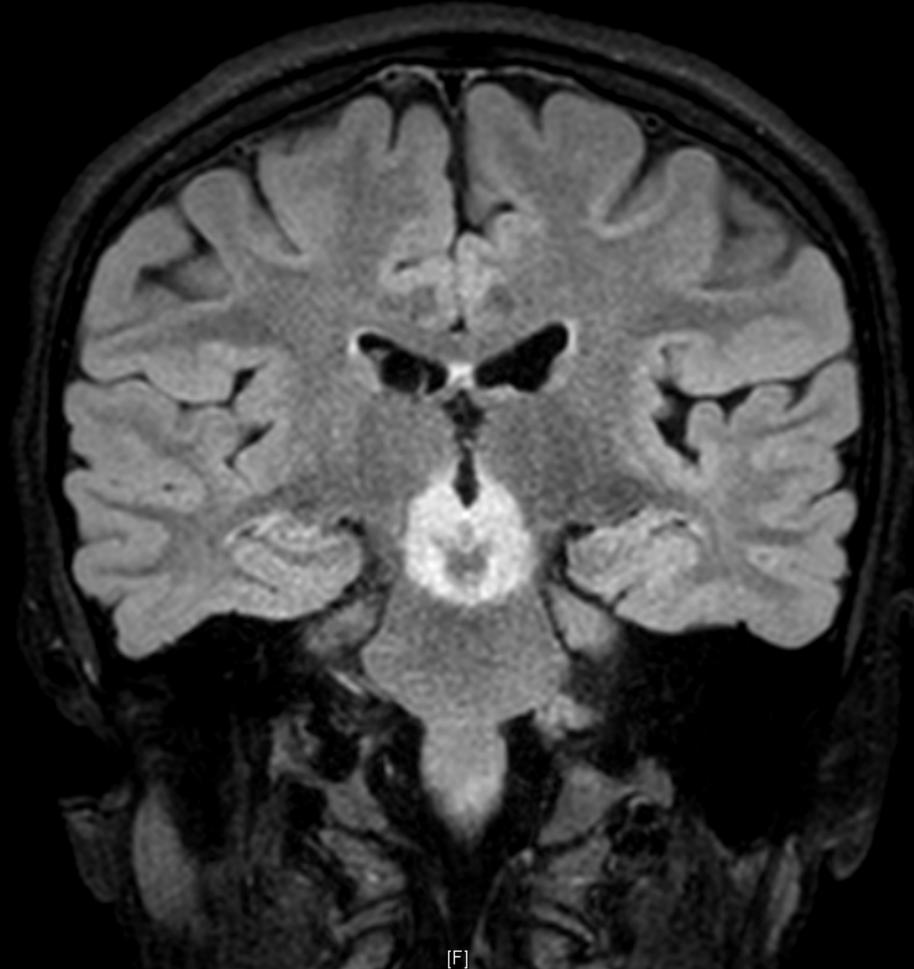
Se:403
Im:33

[H]

[R]

[F]

COR 3D FLAIR





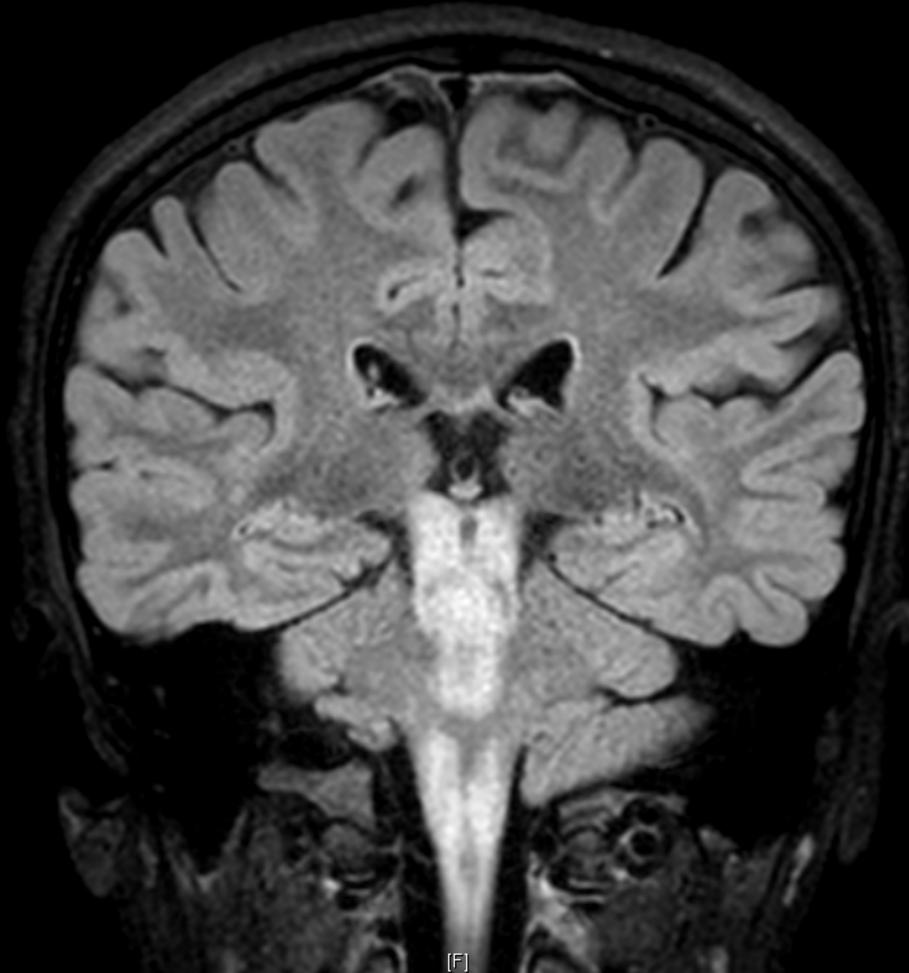
Se:403
Im:35

[H]

[F]

[R]

COR 3D FLAIR



Se:403
Im:36

[H]

[R]

[F]

COR 3D FLAIR



Se:902
Im:7

[HA]

[AF]

[FP]

eT2 Sag SENSE

Se:1201
Im:22

[AF]

[R]

[PH]

Ax T2 SPAIR

Leigh-like Encephalopathy Complicating Leber's Hereditary Optic Neuropathy

Benoît Funalot, MD,¹ Pascal Reynier, MD, PhD,²
Alain Vighetto, MD,³ Danièle Ranoux, MD,¹
Jean-Paul Bonnefont, MD, PhD,⁴
Catherine Godinot, PhD,⁵ Yves Malthierry, MD, PhD,²
and Jean-Louis Mas, MD¹

(Ann Neurol 2002; 52: 374-7)

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and Jean-Louis Mas, MD¹

m.11778G>A

(*Ann Neurol* 2002; 52: 374-7)

A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy

Thomas Klopstock,¹ Patrick Yu-Wai-Man,^{2,3,4} Konstantinos Dimitriadis,¹ Jacinthe Rouleau,⁵ Suzette Heck,¹ Maura Bailie,^{2,3,4} Alaa Atawan,^{2,3,4} Sandip Chattopadhyay,^{2,3,4} Marion Schubert,¹ Aylin Garip,⁶ Marcus Kernt,⁶ Diana Petraki,⁷ Christian Rummey,⁷ Mika Leinonen,⁸ Günther Metz,⁷ Philip G. Griffiths,^{2,3,4} Thomas Meier⁷ and Patrick F. Chinnery^{2,3,4}

(*Brain* 2011; 134: 2677-86)

LETTER TO THE EDITOR

Persistence of the treatment effect of idebenone in Leber's hereditary optic neuropathy

T. Klopstock,¹ G. Metz,² P. Yu-Wai-Man,^{3,4} B. Büchner,¹ C. Gallenmüller,¹ M. Bailie,^{3,4} N. Nwali,^{3,4} P. G. Griffiths,^{3,4} B. von Livonius,⁵ L. Reznicek,⁵ J. Rouleau,⁶ N. Coppard,² T. Meier² and P. F. Chinnery^{3,4}

(*Brain* 2013; 136: 1-5)

OPA-1

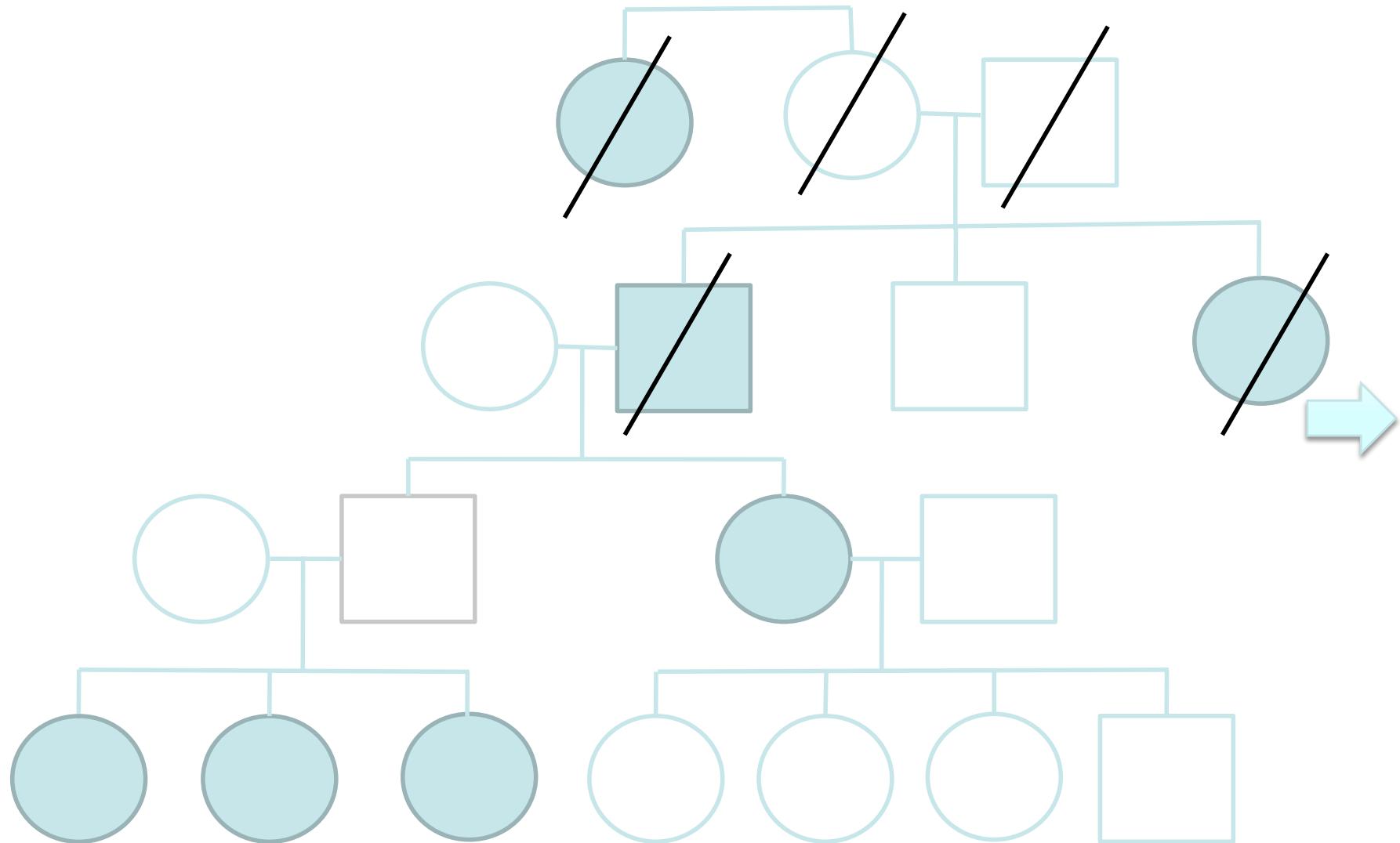
67 yo man with young onset visual and hearing loss. Developed ataxia and painful peripheral neuropathy



Fundoscopy



Family History



OPA-1

Nuclear gene *OPA1*, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy

Cécile Delettre^{1*}, Guy Lenaers^{2*}, Jean-Michel Griffoin¹, Nadine Gigarel³, Corinne Lorenzo², Pascale Belenguer², Laetitia Pelloquin², Josiane Grosgeorge⁴, Claude Turc-Carel⁴, Eric Perret⁵, Catherine Astarie-Dequeker⁶, Laetitia Lasquellec⁷, Bernard Arnaud⁷, Bernard Ducommun², Josseline Kaplan³ & Christian P. Hamel^{1,7}

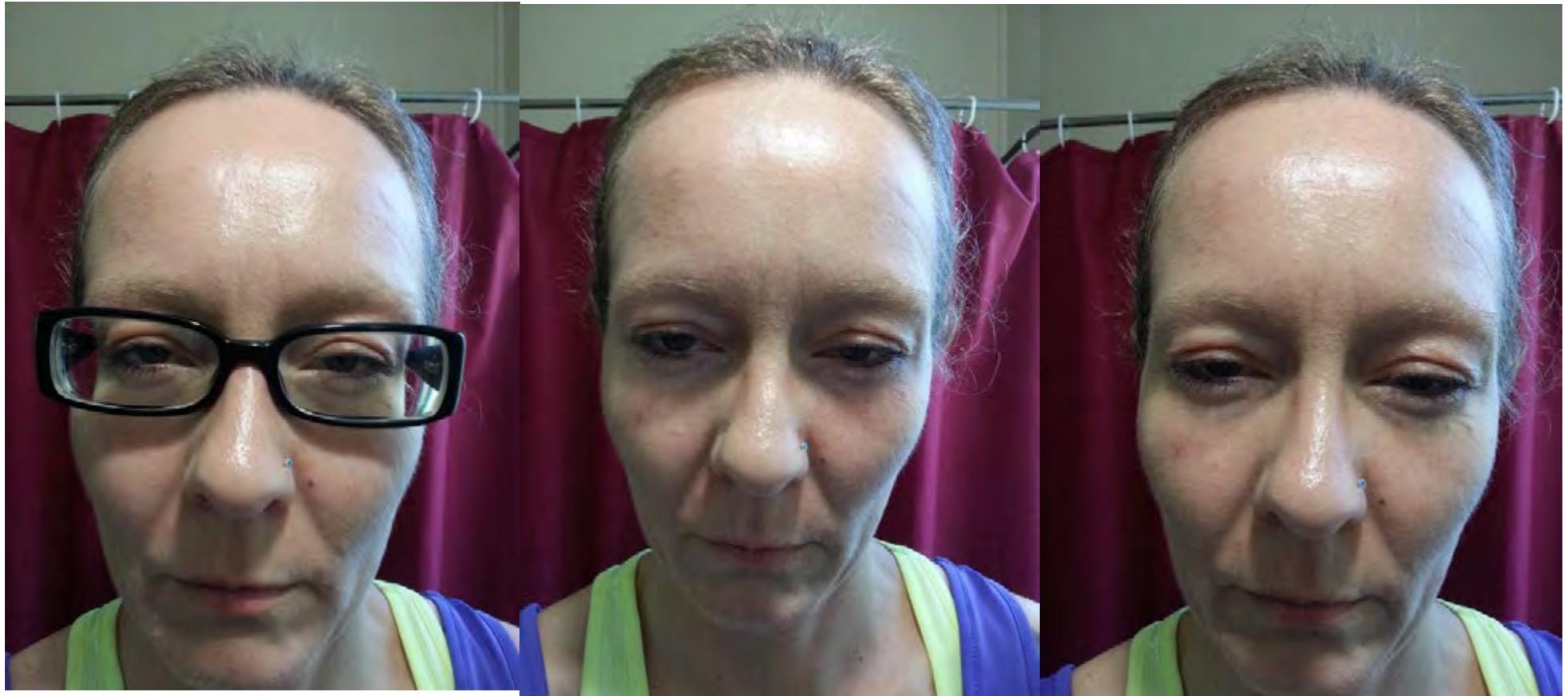
Multi-system neurological disease is common in patients with OPA1 mutations

P. Yu-Wai-Man,^{1,2} P. G. Griffiths,^{1,2} G. S. Gorman,¹ C. M. Lourenco,³ A. F. Wright,⁴ M. Auer-Grumbach,⁵ A. Toscano,⁶ O. Musumeci,⁶ M. L. Valentino,⁷ L. Caporali,⁷ C. Lamperti,⁸ C. M. Tallaksen,⁹ P. Duffey,¹⁰ J. Miller,¹¹ R. G. Whittaker,¹ M. R. Baker,^{11,12} M. J. Jackson,¹¹ M. P. Clarke,² B. Dhillon,¹³ B. Czermin,¹⁴ J. D. Stewart,¹ G. Hudson,¹ P. Reynier,^{15,16} D. Bonneau,^{15,16} W. Marques Jr,³ G. Lenaers,¹⁷ R. McFarland,¹ R. W. Taylor,¹ D. M. Turnbull,¹ M. Votruba,^{18,19} M. Zeviani,⁸ V. Carelli,⁷ L. A. Bindoff,^{20,21} R. Horvath,^{1,22} P. Amati-Bonneau^{15,16} and P. F. Chinnery^{1,23}

Table 3 Major clinical features observed in DOA+ patients

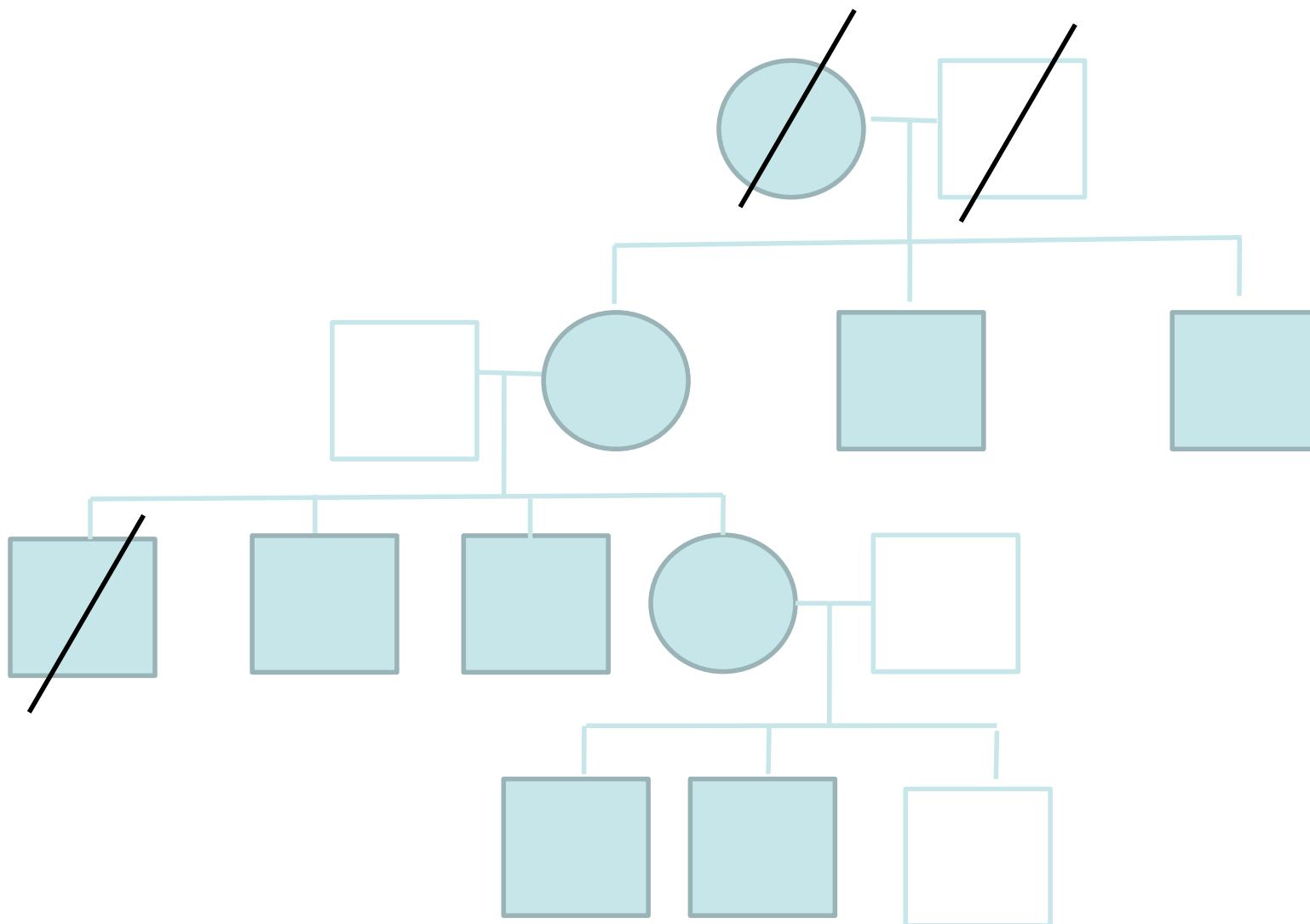
Clinical features ^a	n	%	95% CI	
			Lower	Upper
Optic atrophy	89/104	85.6	77.5	91.2
Deafness	65/104	62.5	52.9	71.2
Ataxia	31/104	29.8	21.8	39.2
Neuropathy	31/104	29.8	21.8	39.2
Myopathy	37/104	35.6	27.0	45.2
Progressive external ophthalmoplegia	48/104	46.2	36.9	55.7

AD PEO



**39 yo woman ptosis, neuropathy, cardiac arrhythmia
and family history of CPEO**

Family History



C10orf2

Twinkle mutations associated with autosomal dominant progressive external ophthalmoplegia lead to impaired helicase function and *in vivo* mtDNA replication stalling

Steffi Goffart¹, Helen M. Cooper¹, Henna Tyynismaa^{2,3}, Sjoerd Wanrooij^{1,†}, Anu Suomalainen^{2,3} and Johannes N. Spelbrink^{1,*}

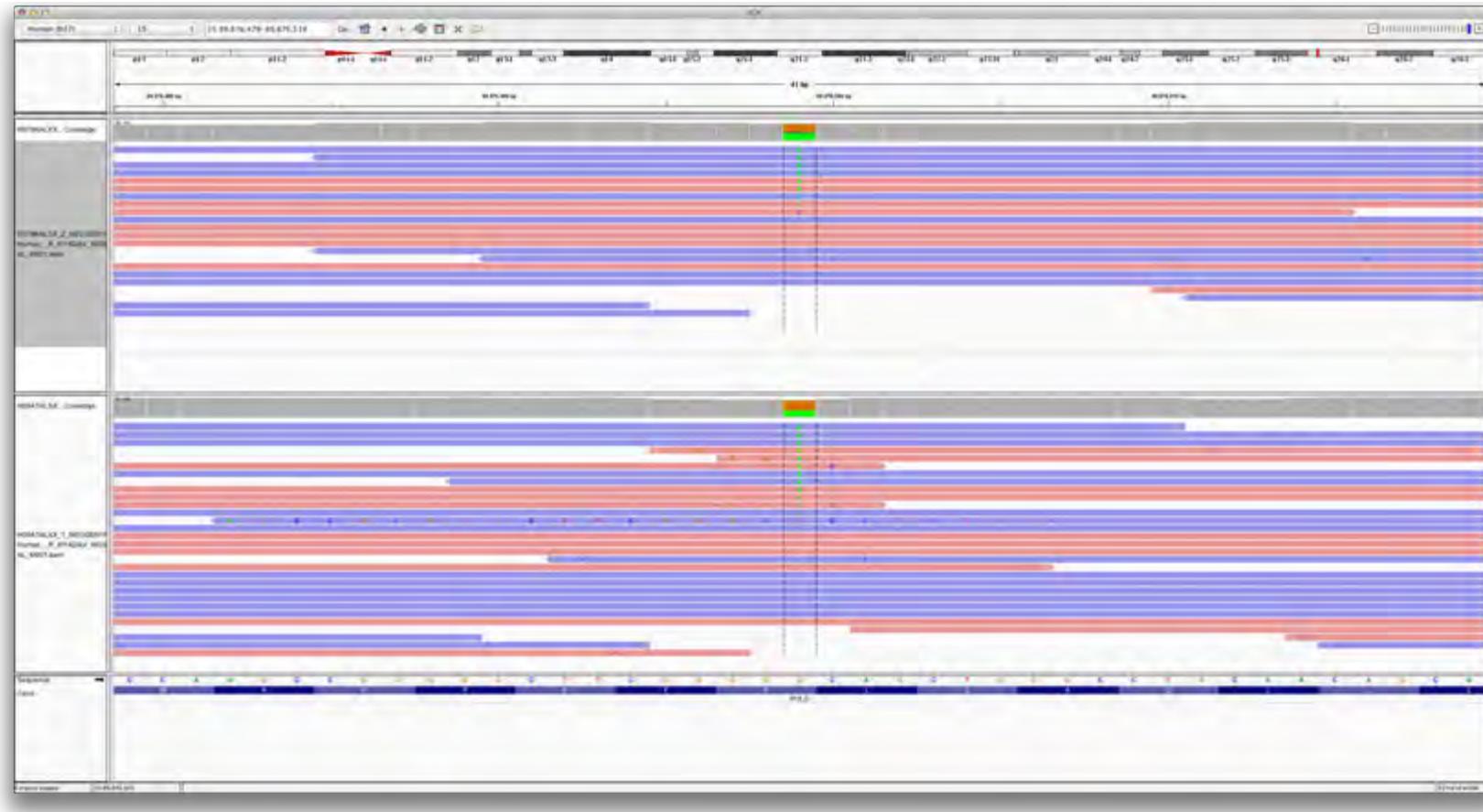
(Hum Mol Genet 2009; 18; 328-40)

Whole genome sequencing

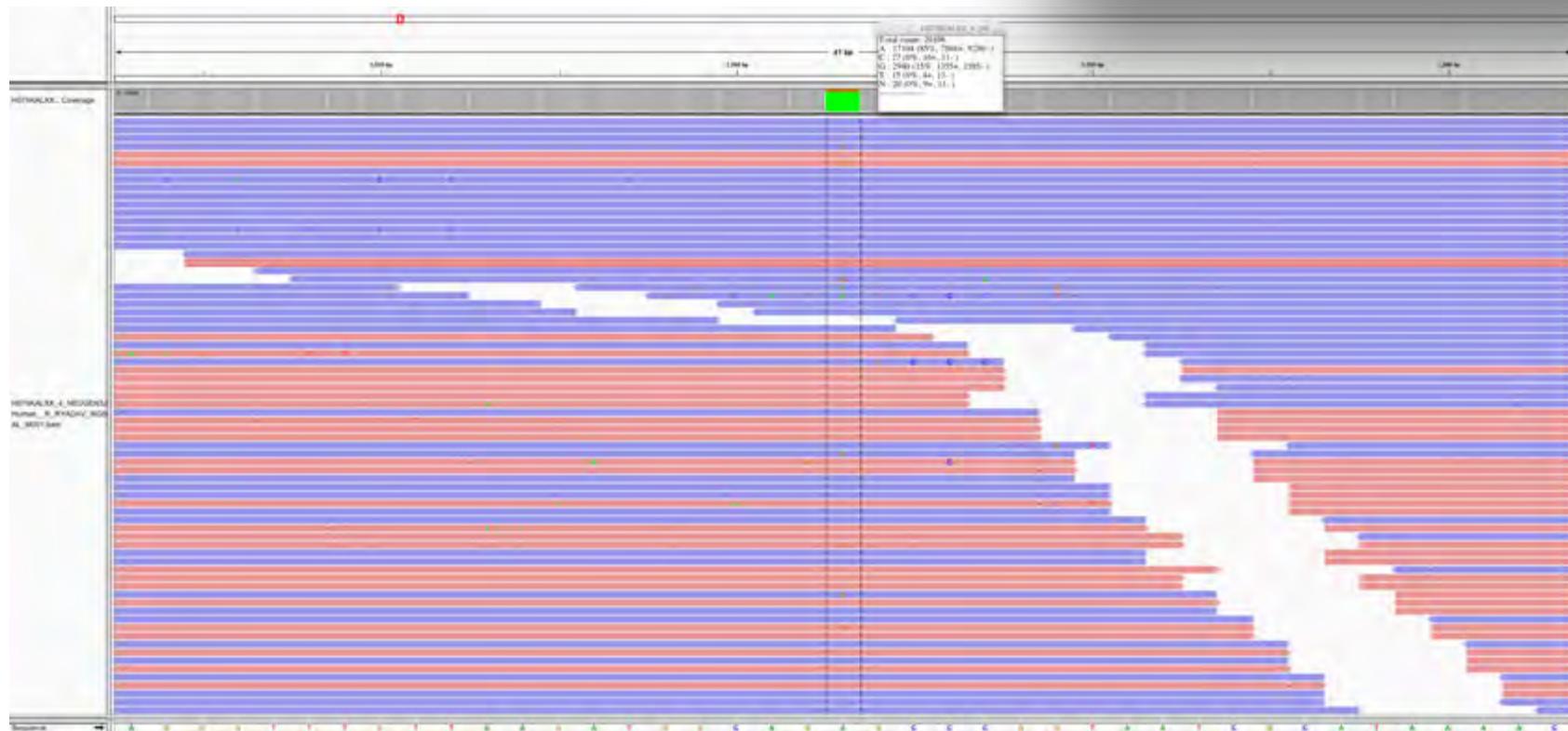
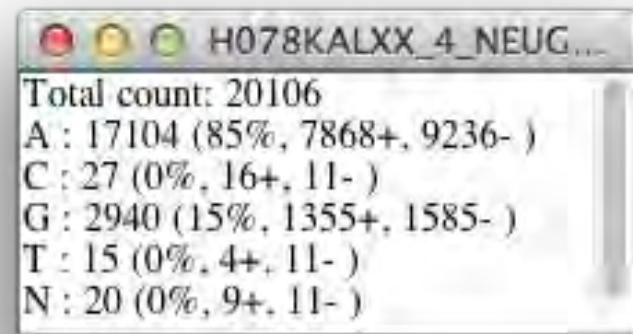


x30 coverage of nuclear genome (1.8 terabites of data in 3 days)

**g.1594C>T, p.P163S,
chr15:89876499G>A**



Mt.3243A>G



15% heteroplasmic load: 2940:17,104





MasterChef Au



Croquembouche



Recipe by: Adriano Zumbo

Ingredients

Choux pastry:
425g Water
520g Milk
20g Sugar
400g Butter
530g Flour
16 Eggs

Method

1. To make the pastry cream, place milk and vanilla bean in a saucepan. Heat gently until the milk almost boils. Remove from the heat, whisk the yolks, sugar and cornflour in a bowl until thick and pale. Gradually whisk in the warm milk. Return mixture to same saucepan and stir over medium heat until the custard boils. Spread over a tray to cool rapidly. Cover the surface of the custard closely with plastic wrap to prevent a skin forming; at 55°C transfer to a bowl and stir through butter and refrigerate to cool completely.
2. Preheat the oven to 210 degrees celsius convection. Lightly grease 4 oven trays and set aside. Combine the butter with water, sugar, milk & salt in a large heavy-based saucepan and bring to the boil. Remove from the heat and using a wooden spoon quickly beat in the flour. Return to the heat and continue beating until the mixture comes together and leaves the side of the pan. Cook, beating over low heat for 1-2 minutes to cook flour. Remove from heat and allow to cool slightly.
3. Transfer to a large bowl. Using a hand mixer, beat the mixture to release any more heat. Gradually add the eggs, one at a time. Beat well between each addition until all the eggs have been added and the mixture is thick and glossy. Beat for a few more minutes, or until thickened.
4. Spoon the mixture, in batches, into a piping bag fitted with a 1.25-1.5cm nozzle. Cover remaining pastry with cling film. Pipe mixture onto trays about 3cm x 2cm high leaving room for spreading. Bake for 25-30 minutes, in batches, or until firm and hollow when tapped. Transfer puffs to wire racks.
5. Put custard into a piping bag with a nozzle less than 1cm. Poke a small hole in the base of each puff and fill with custard.
6. For the caramel, combine water and sugar in a saucepan until it boils, add glucose, and cook until caramel in colour. Remove from the heat and dip the base of the pan in a bowl of water to cool slightly. Grease a cake ring and place ring mould on a baking paper lined tray, pour enough caramel to coat the base 5mm. This is the base for the croquembouche.
7. Dip the puff bases in enough toffee to coat and place upside down on a tray lined with baking paper.
8. To assemble, off the croquembouche cone. Dip the sides of the puff balls in the toffee one at a time and place around the base of the cone. Continue adding balls until the cone is covered.
9. Transfer the base for the croquembouche to a serving plate. Place a small amount of caramel on the base. Grasp croquembouche gently and lift from the cone and place on the caramel base.
10. Re-heat the remaining toffee then dip two forks back to back in it. Spin toffees around the Croquembouche. Decorate with violet.



Summary

- There is a growing number of mitochondrial diseases that are being defined on a molecular basis
- Precise genetic diagnosis enables informed genetic counseling and application of disease specific treatments
- NGS protocols offer a new level of diagnostic accuracy and simplify the diagnosis of mitochondrial disease
 - WGS may provide a “1 stop shop” for patient with mitochondrial disease in the near future.

Acknowledgements

- Clinic Patients
- Referring Doctors
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 - Aleks Filipovska
 - Daniel MacArthur
 - Marcel Dinger
- Funding Sources



- Roula Ghaoui
- Kate Ahmad
- Natasha Gerbis



STAY-IN BED DAY

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Fibroblast growth factor 21 is a sensitive biomarker of mitochondrial disease



(Davis et al., Neurology 2013)

- 54 mitochondrial disease patients with mitochondrial myopathy indicated by muscle biopsy or a known pathogenic mutation
- 20 disease controls with non-mitochondrial neuromuscular disorders (muscular dystrophy, IBM, metabolic myopathy etc)
- 66 control subjects
- Measured CK, Lactate, Pyruvate, BMI, muscle weakness

Results

- Classical markers used to assess mitochondrial disease show limited sensitivity to predict disease
- Sensitivity for FGF-21 to predict disease is almost twice that of the best classical indicator

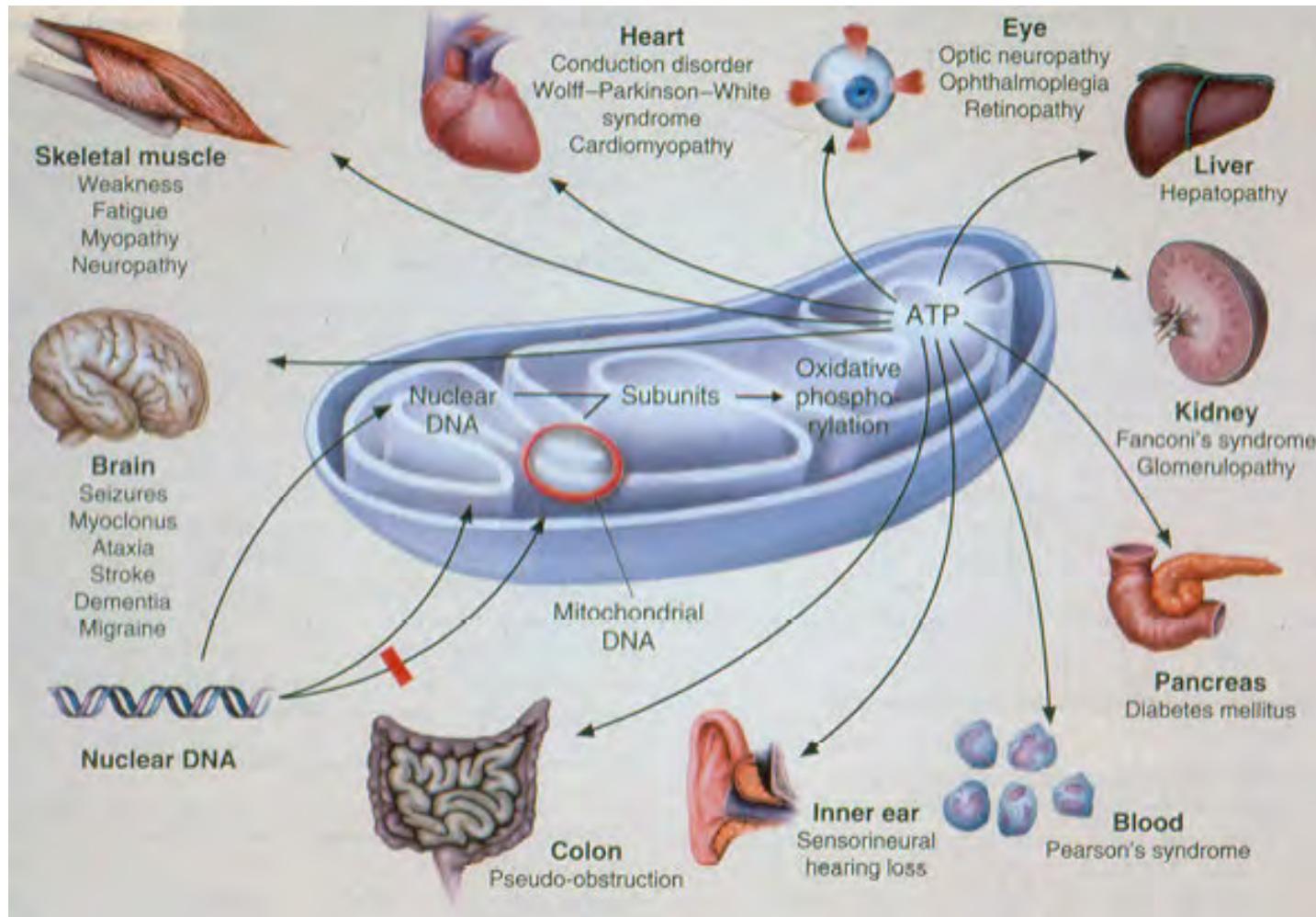
	Disease Control Sensitivity	Mitochondrial Disease Sensitivity
Creatine Kinase	35%	22.2%
Lactate	5%	15.1%
Pyruvate	40%	34.6%
Lactate to Pyruvate ratio	5%	11.5%
FGF-21	35%	68.5%



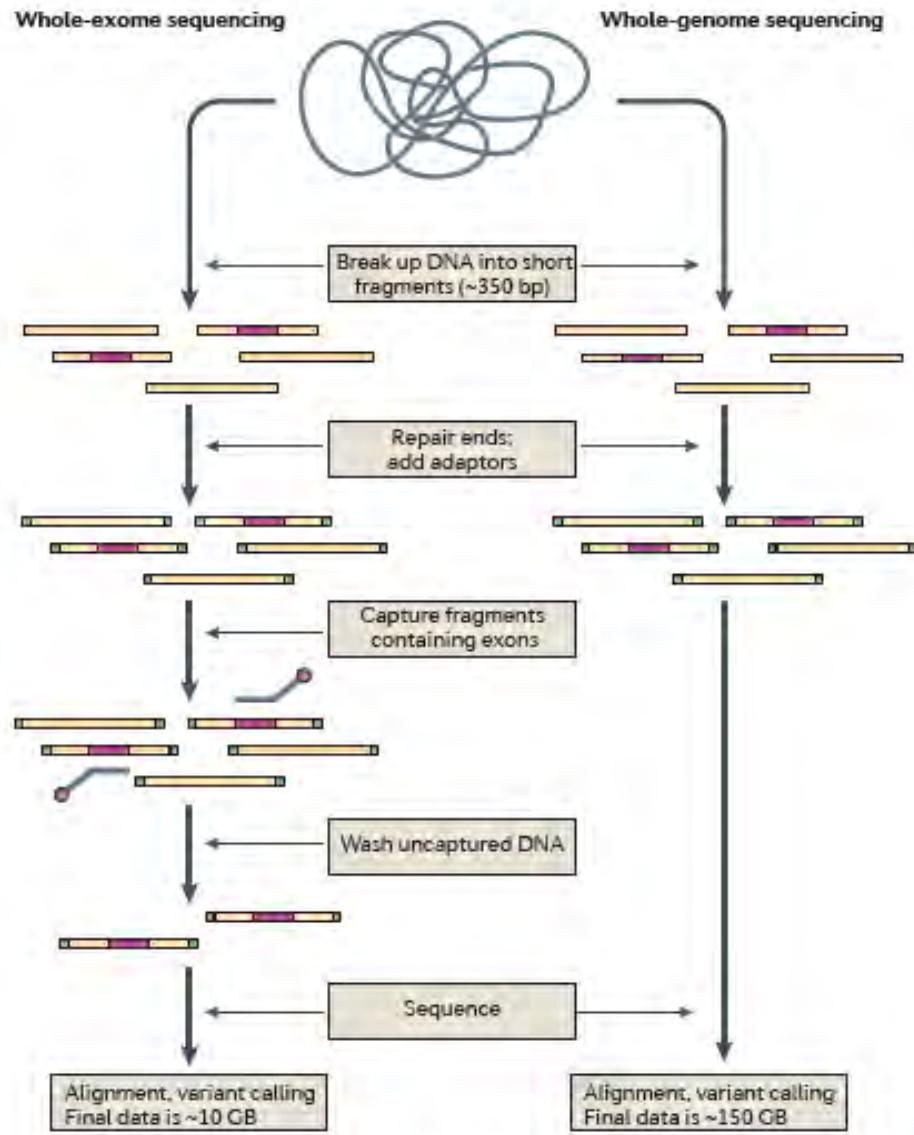
GLOBAL
MITOCHONDRIAL DISEASE
AWARENESS WEEK

15 - 21 September 2013

Mitochondrial Disease



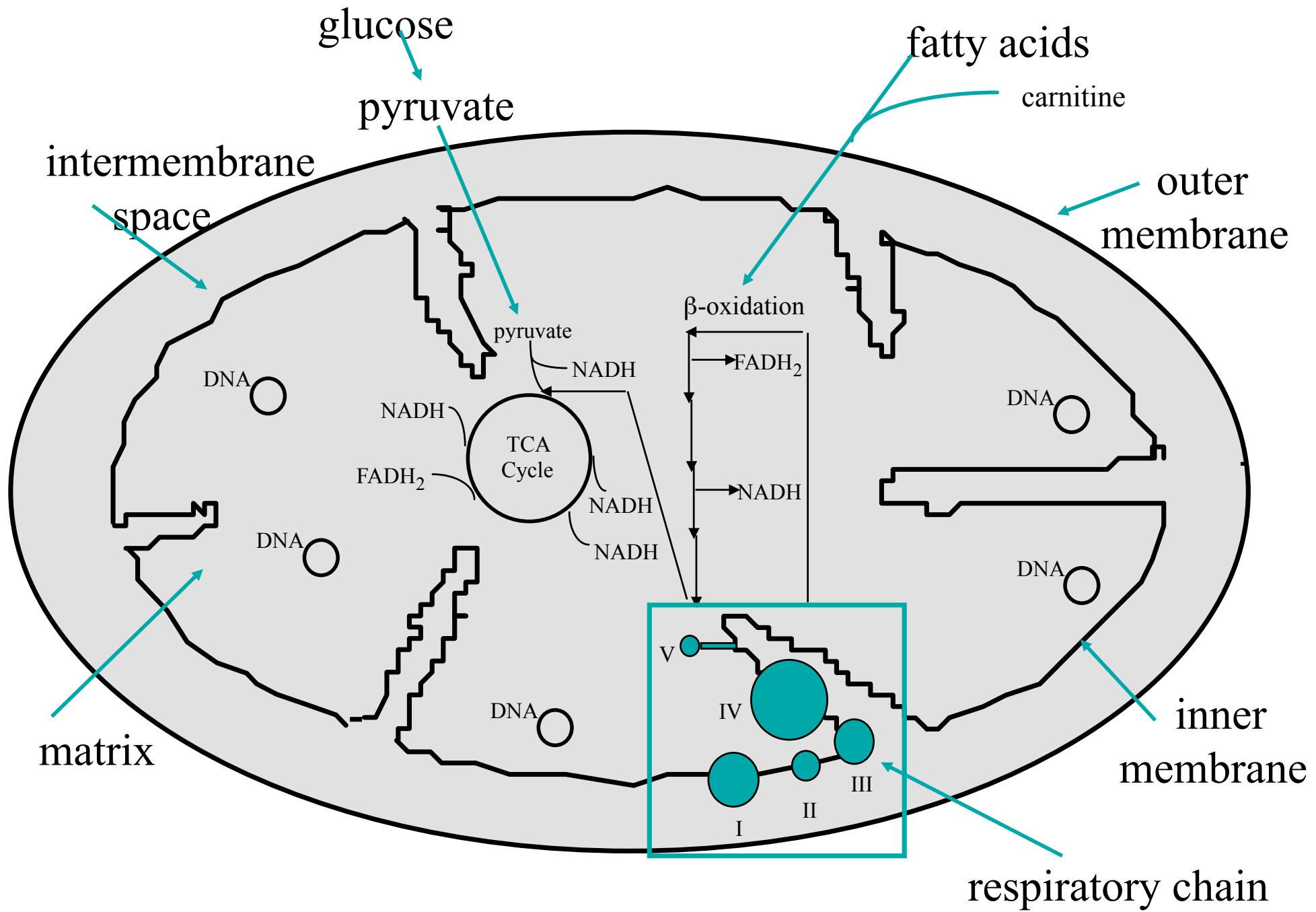
Massively parallel sequencing (NGS)



Human genome project revealed that there are 35 000 genes



**68 yo female with hearing loss, asymmetrical ptosis,
diabetes and proximal weakness**

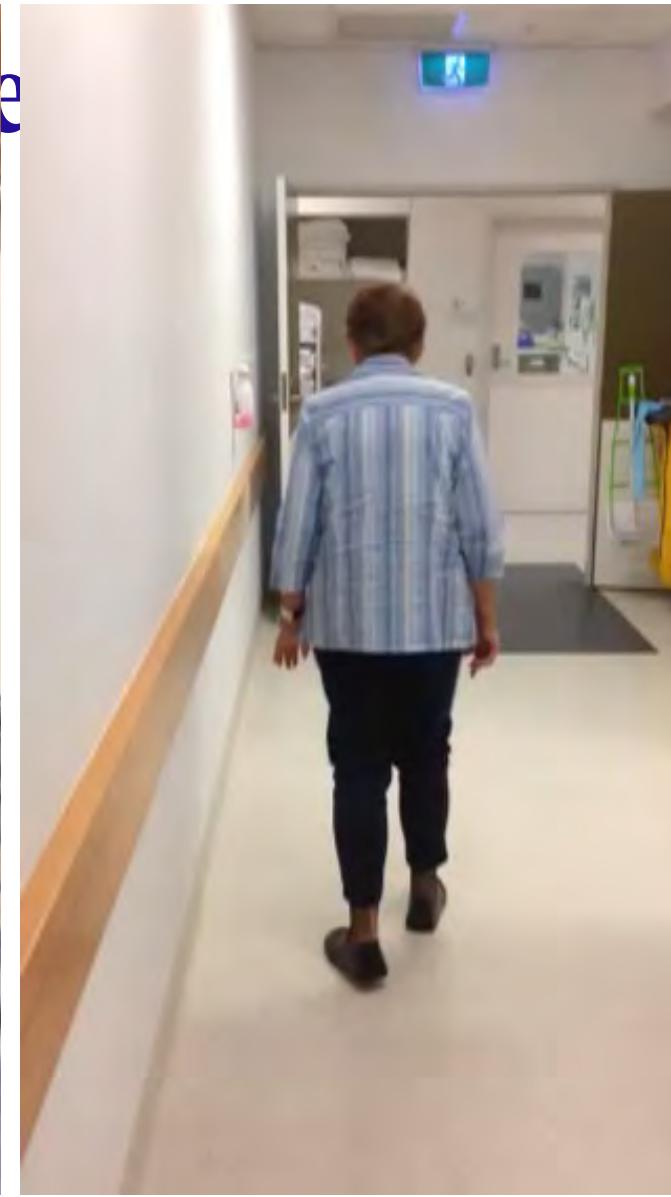
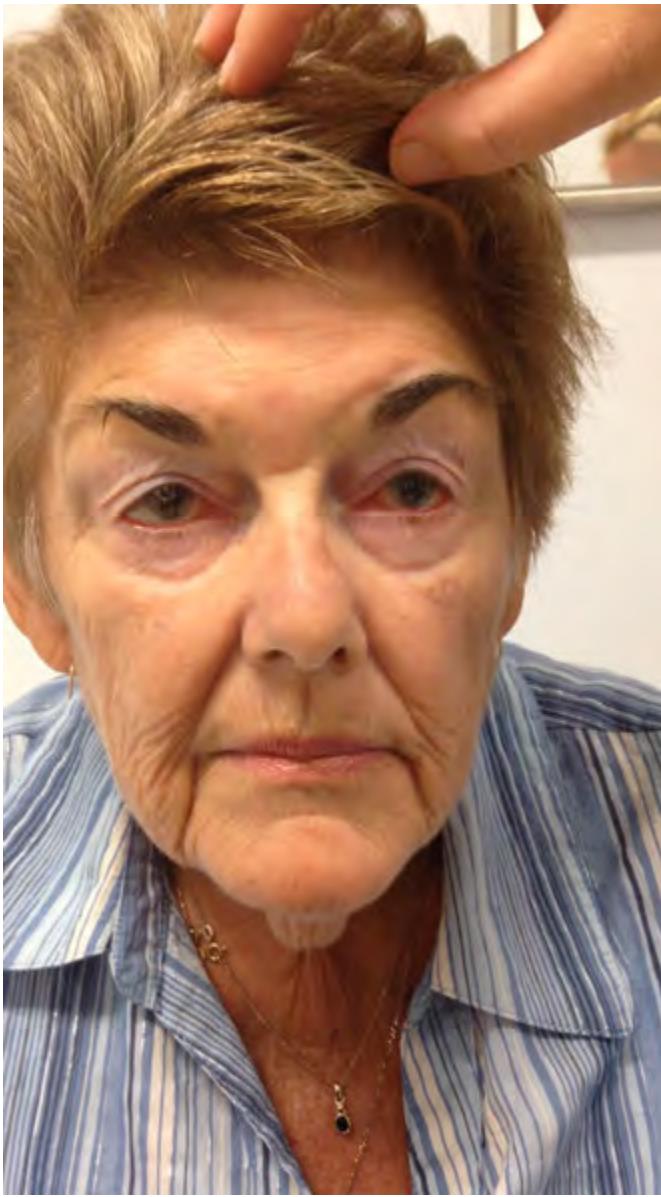




**30 yo male with mild ptosis and proximal weakness
and residual focal seizures**

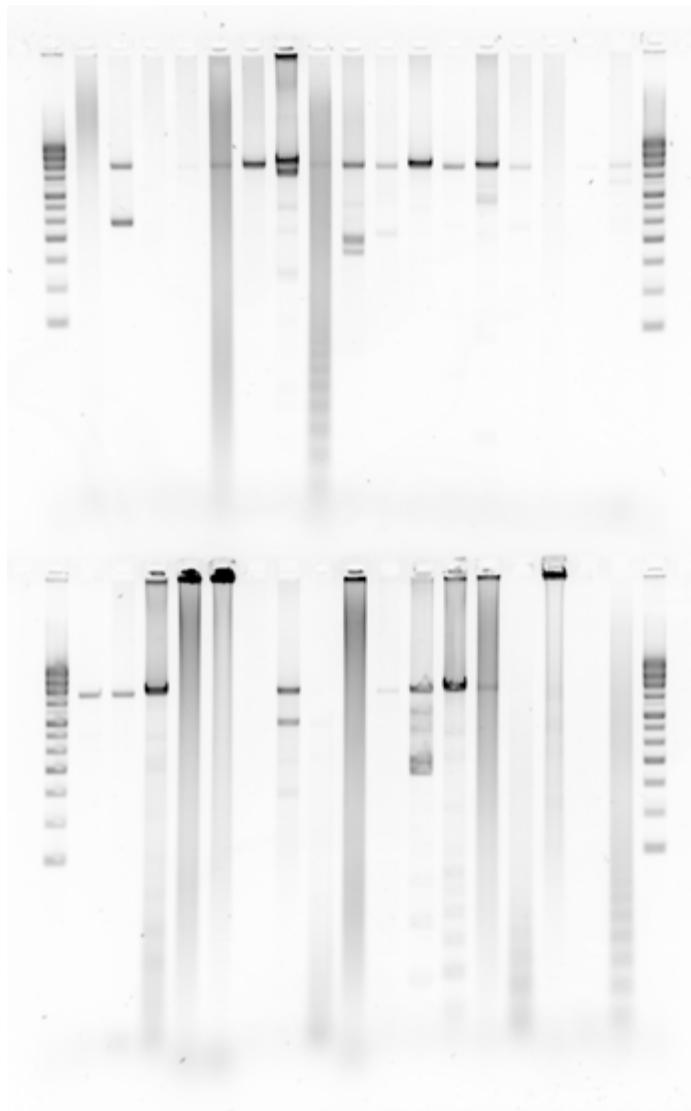


**46 yo woman with proximal weakness, numbness in
the feet and abdominal pain**



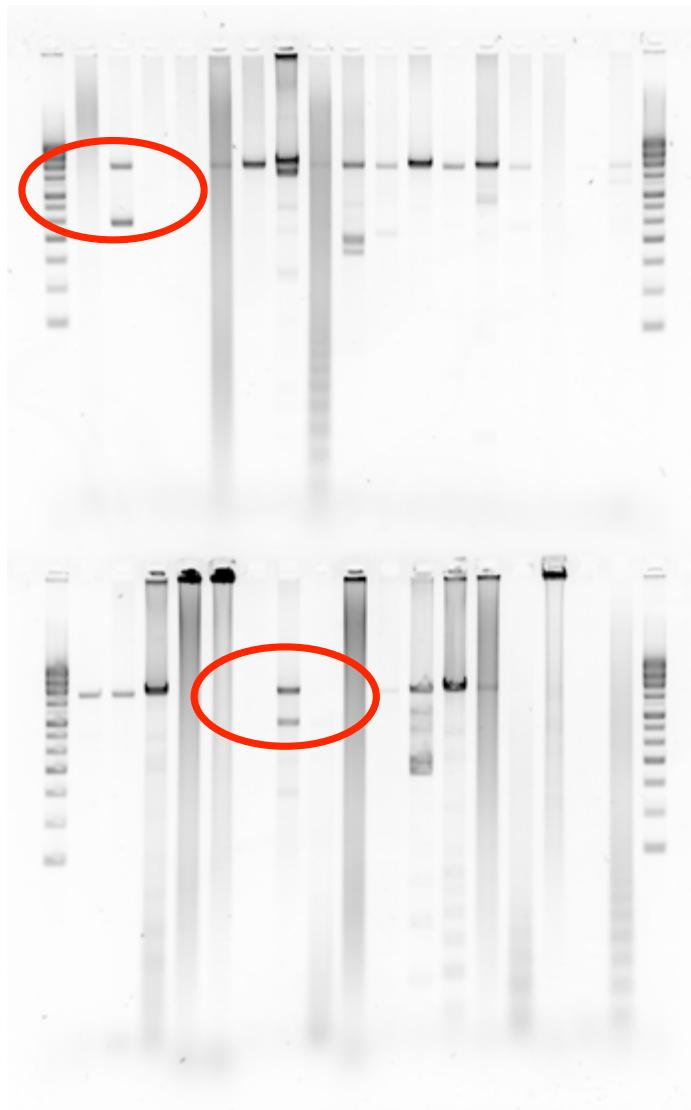
**69 yo woman with visual and hearing loss in 40s.
Developed ataxia**

Long range PCR



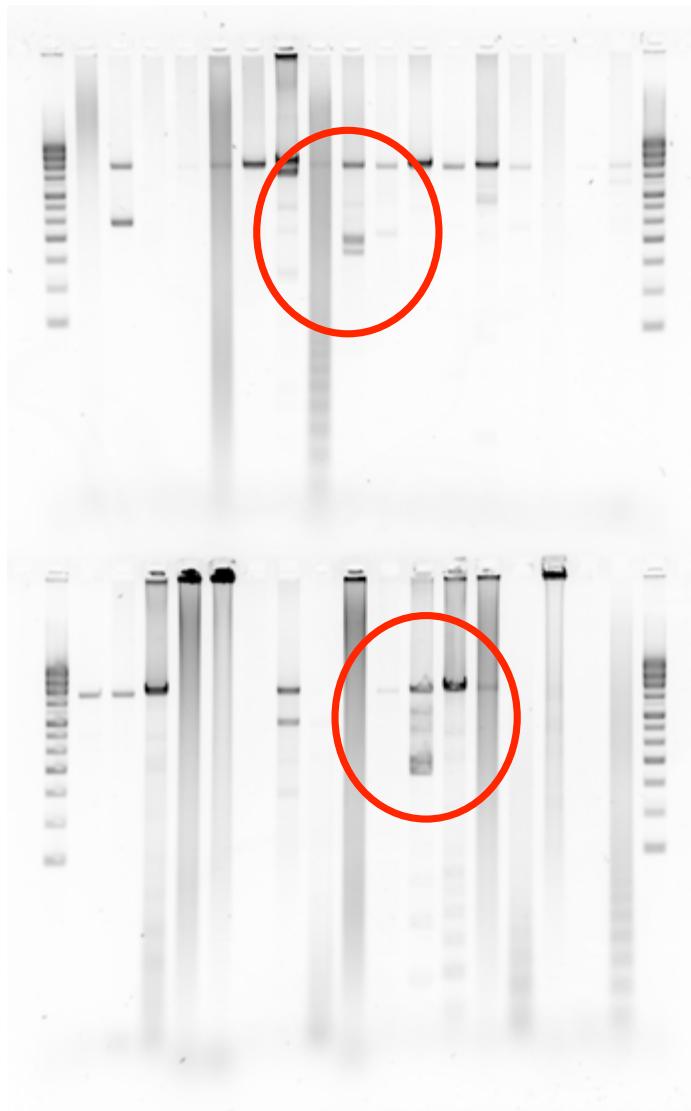
- Analysis of mtDNA
 - Urinary epithelial cells
- Generation of entire genome
 - (16.5kB)
- Detects large scale deletions and multiple deletions
- Can be sequenced by NGS

Long range PCR



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Finding *Twinkle* in the Eyes of a 71-Year-Old Lady: A Case Report and Review of the Genotypic and Phenotypic Spectrum of *TWINKLE*-Related Dominant Disease

Johan L.K. Van Hove,^{1*} Vicki Cunningham,¹ Cathlin Rice,¹ Steven P. Ringel,² Qing Zhang,³
Ping-Chieh Chou,³ Cavatina K. Truong,³ and Lee-Jun C. Wong³

(Calvo et al., Neurology 2013)

Mitochondrial disease



“The great mimicker”

Mitochondrial disease



“The great mimicker”