Omics approaches reveal clues for mitochondrial disease diagnosis and pathogenesis

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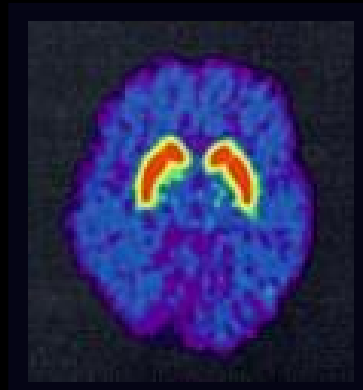
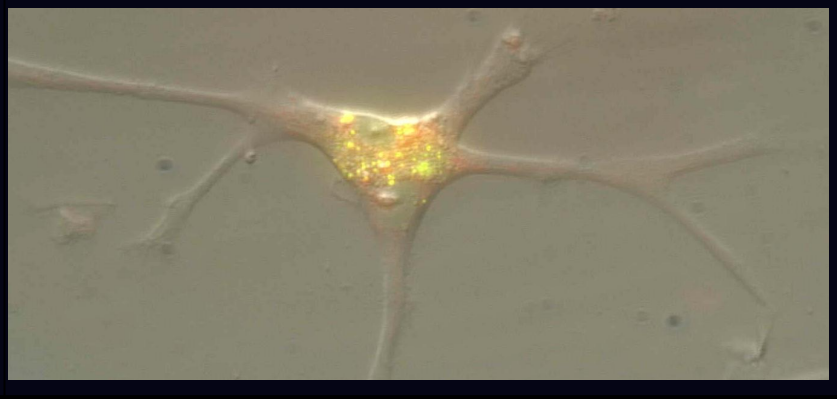
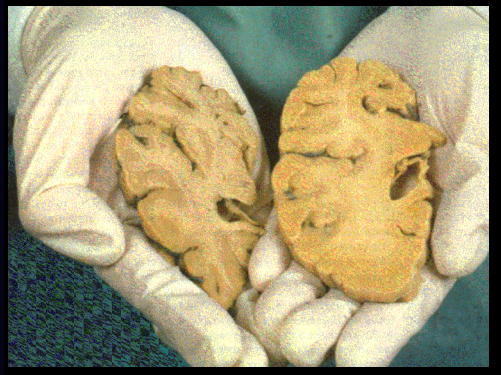
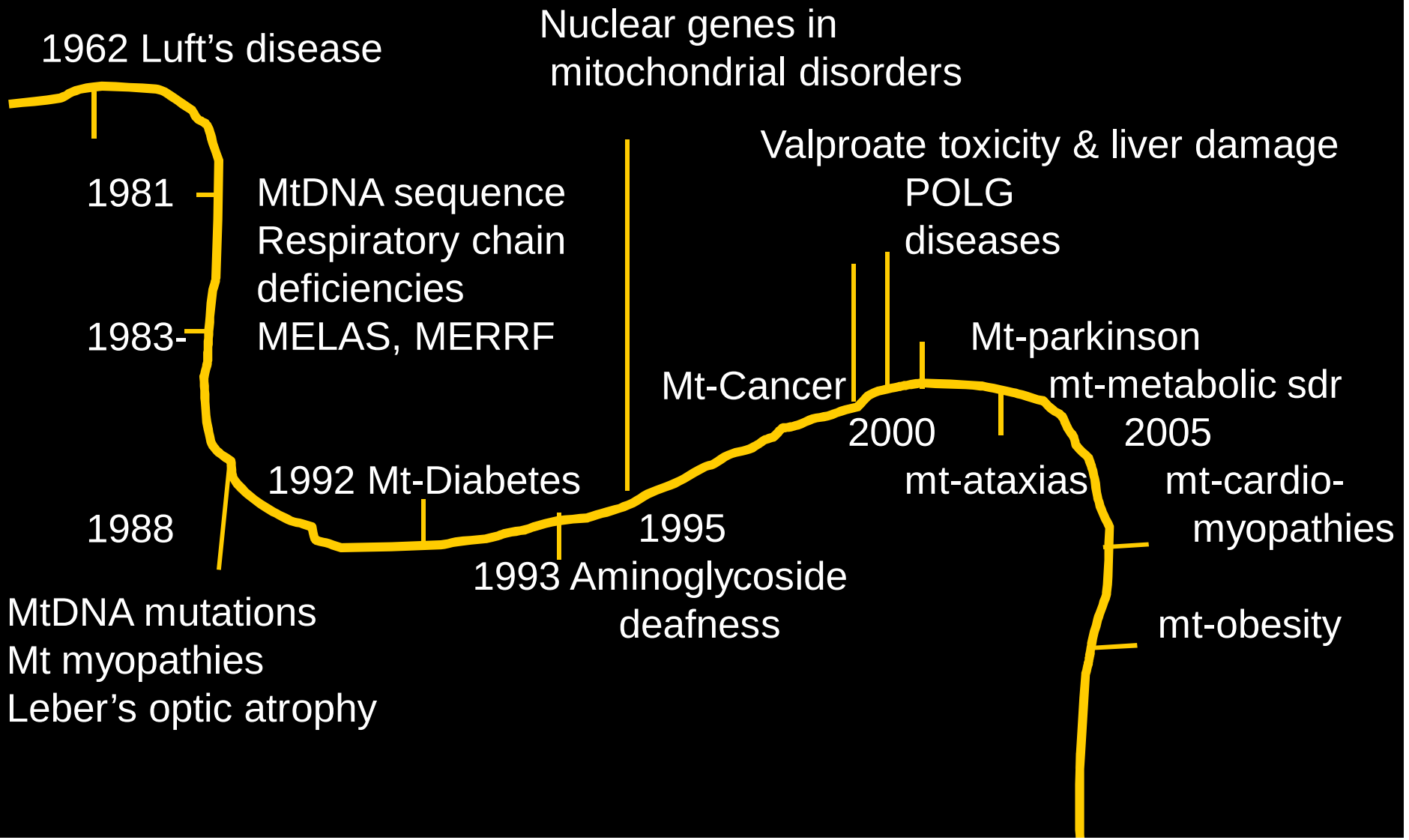
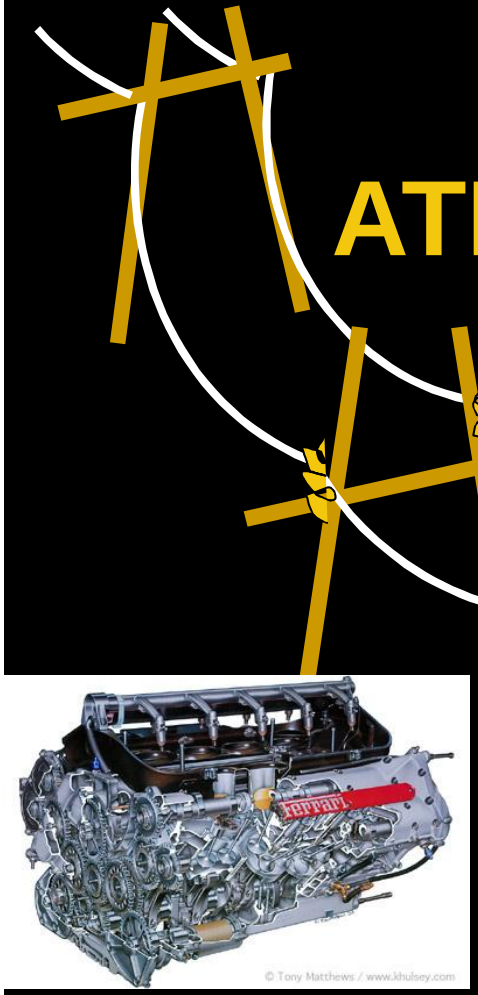




50 years of mitochondrial medicine



Mitochondrial functions



Glucose
Fatty acids
Amino acids

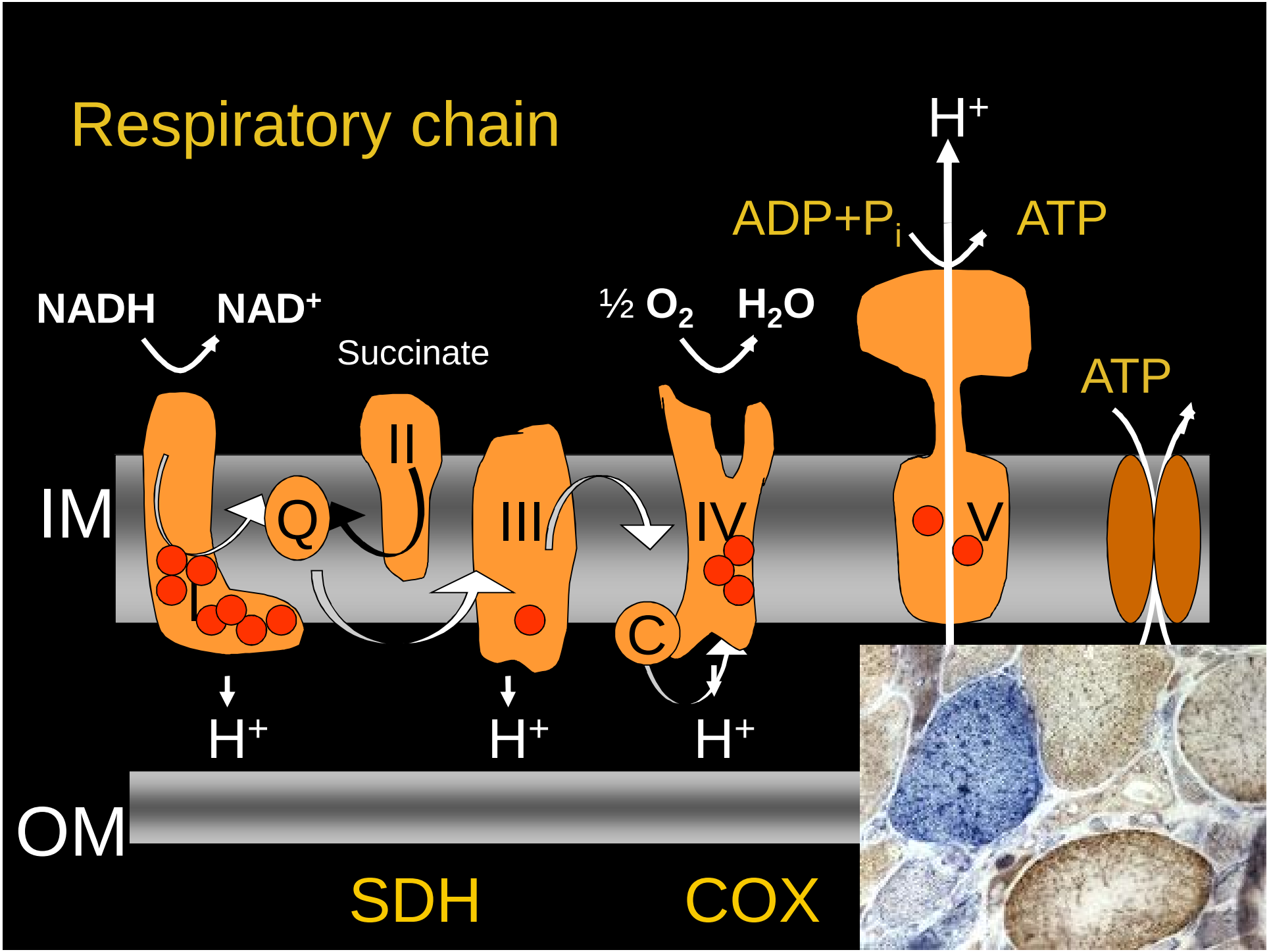
Calcium

Heme, FeS

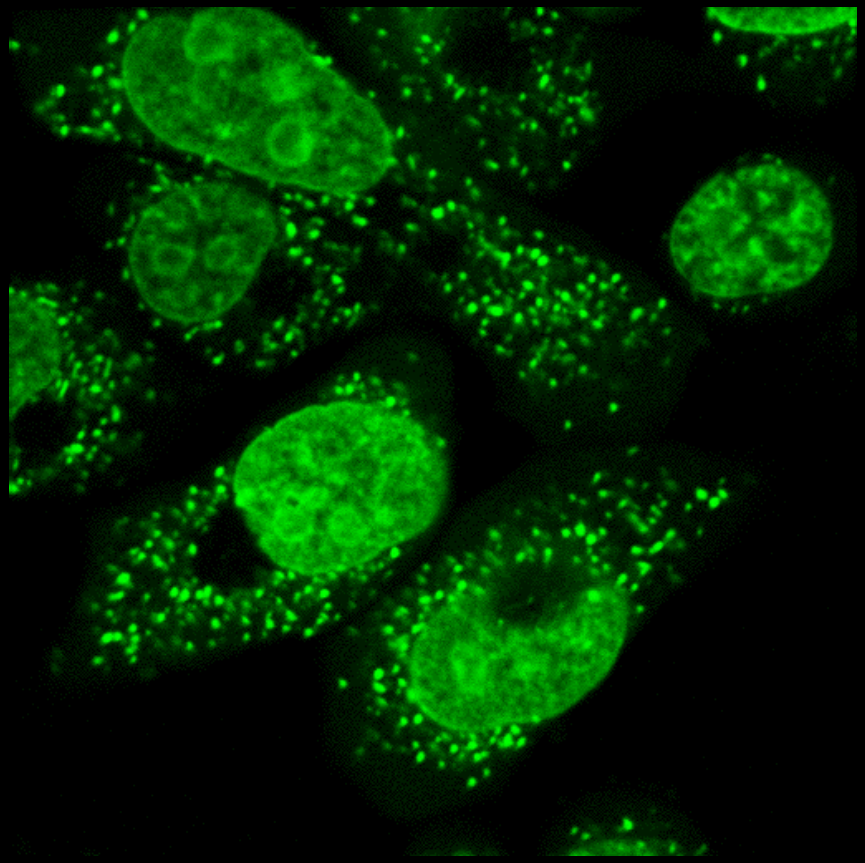
Apoptosis

Steroid synthesis

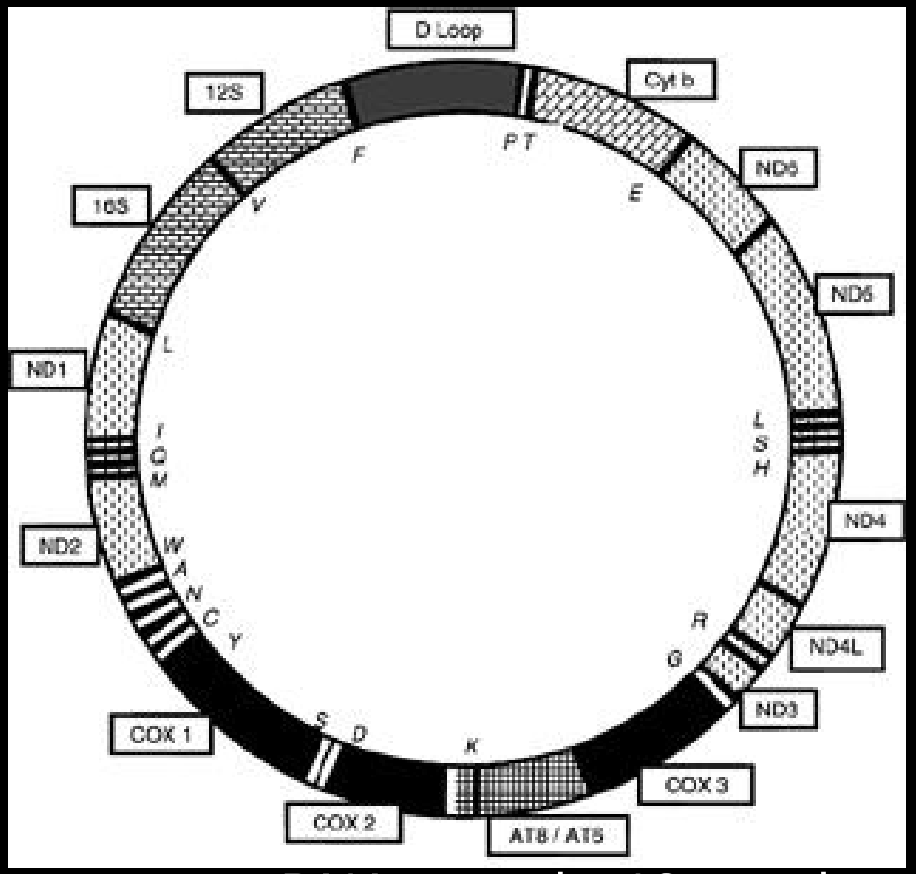
Amino acids, neurotransmitters
CO2 + H2O


Respiratory chain
H+
ADP+Pi
ATP
NADH
NAD+
½ O2
H2O
Succinate
ATP
IM
I
II
III
IV
V
Q
C
H+
H+
H+
OM
SDH
COX

Genetic defect of mitochondrial disease can lie in mitochondrial or nuclear DNA



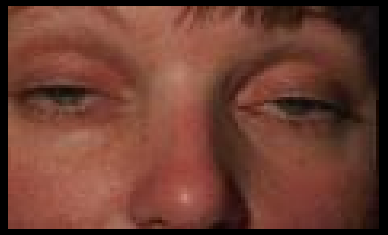
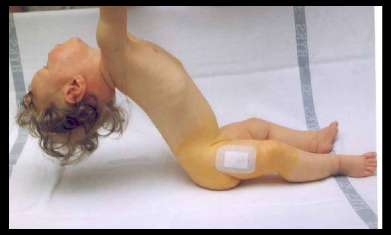
Nuclear genes encode 1500 proteins targeted to mitochondria

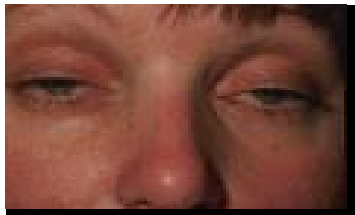


mtDNA: encodes13 proteins

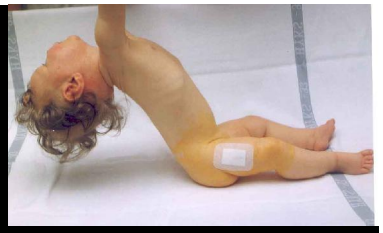
Mitochondrial disorders

Can occur in any tissue, with any symptoms, with any inheritance model



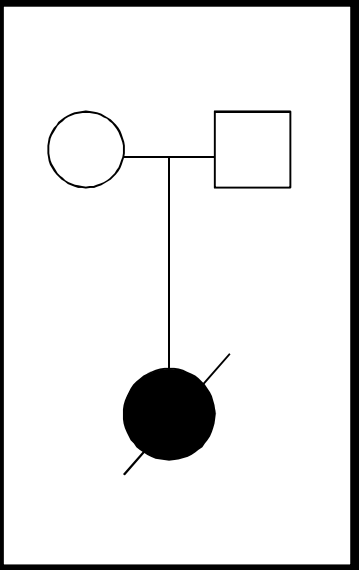
Open questions in mitochondrial diseases



- Why so many different phenotypes?
- Why variable tissue specificity?
- What are the physiological responses to various mitochondrial defects?
- Can any of the processes be affected → treatment

Novel tools for diagnosis

Case report: Infantile hypertrophic cardiomyopathy



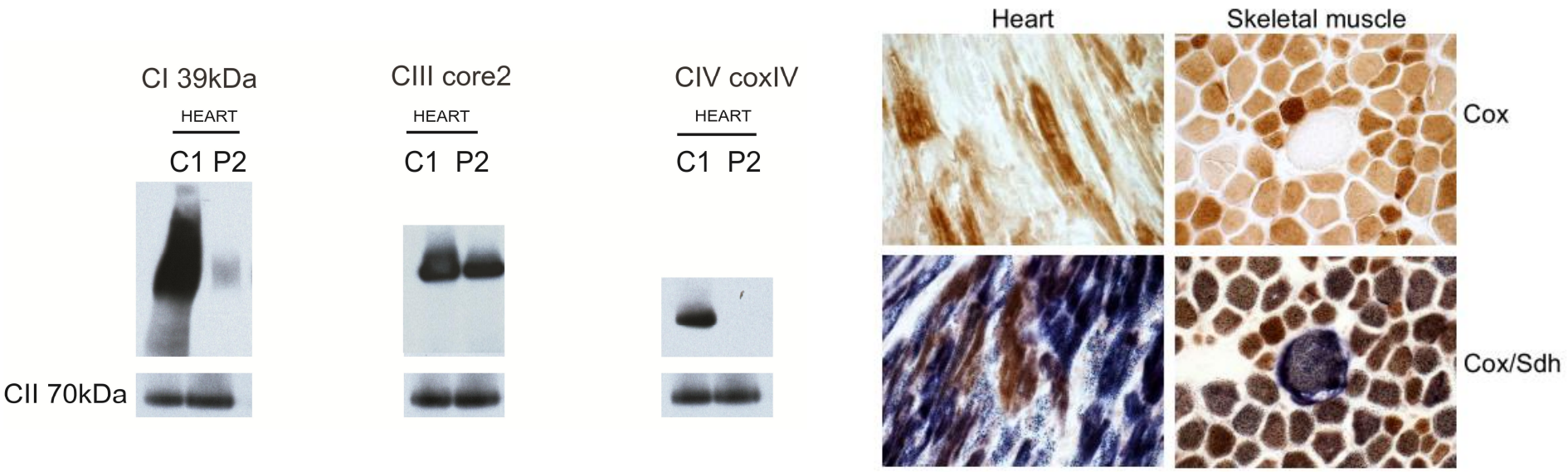
Clinical

First child of healthy parents
Normal pregnancy, neonatal period

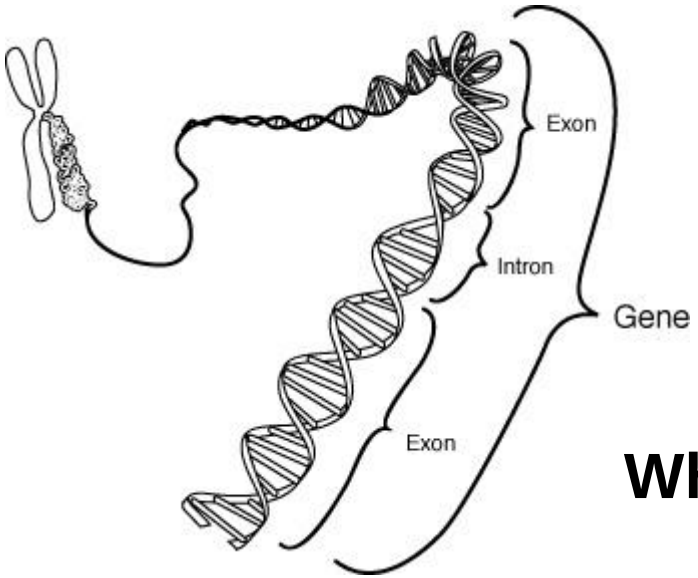
4 months:
- hypertrophic cardiomyopathy, muscle weakness
  lactate levels 2-7mmol/l
- Brain MRI normal, EEG slightly slow

Rapidly progressive disease
10 months: death due to cardiac arrest

Respiratory chain CI, CIII and CIV deficiency in heart



Götz, Tyynismaa et al. Am J Hum Genet 2011



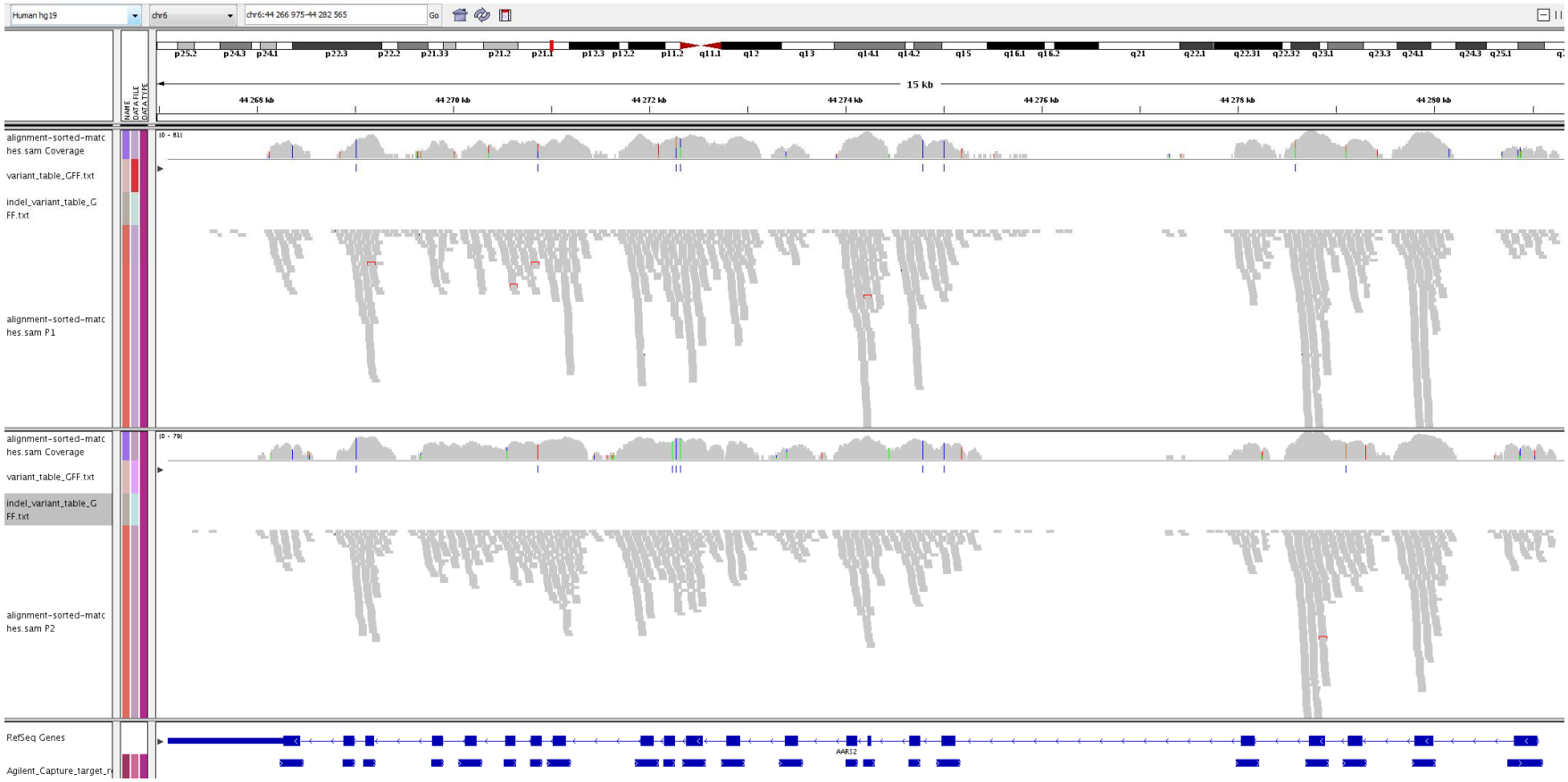
Whole-exome sequencing

Sequence of all coding gene regions of patient's genome

→ 50-60 million short sequence fragments (FIMM)
→ 1.5% of the whole genome =exome (18,000 genes)

Comparison to control genome:
-Known variants vs mutations

Enrichment of sequences to exons

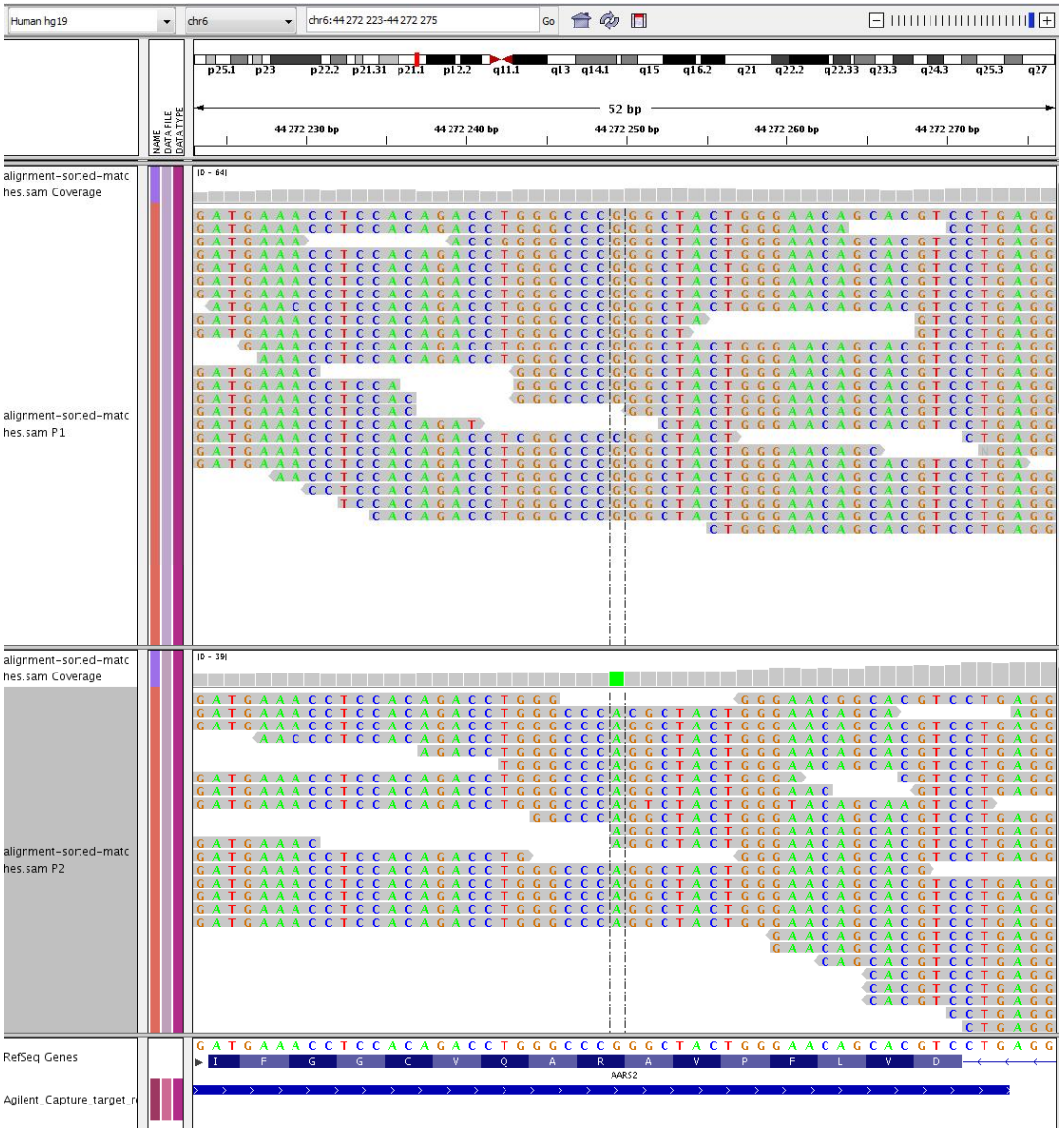


Henna Tyynismaa
Pekka Ellonen
Henrikki Almusa

Results of whole-exome sequencing in the patient

P2	
All variants	65849
Unknown	6323
Map to gene	1549
Homozygous	43
Damaging	7
Mitochondrial	1

Homozygous missense mutation Arg592Trp in the AARS2 gene (putative mitochondrial alanyl-tRNA synthetase)



True disease causing mutation?

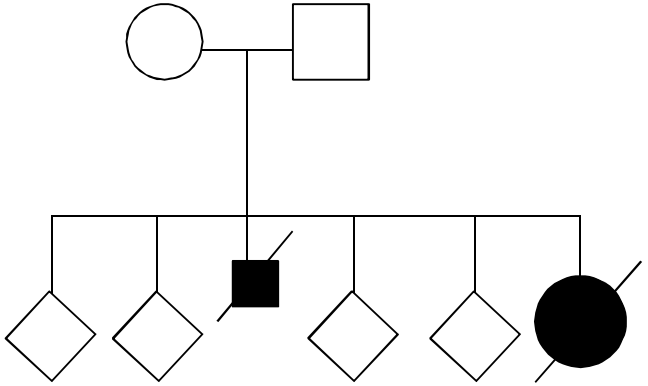
Presence in population

- Absent in 400 control chromosomes

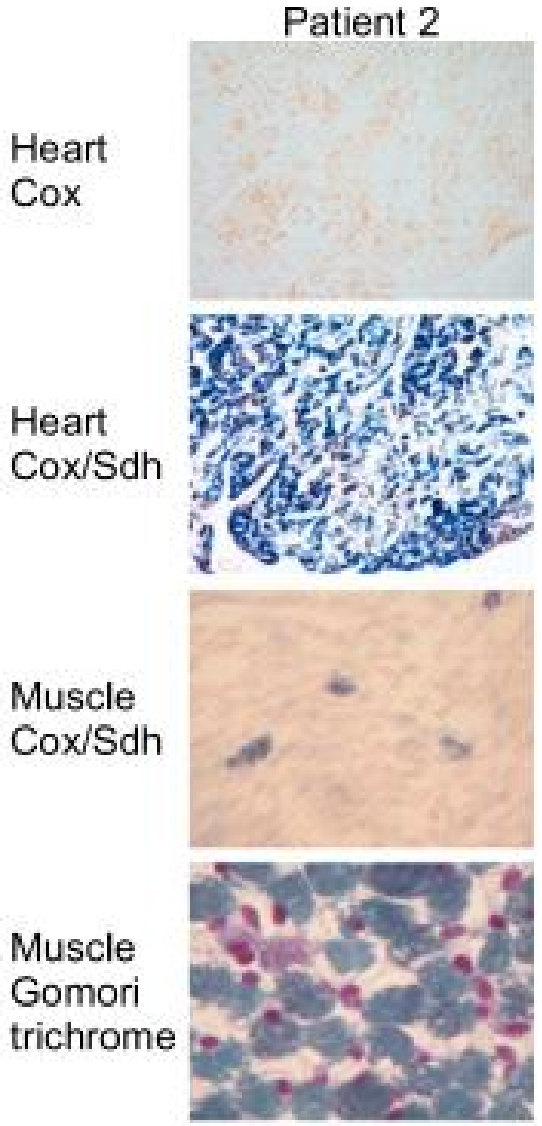
Presence in other CMP families

Functional consequnces

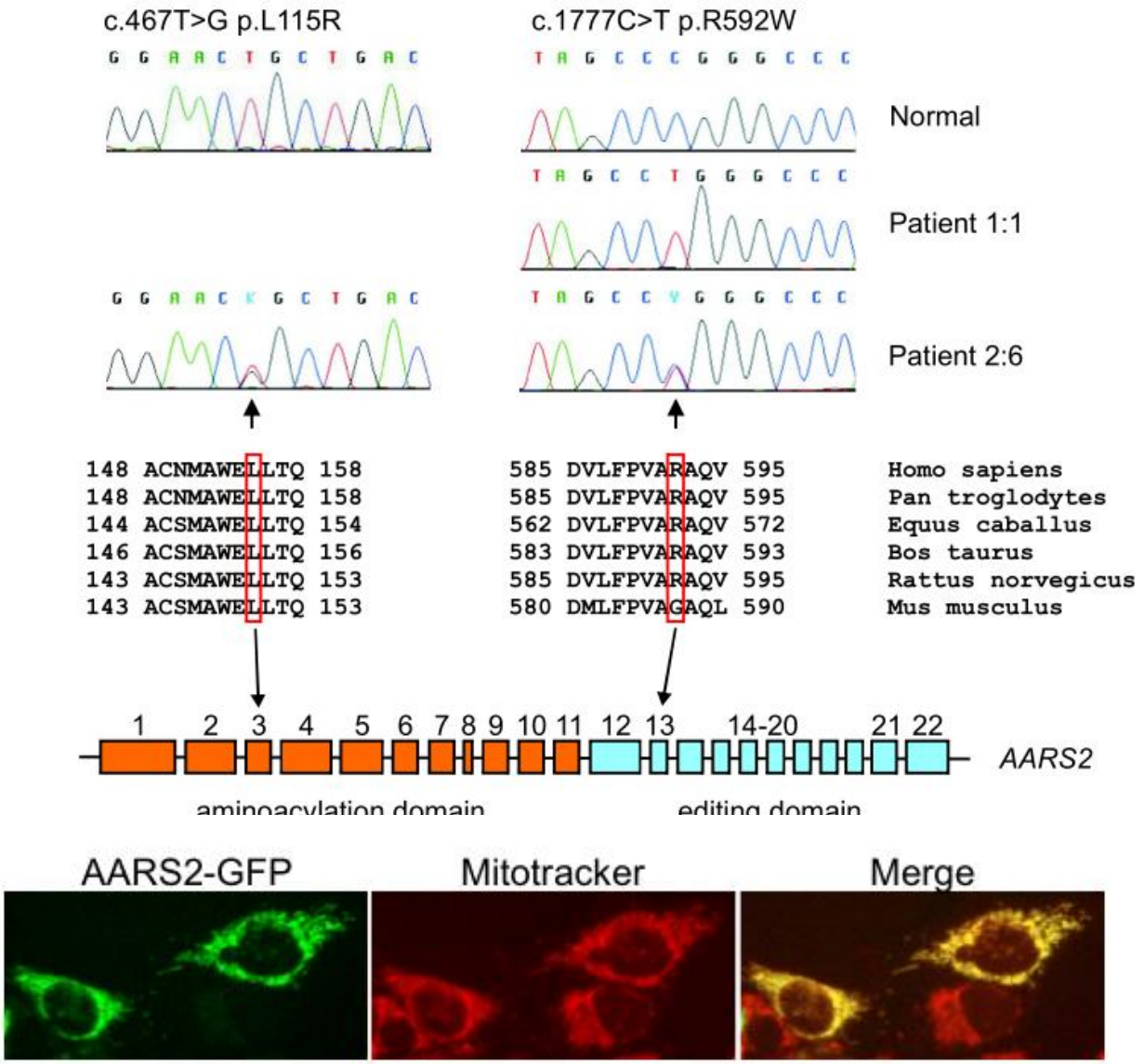
Family 2: prenatal hypertrophic cardiomyopathy



- Prenatal: extrasystolia
- At birth: poor condition, cardiomyopathy, hyperlactacidemia, death at postnatal day 3
- Brother died in utero at week 40
- Compound heterozygosity for two AARS2 mutations



Patient 2 has a homozygous missense mutation R592W in the AARS2 gene (putative mitochondrial alanyl-tRNA synthetase) - shared by another patient

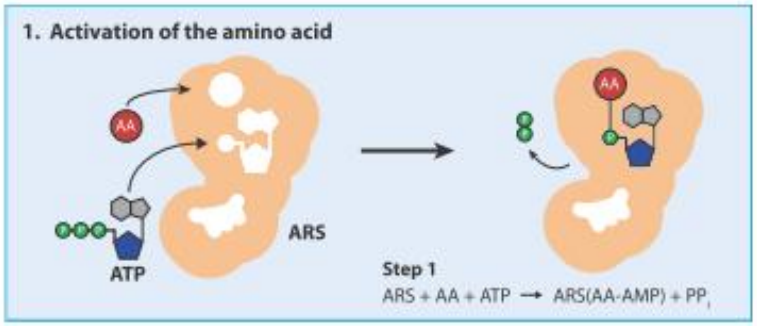


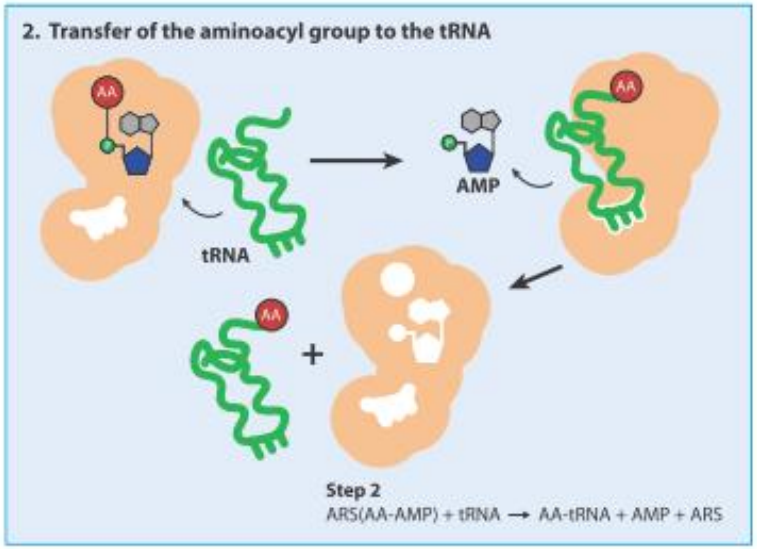
Aminoacyl-tRNA synthetases

Charges a tRNA with aminoacid

Specific synthetases for each tRNA-aa pair; mitochondrial and cytoplasmic

tRNA structure specific; recognition often based on anticodon; amino acid specificity lower





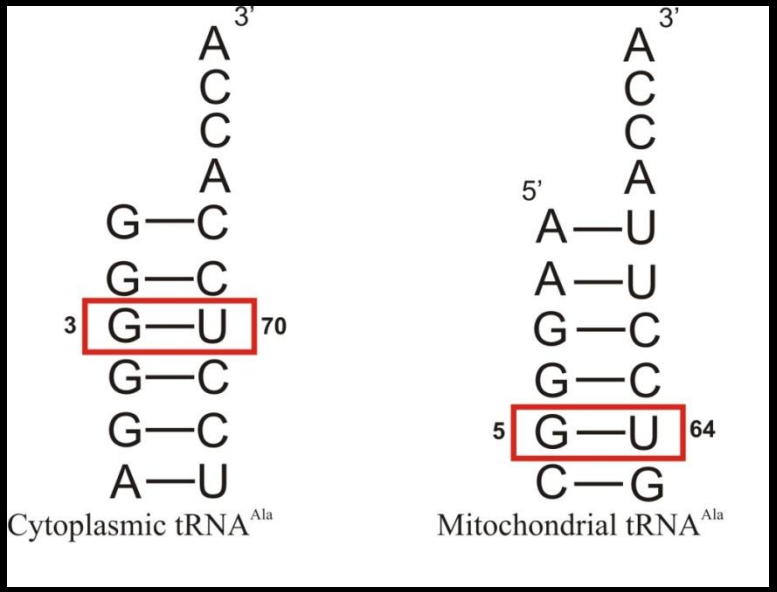
Special features of Ala-tRNA synthetases?

No selectivity between Ala, Ser and Gly in the active site

Removal of incorrect amino acid in the editing domain

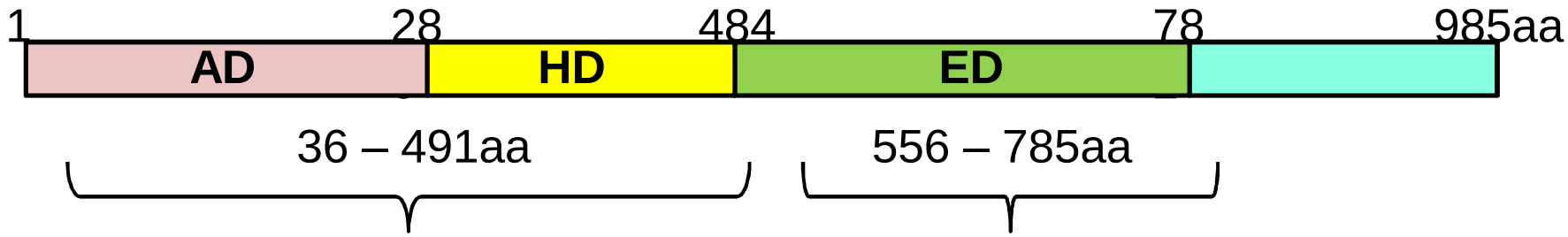
No anticodon binding domain

Enzyme specifically recognizes conserved G:U base pair in the tRNA acceptor stem



Liliya Euro

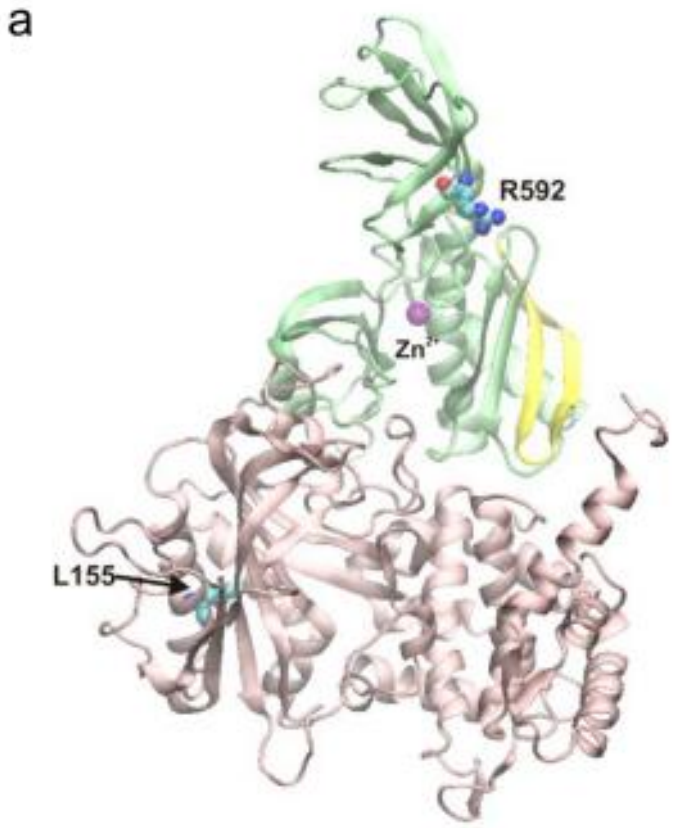
Multiple sequence analysis: human AARS2 structure utilizing known bacterial AlaRS structures as template

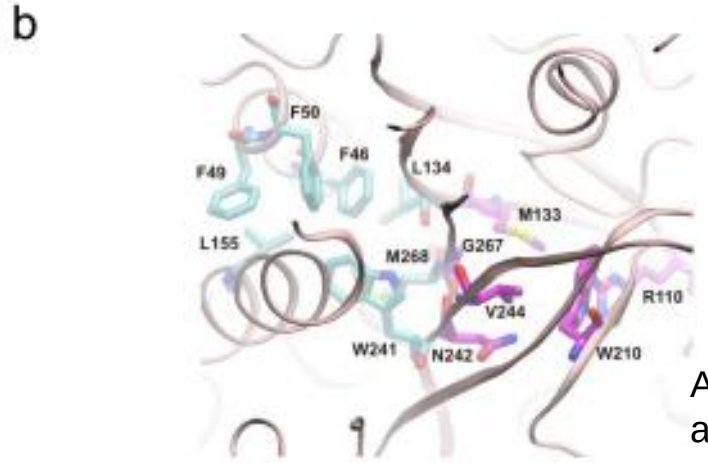


E.coli AlaRS
36.6 % identity
52.6 % similarity

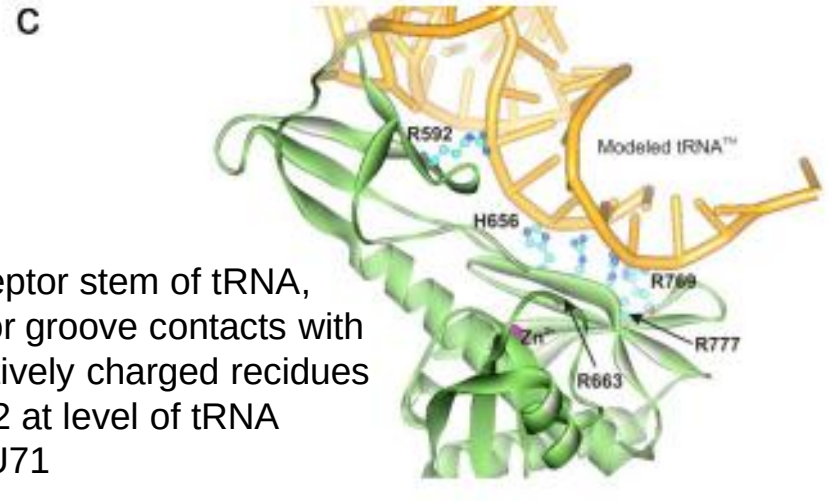
Pyrococcus horikoshii AlaRS
30.1 % identity
42.7 % similarity

L155R affects architecture of the catalytic domain R592W predicted to affect tRNA binding at editing domain



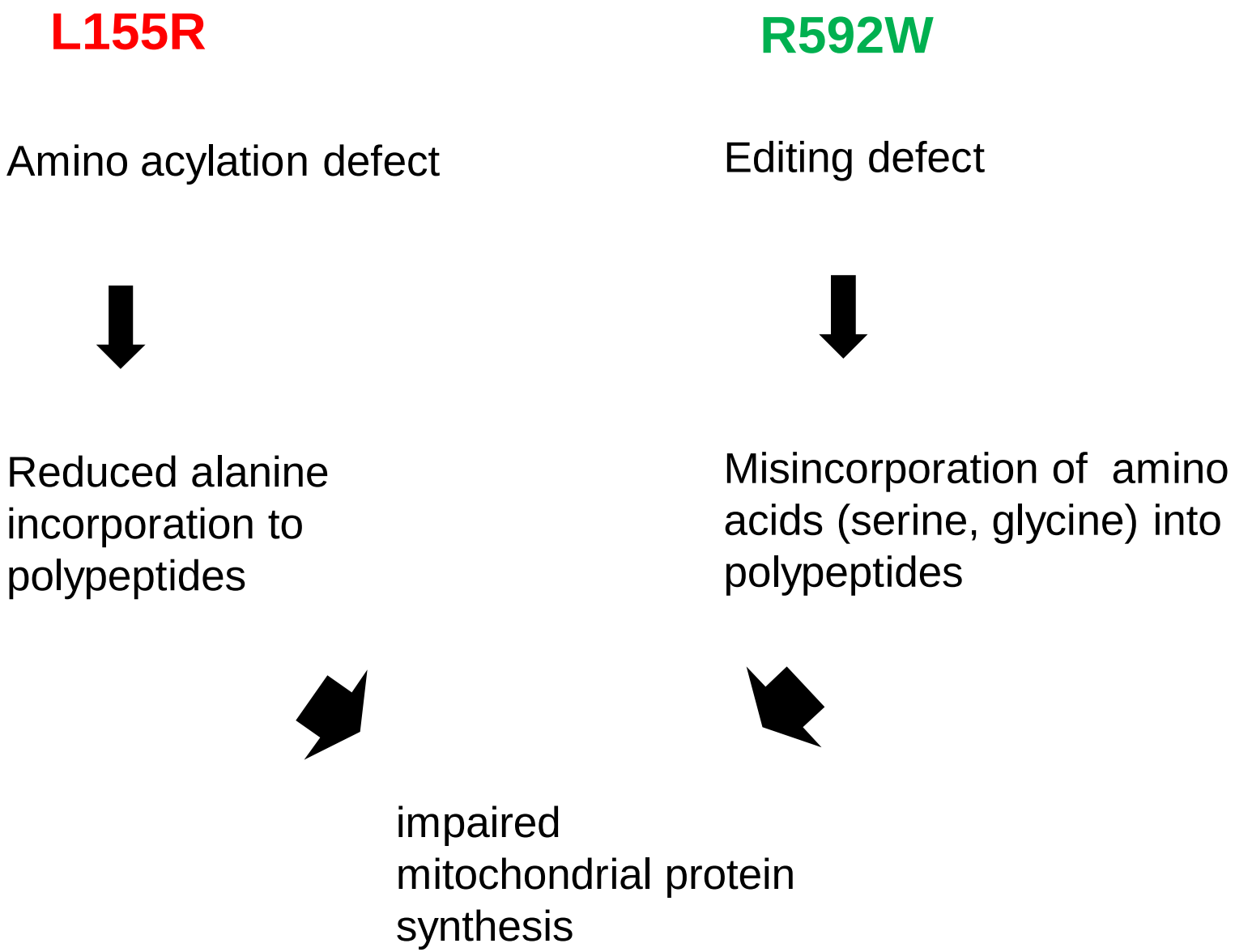


Amino acyl formation affected at catalytic site



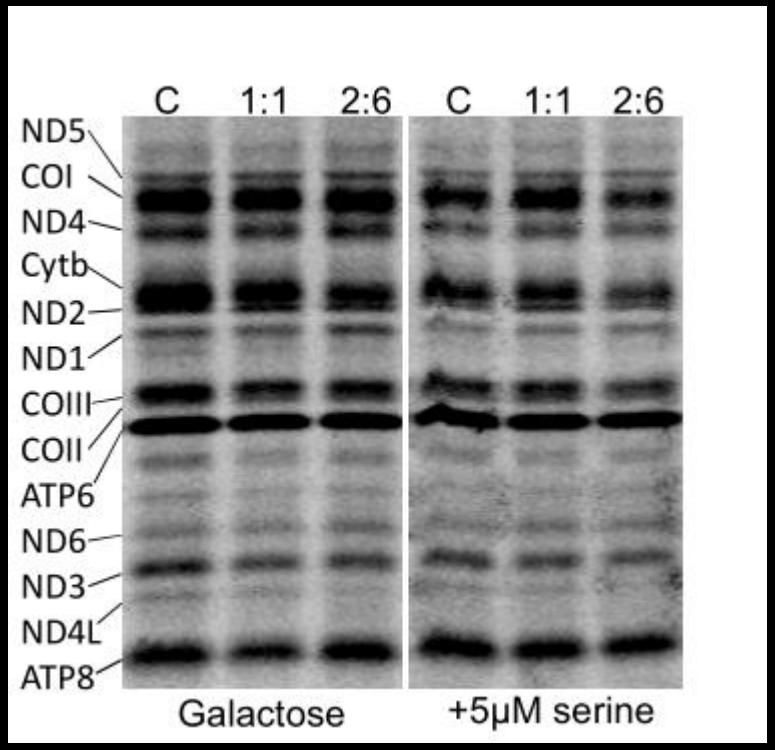
Acceptor stem of tRNA, minor groove contacts with positively charged recidues R592 at level of tRNA G2:U71

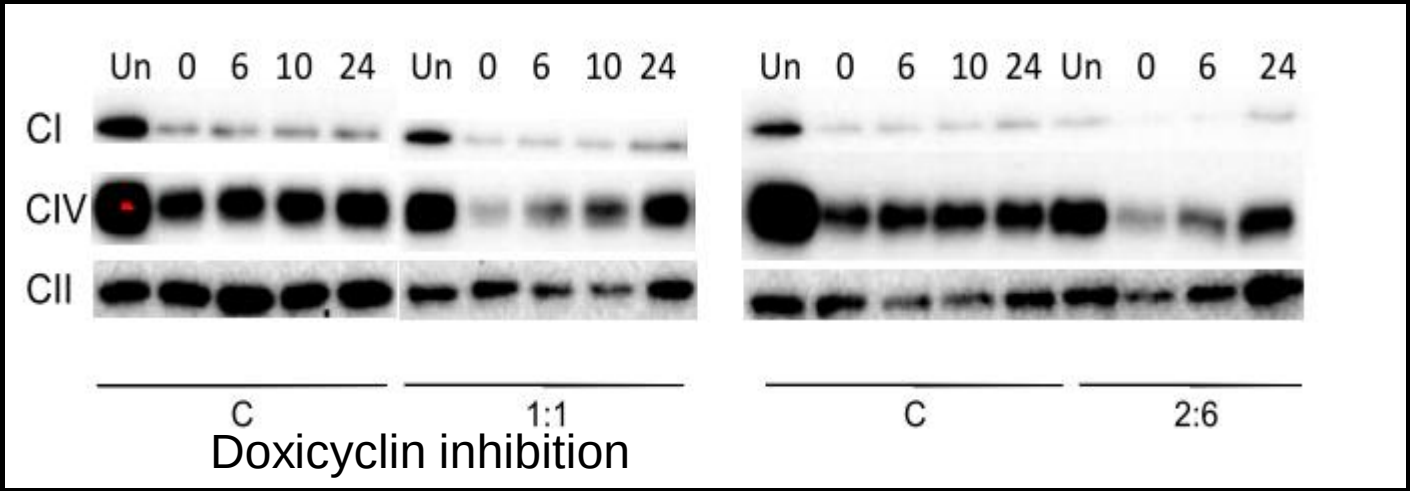
Liliya Euro


L155R
R592W
Amino acylation defect
Editing defect
Reduced alanine incorporation to polypeptides
Misincorporation of amino acids (serine, glycine) into polypeptides
impaired mitochondrial protein synthesis

Minor effect in myotube mitochondrial translation

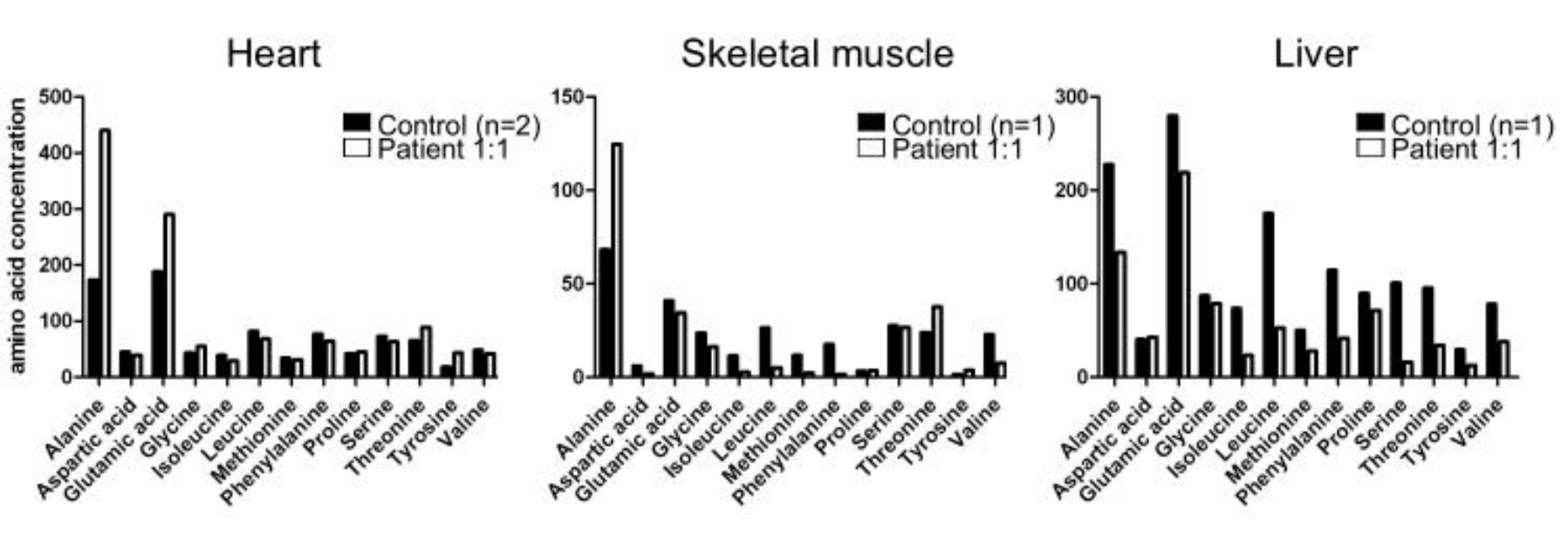
But recovery from translation challenge slow





Tissue specificity?
Can variable levels of amino acids explain cardiac manifestation?

Metabolomic analysis: comprehensive two-dimensional gas chromatography combined withi time-of-flight mass spectrometry (Matej Oresic, VTT, Finland)



Alanine increased,
no major changes in serine / glycine

Mitochondrial aminoacyl-tRNA synthetase mutations are a common cause of inherited disease

DARS2	leucoencephalopathy, lactacidosis, brain stem & spinal cord involvement	2007
RARS2	Infantile encephalopathy, cerebellar degeneration	2010
YARS2	myopathy, lactic acidosis and sideroblastic anemia	2010
AARS2	Cardiomyopathy & myopathy	2011
HARS2	Ovarian dysgenesis & hearing loss	2011
SARS2	hyperuricaemia, pulmonary hypertension, infantile renal failure	2011

Conclusions

Mitochondrial dysfunction: an important cause of childhood / infantile cardiomyopathy – hypertrophic & dilated

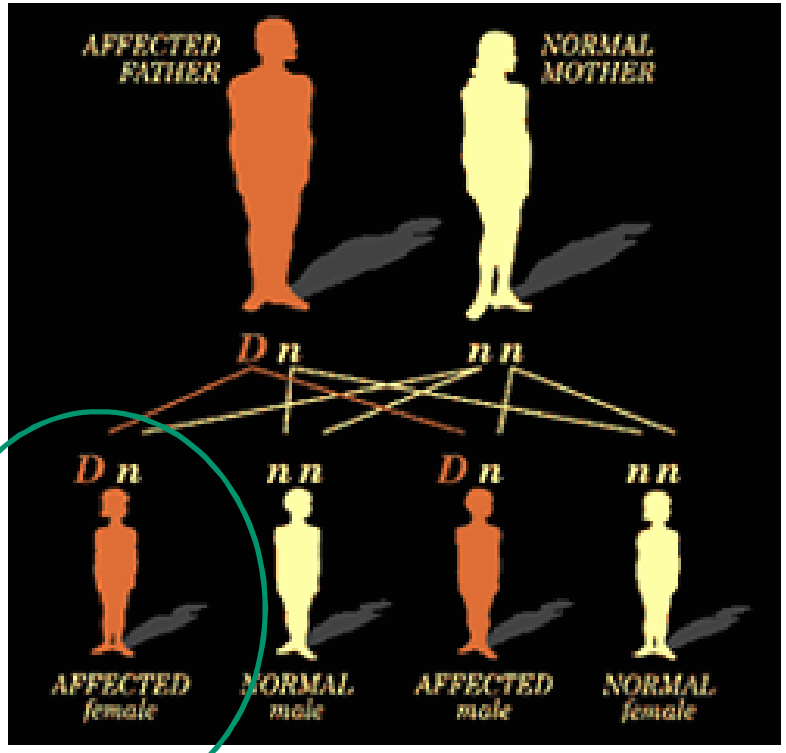
Mitochondrial translation defects may manifest primarily as early cardiomyopathy

Aminoacyl-tRNA synthetase mutations: an important cause of mitochondrial disease, with wide clinical variability – including CMPs

Exome analysis reveals new syndromes with variable clinical manifestations – not previously recognized as single-gene disorders

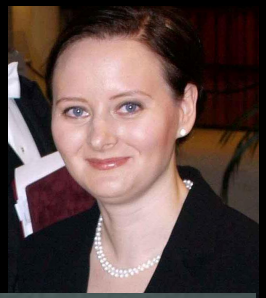
Singleton findings without obvious cell phenotype – subtle phenotype may be found if the pathway challenged

Gene search possible from a single patient
Major progress in DNA diagnosis & research



Confirms diagnosis & inheritance pattern
Provides means for genetic counseling & research of pathogenic mechanism

Thank you







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