Mitochondrial disease and liver

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Outline

- early onset hepatopathies
- later onset hepatopathies
- reversal hepatopathies
- valproate and hepatic failiure in POLG

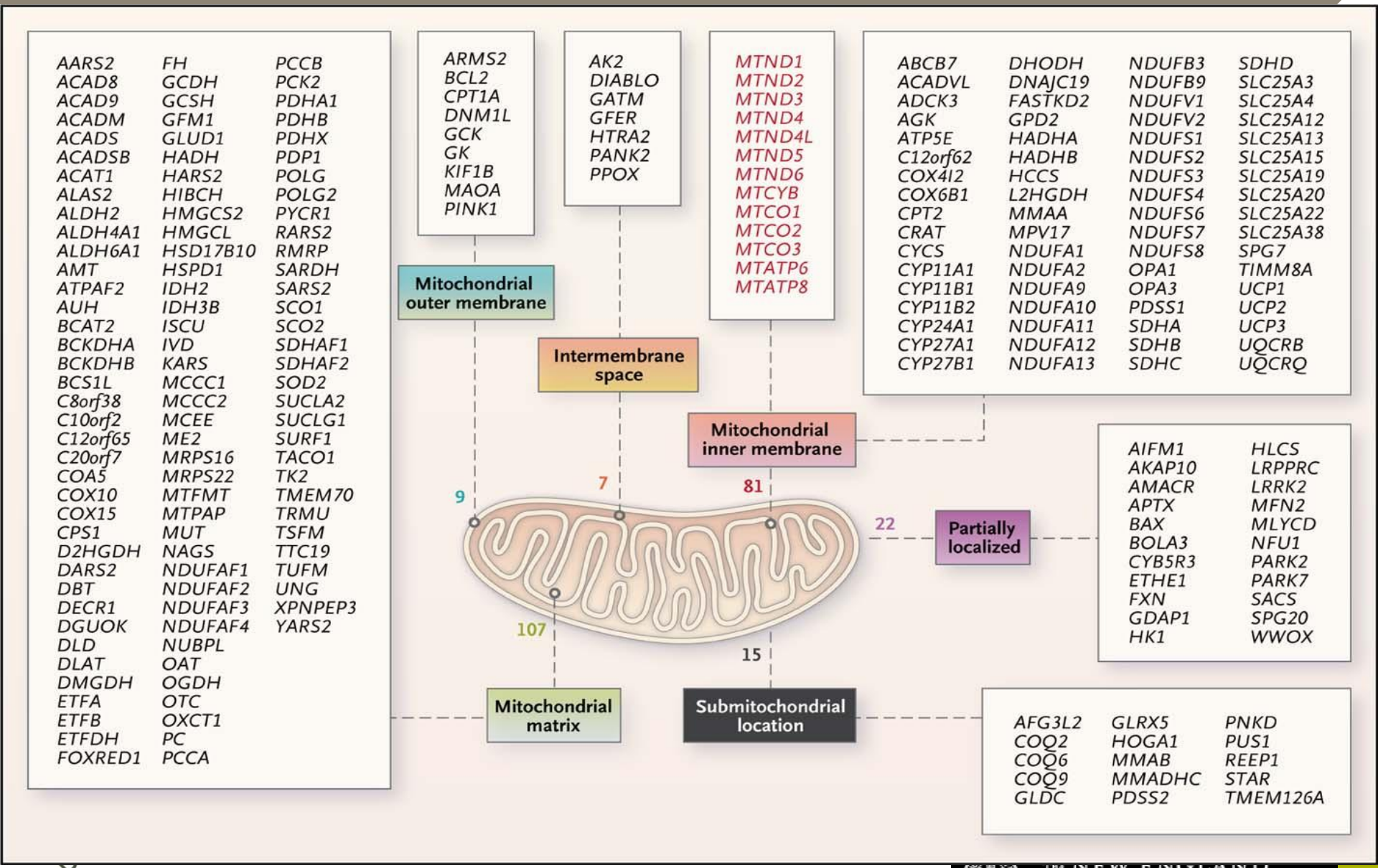
Mitochondrial disorders in neonate

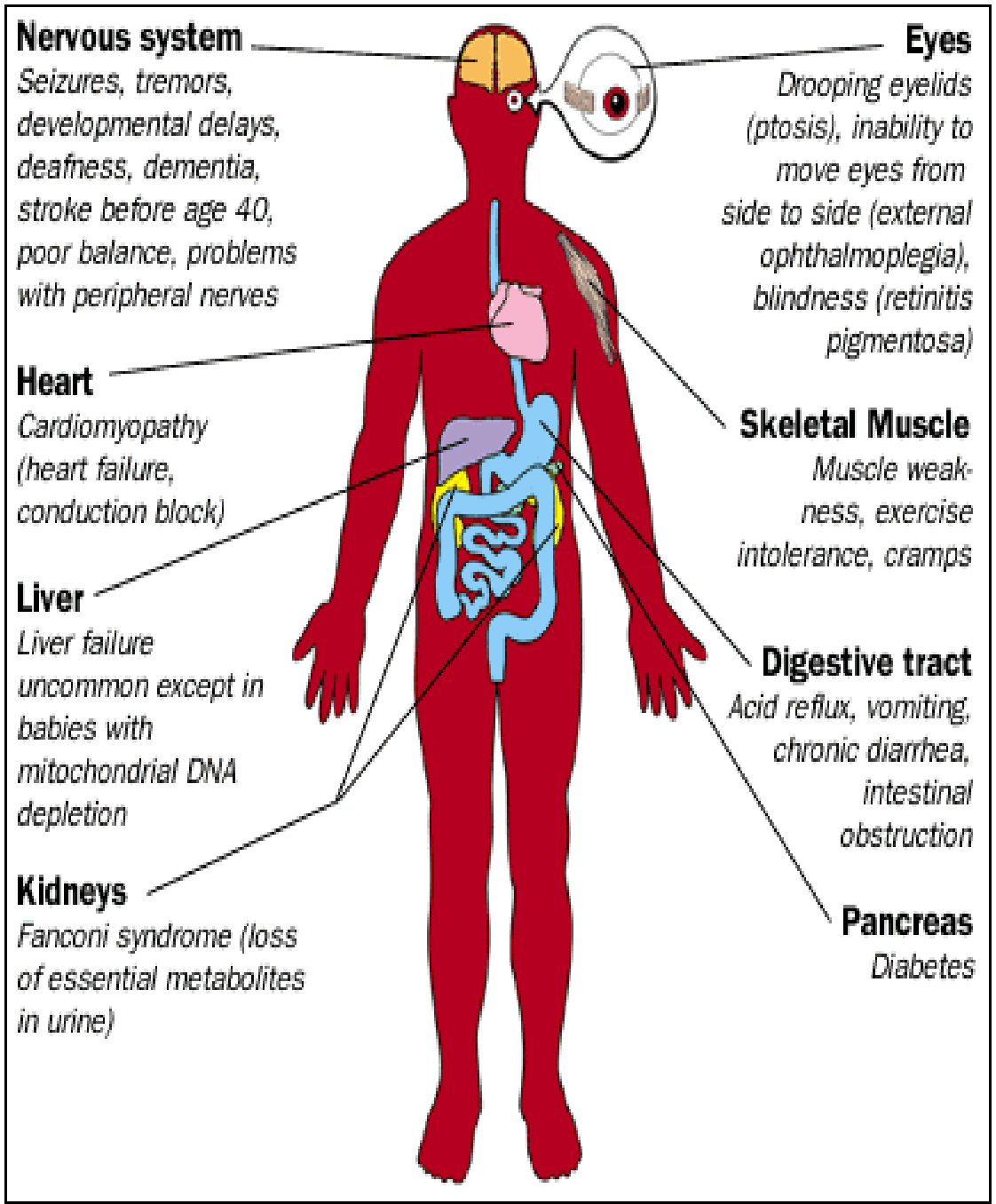
- metabolic crisis combined with one or several organ manifestations
- liver dysfunction due to respiratoy deficiency is commonly overlooked in severely sick newborn
- lactic acidosis, hypoglycemia, elevated serum transaminases and conjugated bilirubin are common signs of mitochondrial hepatopathy
- hepatosplenomegaly in severe cases
- fetal growth restriction, cholestasis in combination with neurological symptoms or renal tubulopathy

Genetic classification

- **Defects in mitochondrial DNA**
  - → Deletions/duplications
  - → Protein synthesis tRNA, rRNA
  - → Protein coding genes
- **Defects in nuclear genes**
  - → Respiratory chain subunits
  - → Assembly genes
  - → Genes involved in intergenomic communication
    - mitochondrial DNA depletion
    - mtDNA translation
    - MIM lipid milieu
    - mitochondrial dynamics

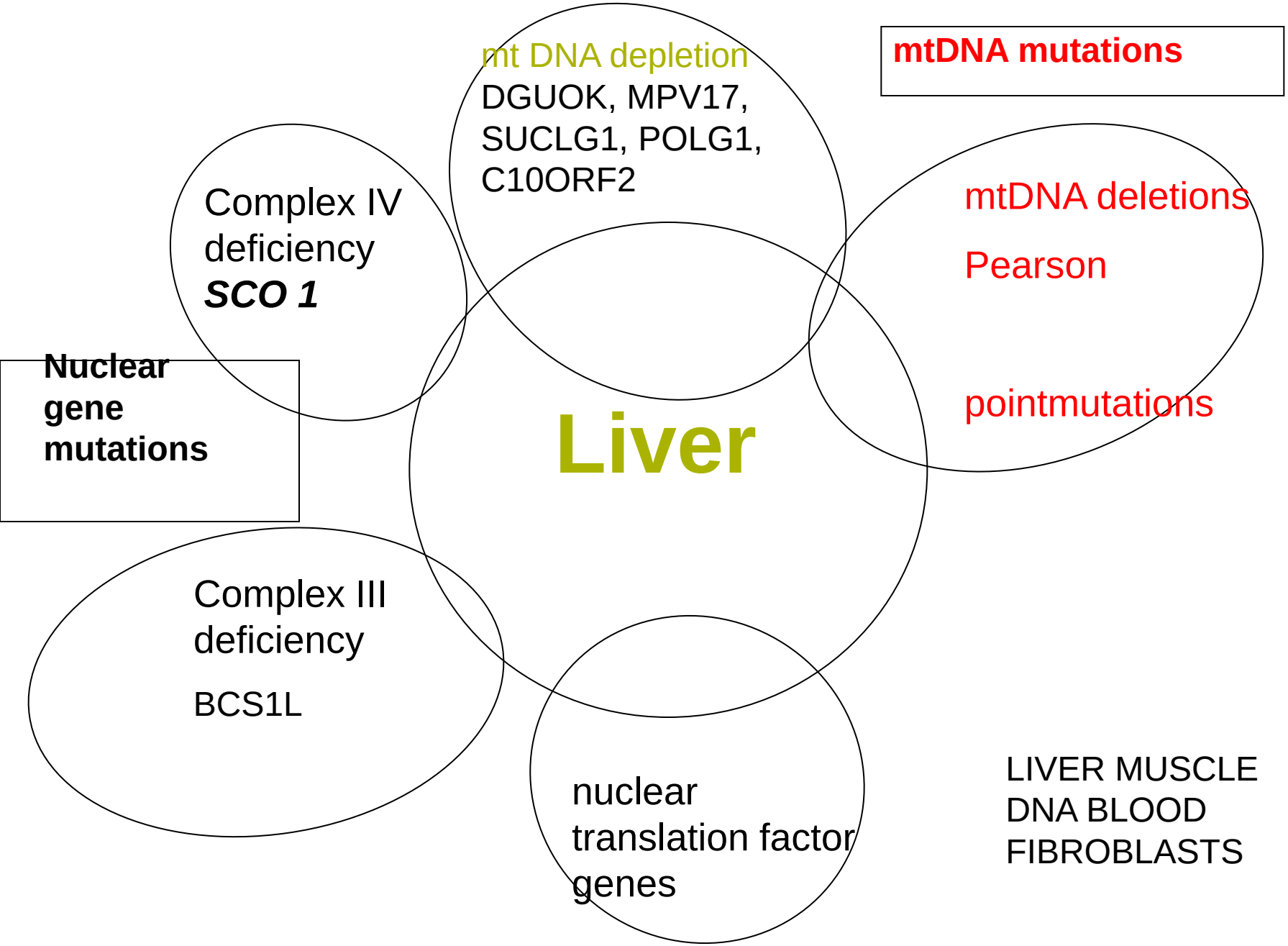
Genes of Mitochondria-Localized Proteins Linked to Disease in Humans.




Nervous system
Seizures, tremors, developmental delays, deafness, dementia, stroke before age 40, poor balance, problems with peripheral nerves
Heart
Cardiomyopathy (heart failure, conduction block)
Liver
Liver failure uncommon except in babies with mitochondrial DNA depletion
Kidneys
Fanconi syndrome (loss of essential metabolites in urine)
Eyes
Drooping eyelids (ptosis), inability to move eyes from side to side (external ophthalmoplegia), blindness (retinitis pigmentosa)
Skeletal Muscle
Muscle weakness, exercise intolerance, cramps
Digestive tract
Acid reflux, vomiting, chronic diarrhea, intestinal obstruction
Pancreas
Diabetes

Mitochondrial syndromes

- Leigh
- Alpers-Huttenlocher
- Pearson
- Kears Sayre
- Sengers
- Megdel
- LHON
- MIDD
- MELAS
- NARP
- MERRF
- Ataxia neuropathy spectrum
- CPEO
- MNGIE


mt DNA depletion
DGUOK, MPV17, SUCLG1, POLG1, C10ORF2
mtDNA mutations
Complex IV deficiency
SCO 1
mtDNA deletions
Pearson
Nuclear gene mutations
Liver
pointmutations
Complex III deficiency
BCS1L
nuclear translation factor genes
LIVER MUSCLE
DNA BLOOD
FIBROBLASTS

Mitochondrial hepatopathy

- mitochondrial DNA (deletion) Pearson syndrome
- mitochondrial depletion syndrome
  → DGUOK, MPV17, SUCLG1, POLG1, C10ORF2
- combination of lactic acidosis, liver involvement, and Fanconi type renal tubulopathy complex III assembly factor BCS1L
- assembly COX
- mutations in nuclear translation factor genes (TRMU, EFG1, and EFTu)

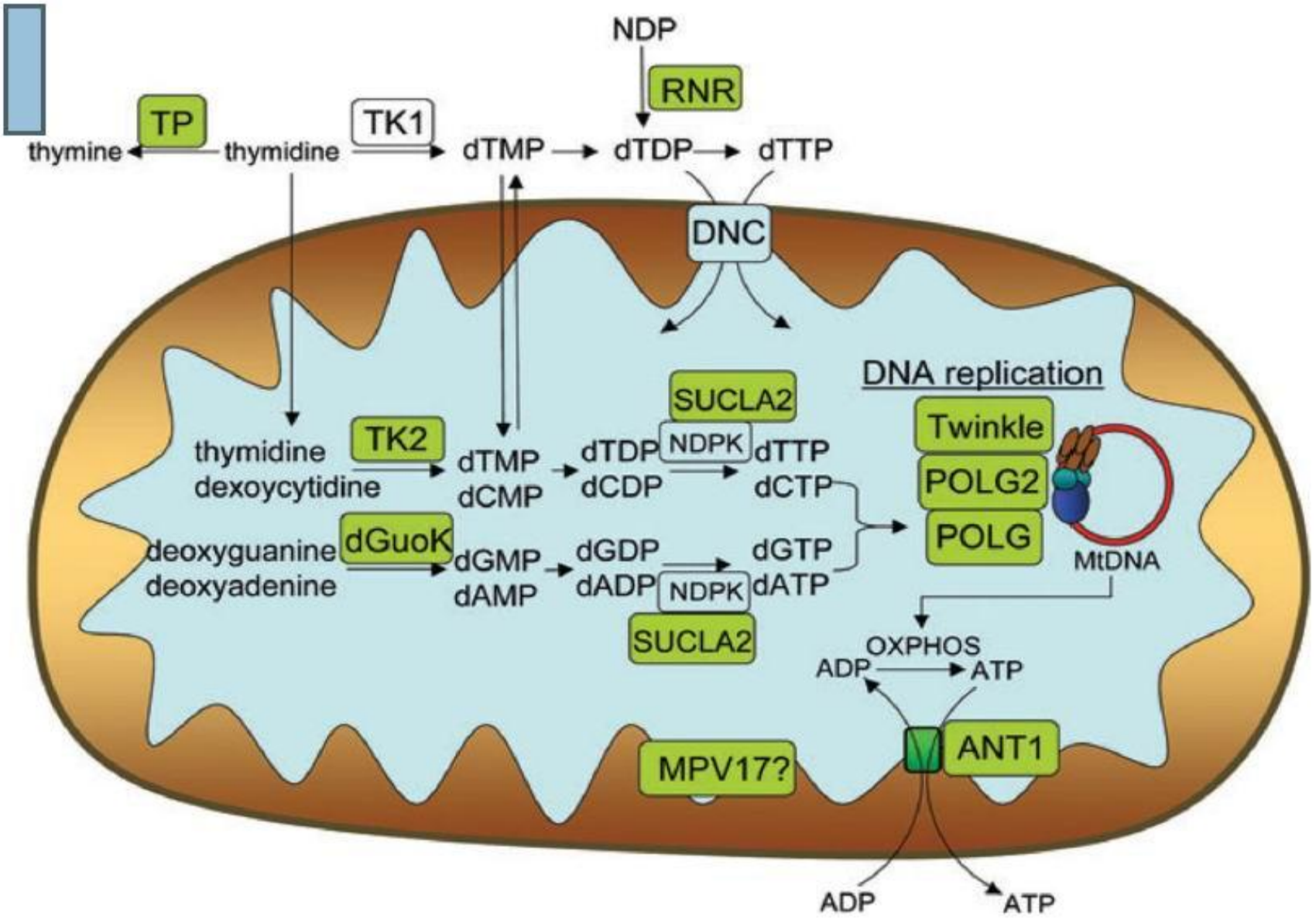
Pearson syndrome mtDNA deletion

- severe macrocytic anemia, neutropenia and thrombocytopenia
- vacuolisation of marrow precursors
- ringed sideroblasts in bone marrow
- pancreatic insufficiency: fat malabsorption diarrhoea
- hepatomegaly, steatosis, cirrhosis liver failure
- renal tubular disease, skin lesions, diabetes mellitus, myopathy, retinopathy

Mt depletion syndrome

- Described first in 1991 by Moraes
- Infants with poor intake and failure to thrive
- Liverdysfunction, lactic acidosis, ketotic hypoglycemia
- Or hypotonia, nystagmus,weakness
- Autosomal recessive
- Microvesicular steatosis, canalicular cholestasis, hepatocellular cholestasis
- elevated α-foetoprotein

Mitochondrial DNA depletion



Mitochondral DNA depletion syndromes

- Autosomal recessive disorders
- Defects in maintenance in mtDNA
  - → Mitochondrial deoxyribonucleoside triphosphate (dNTP) synthesis depleting mitochondrial DNA building blocks
  - → mtDNA replication leading to insufficient mtDNA synthesis
- Heterogenous group affect specific organ or combination of organs
- mtDNA replicates normally continously using salvage pathway to dNTPs

Classification

- Myopathic form TK2
- Encephalomyopathic form SUCLA2, SUCLG1, RRM2B
- Hepatocerebral form DGUOK, MPV17, POLG, C10orf2
- Neurogastrointestinal form TYMP

Mitochondrial DNA depletion syndromes

MPV17	• Early onset liver failure, leukoencephalopathy, polyneuropahy, increased lactate
DGUOK	• Early onset leukoencephalopahy, increased lactate • hepatopathy
TK2	• Infantile myopathy with respiratory failure, increased lactate and CK, RRF; isolated myopathy; SMA-like
SUCLA2	• Leigh like, dystonia, deafness, mild MMA
SUCLG1	• Fatal infantile LA, liver and CNS, mild MMA
RRN2B	• Fatal infantile LA, renal and CNS disease

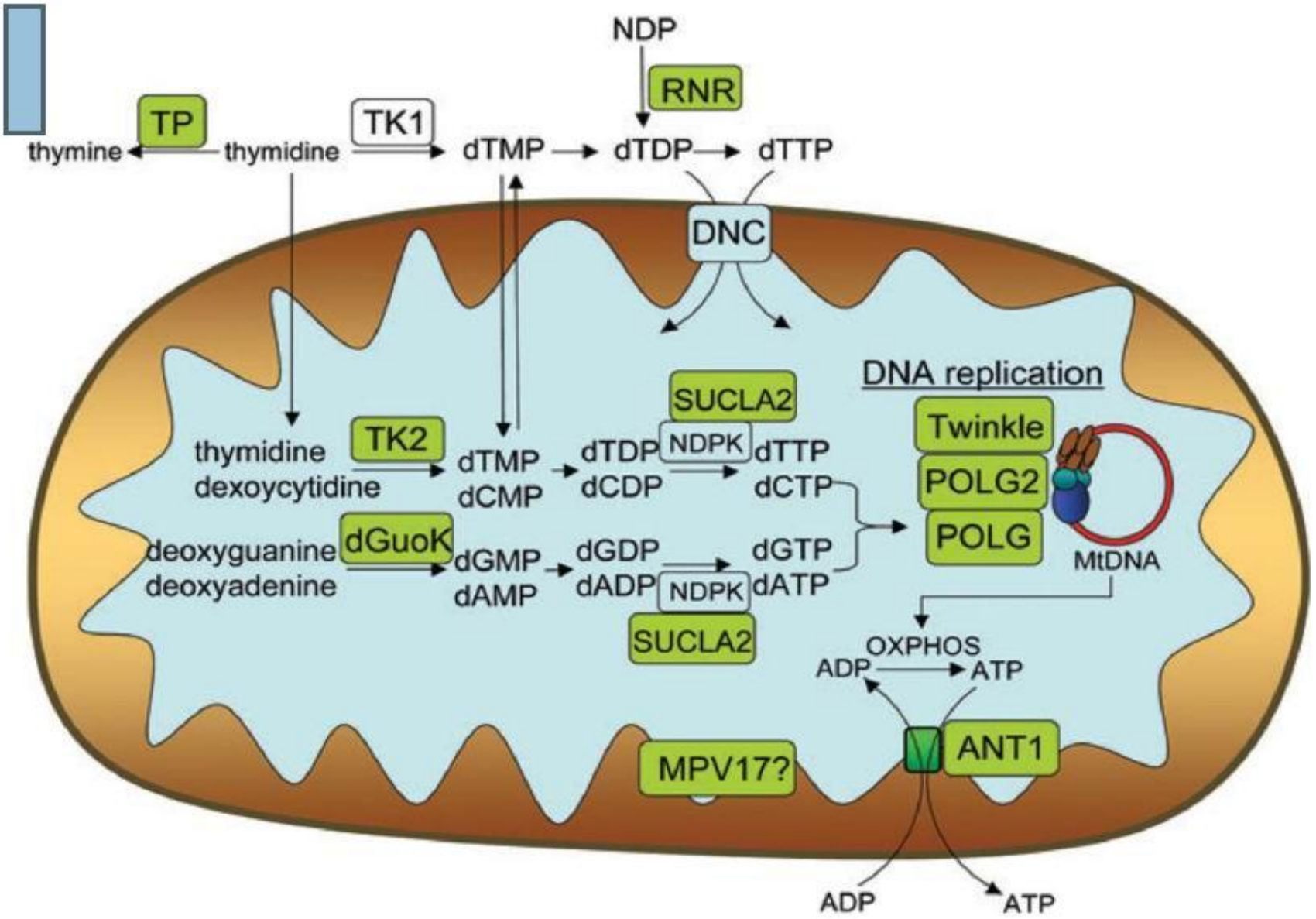
Mitochondrial DNA depletion syndromes

- POLG
  - Infants, children:
    - → Alpers, myoclonus epilepsy/encephalopathy; isolated therapy refractory epilepsy
  - Adults
    - → progressive external ophthalmoplegia, ataxia, Parkinson, polyneuropathy and others; epilepsy
- TP
  - MNGIE
- Twinkle
  - hepatocerebral form of MDS

DGUOK

- expressed in high abundance in liver, muscle, brain
- dGK protein is involved in maintenance of balanced mitochondrial deoxyribonucleotide pool for mtDNA synthesis
- reduced activities of respiratory chain complexes I, III and IV

Mitochondrial DNA depletion



DGUOK

- Two forms

1. Multi-organ disease in neonates

    Majority neonatal form with lactic acidosis, hypoglycemia, cholestasis

    Associated with myopathy, regression, typical rotatory nystagmus

    Progressive liver failure

DGUOK

- 2.isolated hepatic disease in infancy or childhood

  mild hypotonia, renal involvement with proteinuria and aminoaciduria

- 3. recently neonatal hemochromatosis

  and adult-onset mitochondrial myopathy with multiple deletions in skeletal muscle

DGUOK

- newborn infants hepatic failure, severe failure to thrive, oscillating eye movements and neurological abnormalities
- lactic acidosis, hypoglycemia and elevated a-fetoprotein
- iron overload with elevated transferrin saturation, high ferritin levels, and iron accumutation in hepatocytes and Kupffer cells
- death before one year of age

MPV17

- MPV17 encodes mitochondrial inner membrane protein controlling mtDNA maintenance and OXPHOS activity
- rapidly progressive liver disorder early after birth
- hypoglycemia + lactic acidosis
- failure to thrive and jaundice
- severe liver failure with hepatosplenomegaly
- liver transplantation, resulting in prolonged survival, but neurological symptoms later

case

- neonate D1 hypoglycemia, lactic acidosis and hypothermia
- consanguinous parents
- early onset liver dysfunction with severe steatosis and terminal hepatocellular failure
- delayed development no visual fixation
- elevated alphafoetoproteins
- mtDNA 60% depletion in muscle
- liver 30% decreased activities of I, III, IV
- homozygous mutation in MPV17

Navajo neurohepatopathy

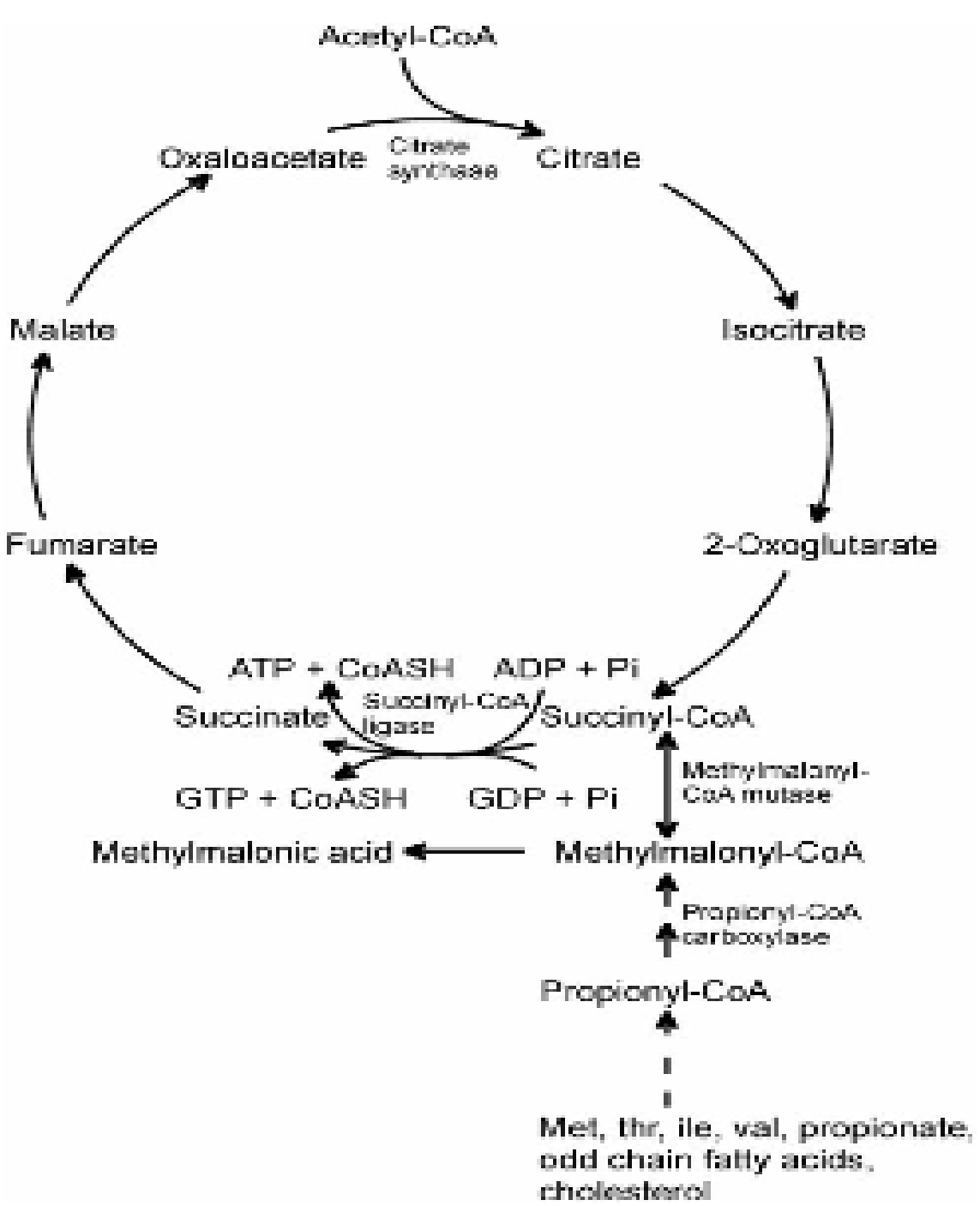
- sensorimotor neuropathy + progressive liver disease
- development of weakness, hypotonia, areflexia, loss of sensation in the extremities
- corneal ulceration, poor growth, short stature
- serious systemic infections
- MRI progressive white matter lesions
- peripheral nerve loss of myelinated fibers
- liver involvement Reye-like syndrome episodes cholestasis, cirrhosis

Navajo neurohepatopathy

- → infantile presentation failure to thrive, jaundice progressing to hepatic failure and death within 2 years of life, with or without neuro
- → childhood 1 and 5 years rapid development of liver failure within 6 months
- → classic form progressive neurologic findings dominate plus liver dysfunction
- → liver histology portal fibrosis or micronodular cirrhosis, macrovesicular and microvesicular steatosis, pseudoacinar formation, multinucleated giant cells, cholestasis, periportal inflammation

SUCLA2 and SUCLG1

- encode subunits of succinyl CoA ligase
- catalyzes conversion of succinyl-CoA and ADP or GDP to succinate and adenosie triphosphate or guanosine triphosphate
- SUCL alpha unit and beta unit
- makes a complex with mitochondrial nucleoside diphosphate kinase, resulting in decreased mt DNA synthesis


Acetyl-CoA
Oxaloacetate
Citrate synthase
Citrate
Isocitrate
2-Oxoglutarate
ATP + CoASH
ADP + Pi
Succinyl-CoA ligase
Succinate
Succinyl-CoA
GTP + CoASH
GDP + Pi
Methylmalonyl-CoA mutase
Fumarate
Malate
Methylmalonic acid
Methylmalonyl-CoA
Propionyl-CoA carboxylase
Propionyl-CoA
Met, thr, ile, val, propionate, odd chain fatty acids, cholesterol

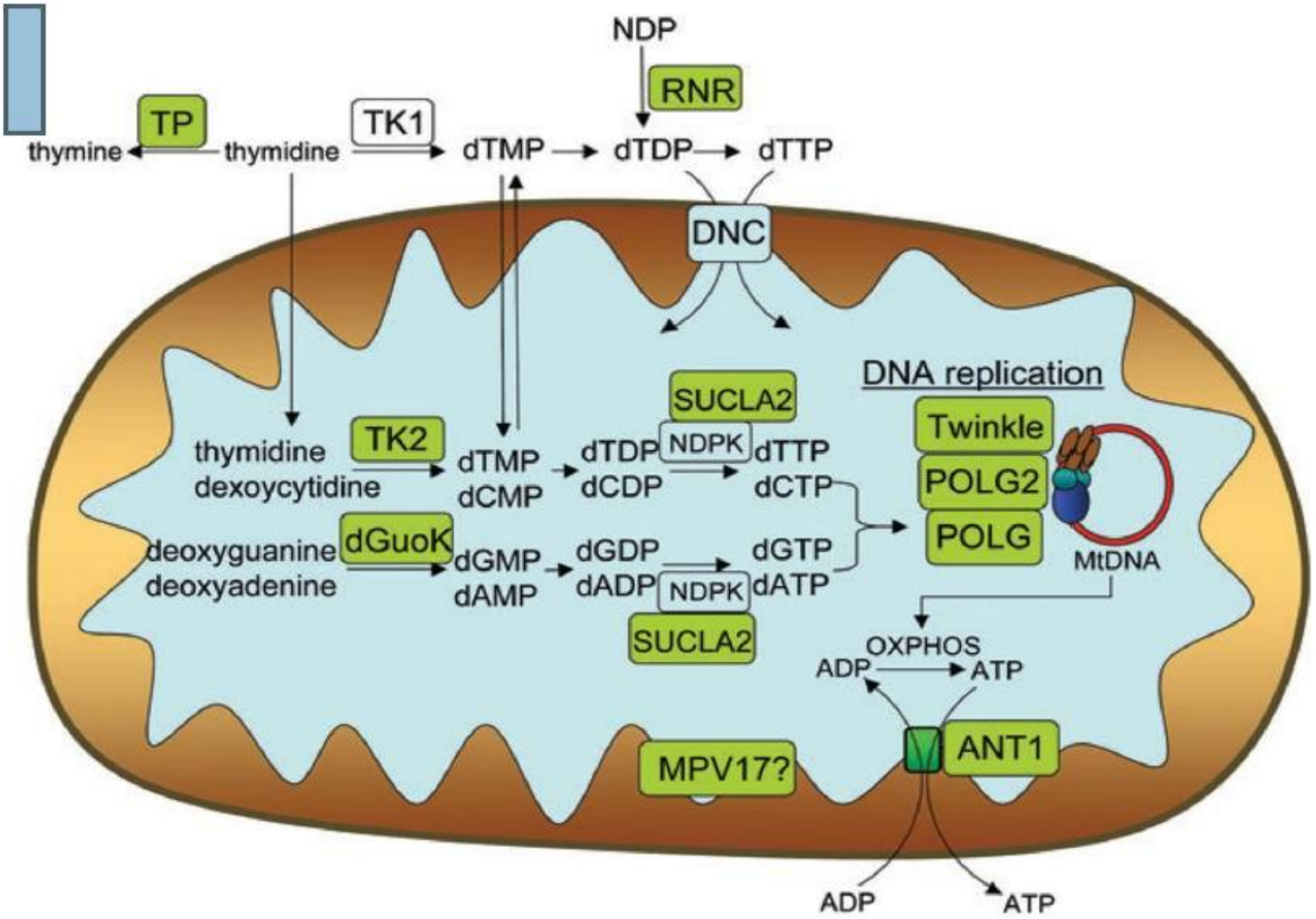
SUCLG1

- succinyl-CoA ligase enzyme in Krebs cycle
- hepatoencephalomyopathy
- fetal growth restriction
- severe lactic acidosis soon after birth
- infant can survive for months, myopathy, hearing loss, Leigh disease-like, progressive liver disease
- mild MMA in urine

Mitochondrial depletion syndromes and replication

- POLG
  - Infants, children:
    → Alpers, myoclonus epilepsy/encephalopathy; isolated therapy refractory epilepsy
  - Adults
    → progressive external ophthalmoplegia, ataxia, Parkinson, polyneuropathy and others
- TP
  - MNGIE
- Twinkle
  - hepatocerebral form of MDS

Mitochondrial DNA depletion



Alpers disease

- Initial normal development
- Early onset intractable seizures
- Cortical blindness
- Liver failure with micronodular cirrhosis
- Typical EEG changes with assymetry and slowing
- Sensitivity to valproate
- High CSF protein
- Increased alpha foetoprotein
- **Diagnosis**
  - → Muscle biopsy
  - → Biochemistry; RT-PCR for mtDNA depletion
  - → Mutation in mitochondrial DNA polymerase γ

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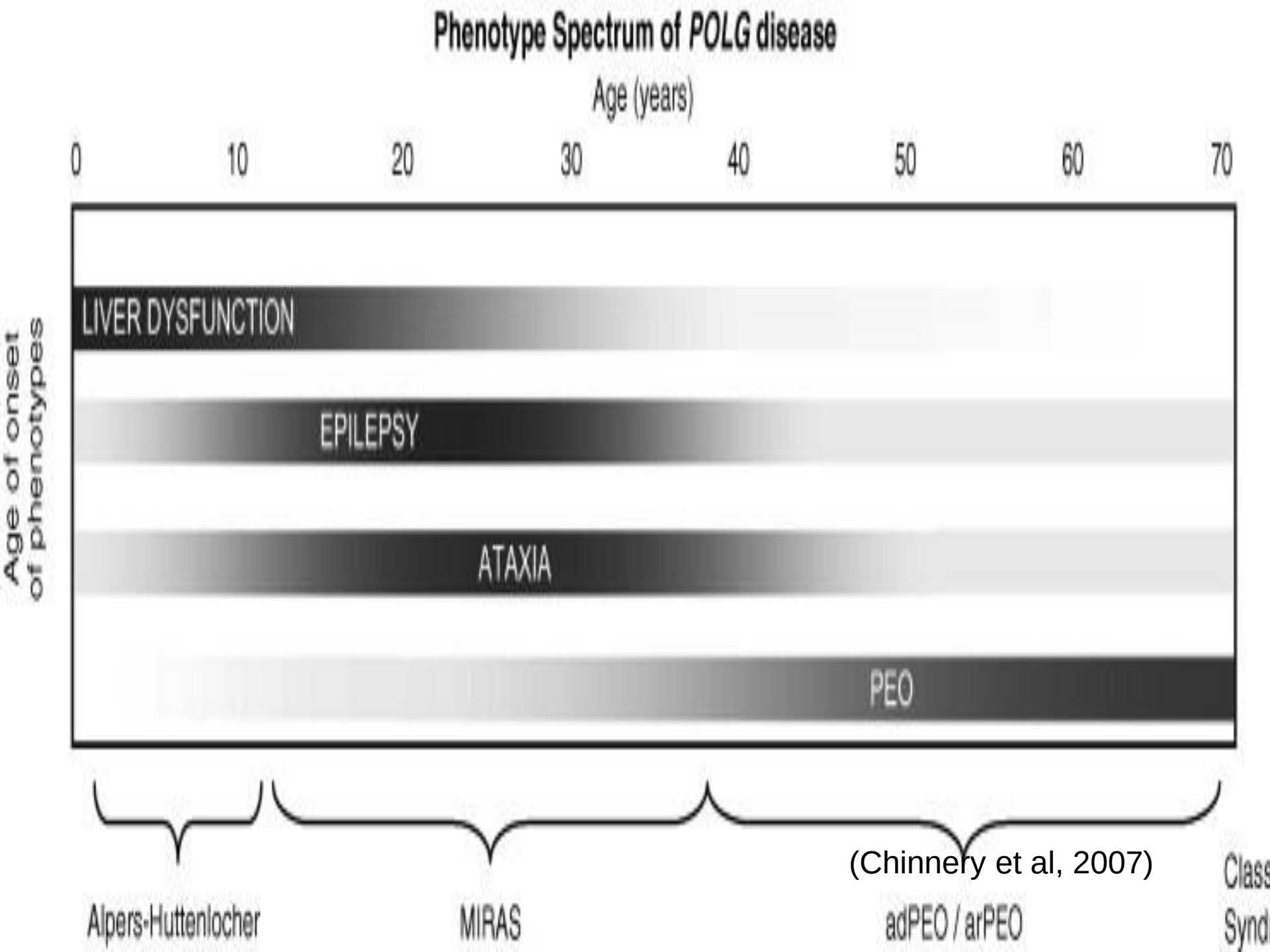


(Chinnery et al, 2007)

Clinical syndromes *POLG* mutations

- **Defined Alpers–Huttenlocher syndrome (AHS)**
- Chronic progressive external ophthalmoplegia (CPEO)
- **Infantile hypotonia/spinal muscular atrophy (SMA)**
- Mitochondrial encephalomyopathy with ragged-red fibres (MERRF)
- Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)
- Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)
- Mitochondrial recessive ataxia syndrome (MIRAS)
- Sensory ataxic neuropathy with dysarthria and ophthalmoparesis (SANDO)
- **Epileptic syndrome with initial features of occipital lobe epilepsy in adolescence**

Alpers hepatoencephalopathic poliodystrophy

(Blackwood *et al*., 1963 ; Huttenlocher *et al*., 1976)

- refractory seizures, in particular epilepsia partialis continua
- progressive neurological deterioration with poliodystrophic changes
- progressive hepatic failure

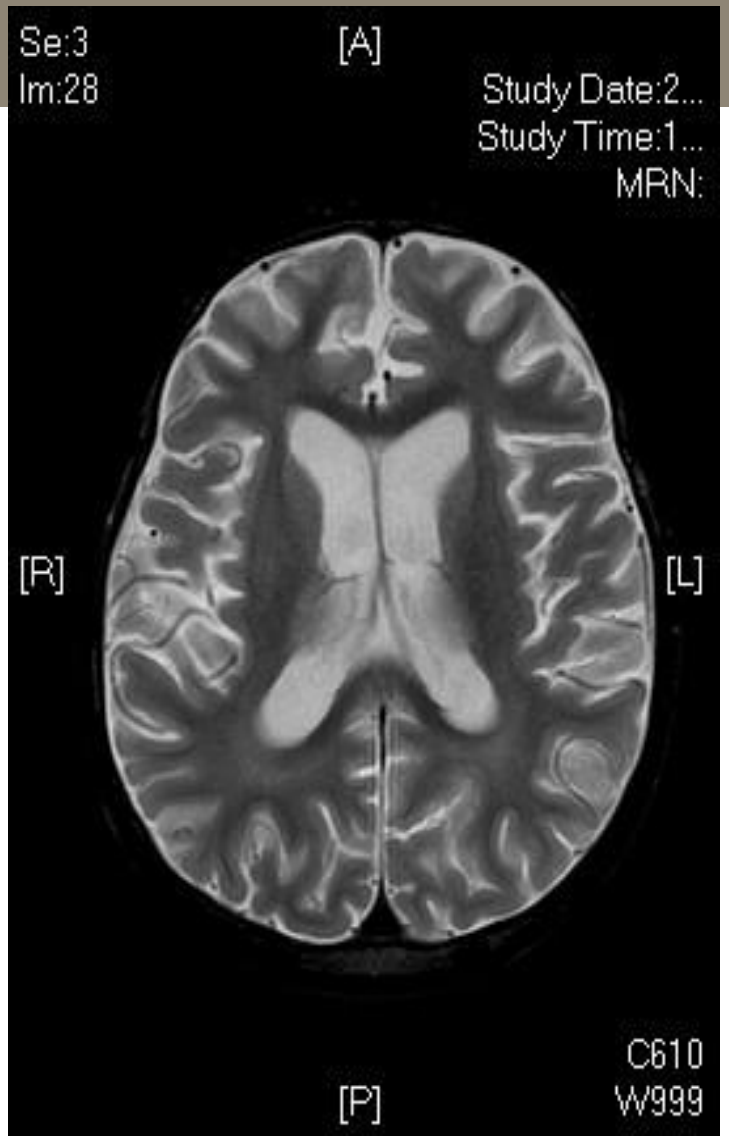
Alpers-Huttenlocher Syndrome

- onset between 2 months and 8 years
- hepatomegaly, jaundice, coagulopathy, hypoglycemia
- liver failure preceded by hypotonia, feeding difficulties, gastroesophageal reflux or intractable vomiting, failure to thrive, ataxia
- Followed onset of refractory partial motor epilepsy or multifocal myoclonus
- multiple anticonvulsants for seizure controle
- valproic acid precipitate liver failure

Alpers Huttenlocher syndrome

- neurologic features less severe or later onset
- elevated blood or cerebrospinal fluid (CSF) lactate and pyruvate
- increased CSF protein
- characteristic EEG

  → high-amplitude slow activity with polyspikes
- asymetric abnormal VER
- MRI occipital and temporal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Study Time:1...
MRN:
[R]
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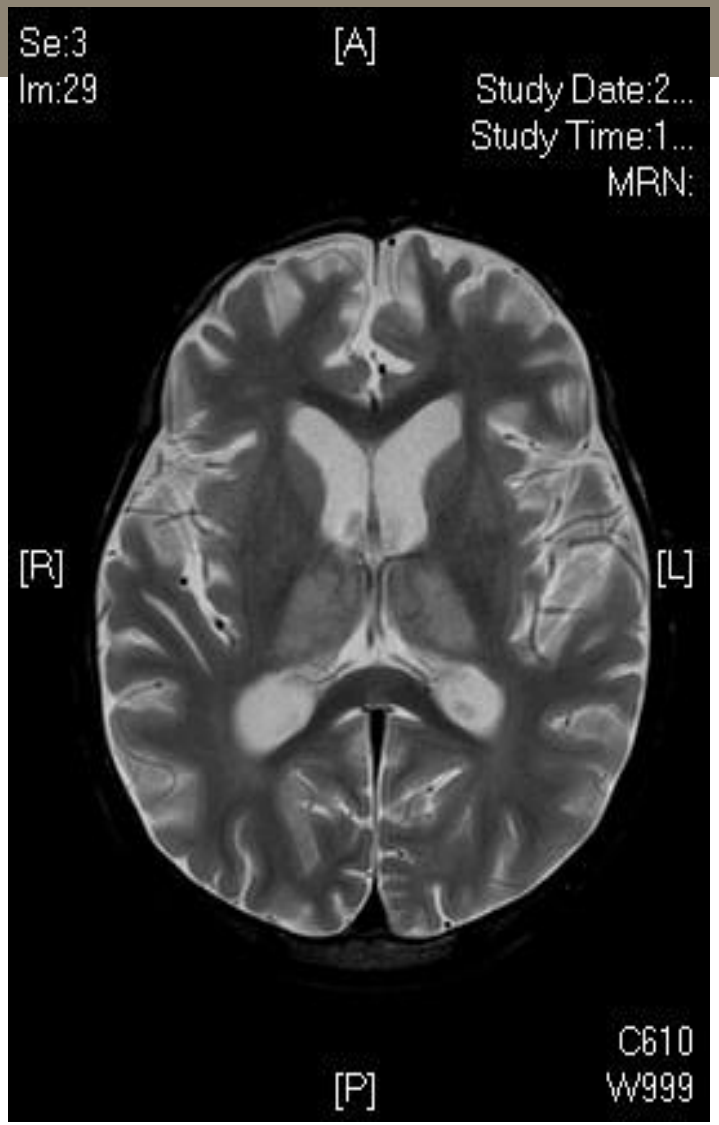
[P]
C610
W999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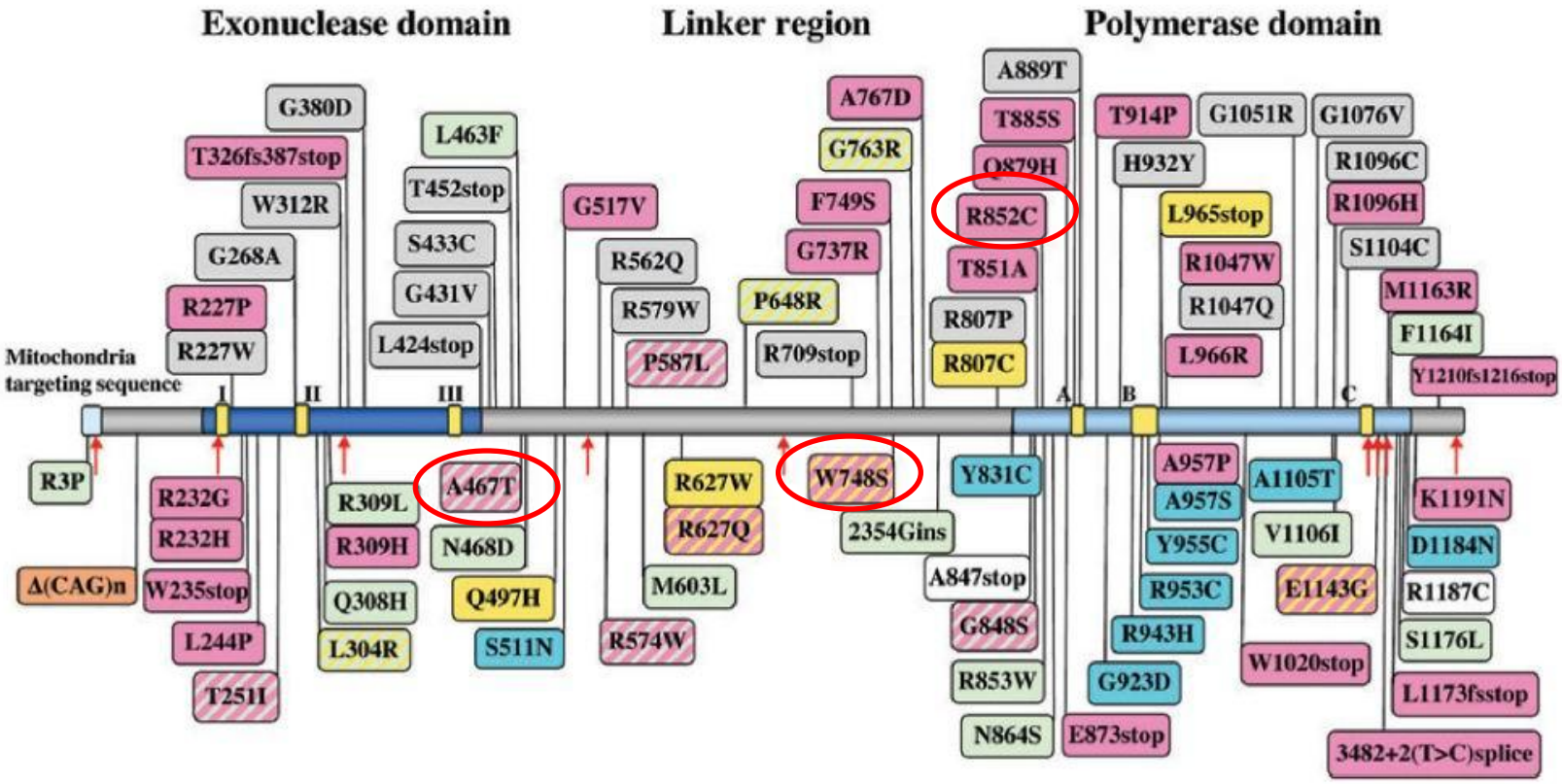
DNA polymerase gamma (POLG)



pink-= Alpers

W. Copeland, 2007)

Mutations POLG

- homozygous A467T
- homozygous W748S
- compound heterozygotes A467T/W748S

Twinkle replicate helicase

- early and late onset neuromuscular phenotypes
- early manifestation with hepatocerebral phenotype after birth
- infant neurological distress, lactic acidosis, hypoglycemia, first week of life
- hepatomegaly, axial hypotonia, peripheral hypertonia, cholestasis
- progressive neurological symptoms

Clues to Mt DNA depletion

- Autosomal recessive
- Tissue-specific reduction in mtDNA copy number
- Decreased activity of the mt-DNA encoded respiratory chain defects (I, III, IV,V)
- Decreased mt DNA on Southern blot
- Real-time quantitative PCR necessary

Clinical and Molecular Characteristics of Mitochondrial DNA Depletion Syndrome Associated with Neonatal Cholestasis and Liver Failure
Abdulrahman Al-Hussaini, et al, J Ped 2014

- 20 infants suspected hepatocerebral MDS 2007 -2013
- Genomic DNA isolated from blood leukocytes, liver, and/or skeletal muscle
- Mitochondrial DNA copy number relative to nuclear DNA levels in muscle and/ or liver DNA using real-time quantitative polymerase chain reaction and compared with age-matched controls
- Nuclear candidate genes, including polymerase γ, MPV17, and DGUOK sequenced

Abdulrahman Al-Hussaini, et al, Journal of Pediatrics 2014

- pathogenic MPV17 and DGUOK mutations in 11 infants (6 females) 2.5% of the 450 cases of infantile cholestasis and 22% of 50 cases of infantile liver failure referred
- all of 11 patients cholestasis followed by rapidly progressive liver failure and death before 2yrs
- mitochondrial DNA depletion in liver or muscle 8/11
  - → 7 MPV17
  - → 4 DGUOK

BCS1L

- assembly factor BCS1L gene (bcs1-like) encodes mitochondrial protein member of AAA-family ATPases
- chaperone for incorporating Rieske iron sulfur protein (RISP) into complex III
- homozygous 232A>G, serine to glycine (S78G) in BCS1L GRACILE syndrome ‘Finnish disease heritage’

Gracile syndrome

- growth restriction (fetal)
- aminoaciduria (due to Fanconi type tubulopathy)
- cholestasis (with steatosis and cirrhosis)
- iron overload
- lactic acidosis,
- early death

Sib 1 (1994)

- → Girl BW 2.290kg L 40 cm PC 32 cm
- → D_0 3 hours hypoglycemia (14mg/dl) after first feeding, followed by increased LA
- → Thick meconial mass was pulled out, with a transparent envelope
- → Liver disturbances with coagulopathy
- → Urine hyperaminoaciduria; lactic aciduria

Sib 2 (2001)

- → Boy 38 weeks 2kg380, 48cm, 33.5cm.
- → Severe lactic acidosis within the first days with hypoglycemia
- → Meconium of particular aspect:
  - elastic, dark green, covered with a white shell

BCS1L
assembly gene complex III

- Complex III deficiency was found in liver (R Van Coster , Gent
- Two mutations in BCS1L gene
  → R45C (exon 1)
  → R56X (stop in exon 1)
- parents were each carrier of one mutation

BCS1L (assembly complex III)

- Dysmaturity
- Early onset lactic acidosis
- Feeding difficulties and FTT
- Chronic liver failure
- Tubulopathy; Toni-Debré-Fanconi syndrome
- Encephalopathy

SCO1

- nDNA-encoded protein in COX assembly
- SCO1 gene,17p13.1, encodes protein functioning as copper chaperone
- transfers copper from Cox17p, a copper-binding protein of cytosol and mitochondrial intermembrane space to mitochondrial COX subunit II
- COX deficiency

Case SCO1

- small- for-gestational-age newborn
- hypotonia
- severe metabolic acidosis
- hypoglycemia, hyperlactatemia
- hepatomegaly
- swollen hepatocytes and steatosis
- infant died at two months of age

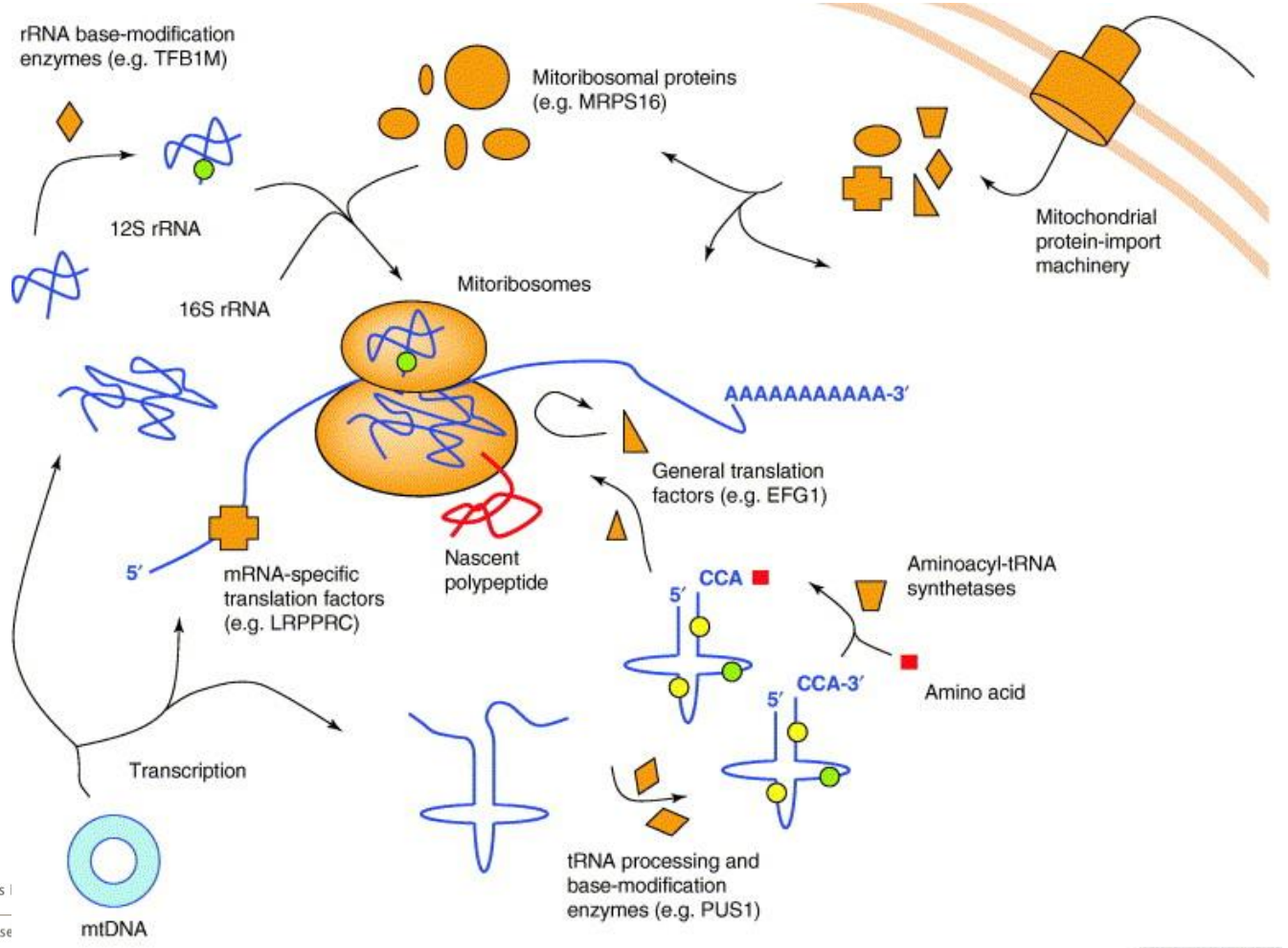
Defects in mtDNA translation

- Initiation phase start site of mRNA is selected, initiator tRNA (fMet-tRNA) is base paired to mRNA intiation factors IF2, IF3
- Elongation phase mRNA codons are read sequentially EF-Ty, EF-TS , EF-G1
- Aminoacid are incorporated by aminoacyl-tRNA synthetases
- Complete polypeptide is released and ribosome complex is available in recycling phase eRF1, ICT1

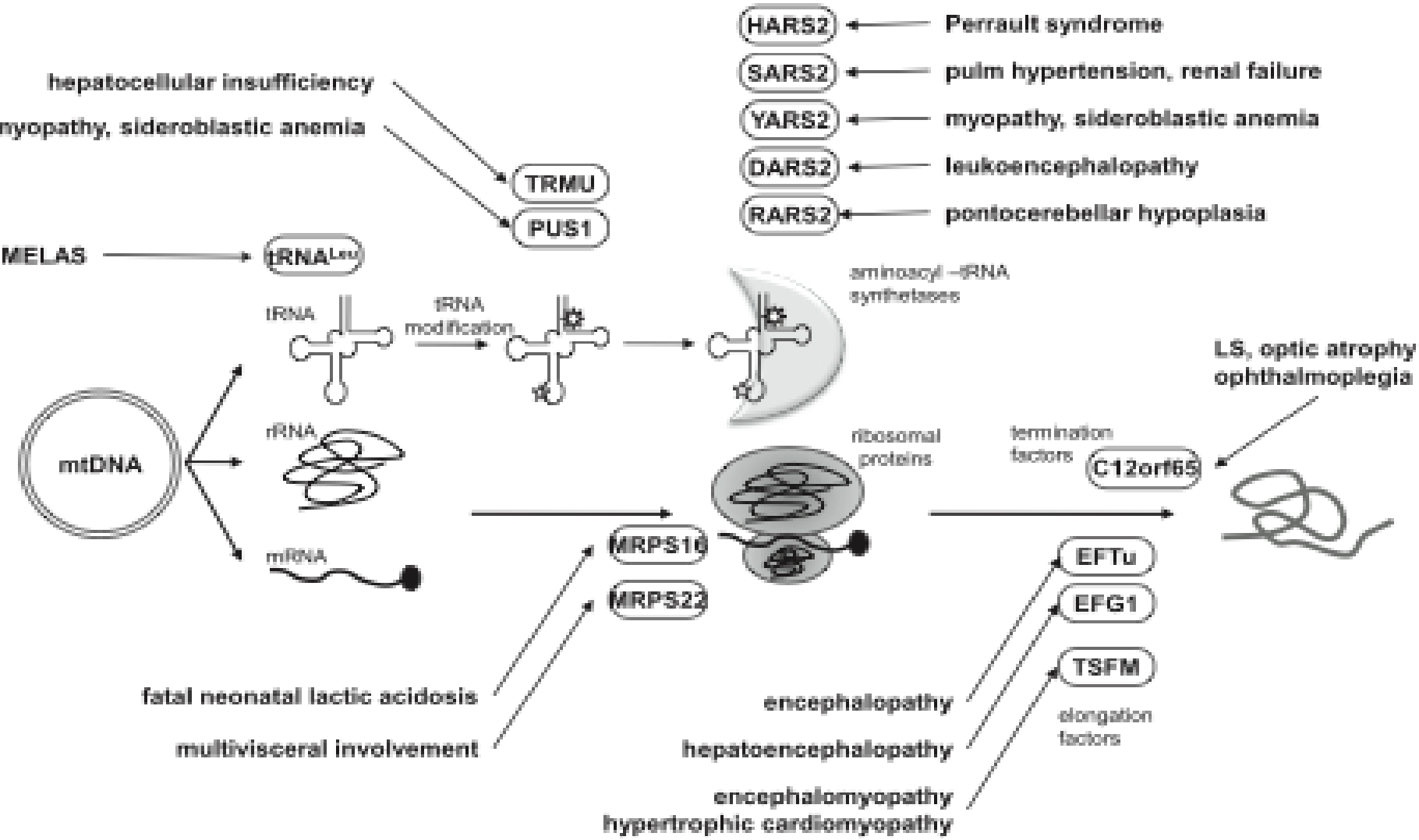
mtRNA translation

- Further several translational acitivators TACO1, LRP130
- Specific base modifiers TRMU and PUS1
- Mutations in mRNA polyadenylation f-vectors, ribosomal proteins, ribosomal assembly factors, amino-acyl-tRNA modifiers

Mitochondrial RNA translation



Rotig Biochim Biophys Acta1807 (2011) 1198-1205



2. The schematic steps of mitochondrial protein synthesis. The gene mutations as well as the associated clinical phenotypes are shown. (LS: Leigh syndrome).

Elongation

- elongation factor EFG1 (gene GFM1)
  - → Newborn fetal growth restriction, lactic acidosis, liver dysfunction within 10 days into liver failure and death two weeks later
  - → Decreased activities in complexes I, III, and IV
  - → Sibling same mutation less severe disorder neurological symptoms and no liver dysfunction
- elongation factor EFTu
  - → Severe lactic acidosis lethal encephalopathy
  - → Mild hepatic involvement

Nuclear translation factor genes

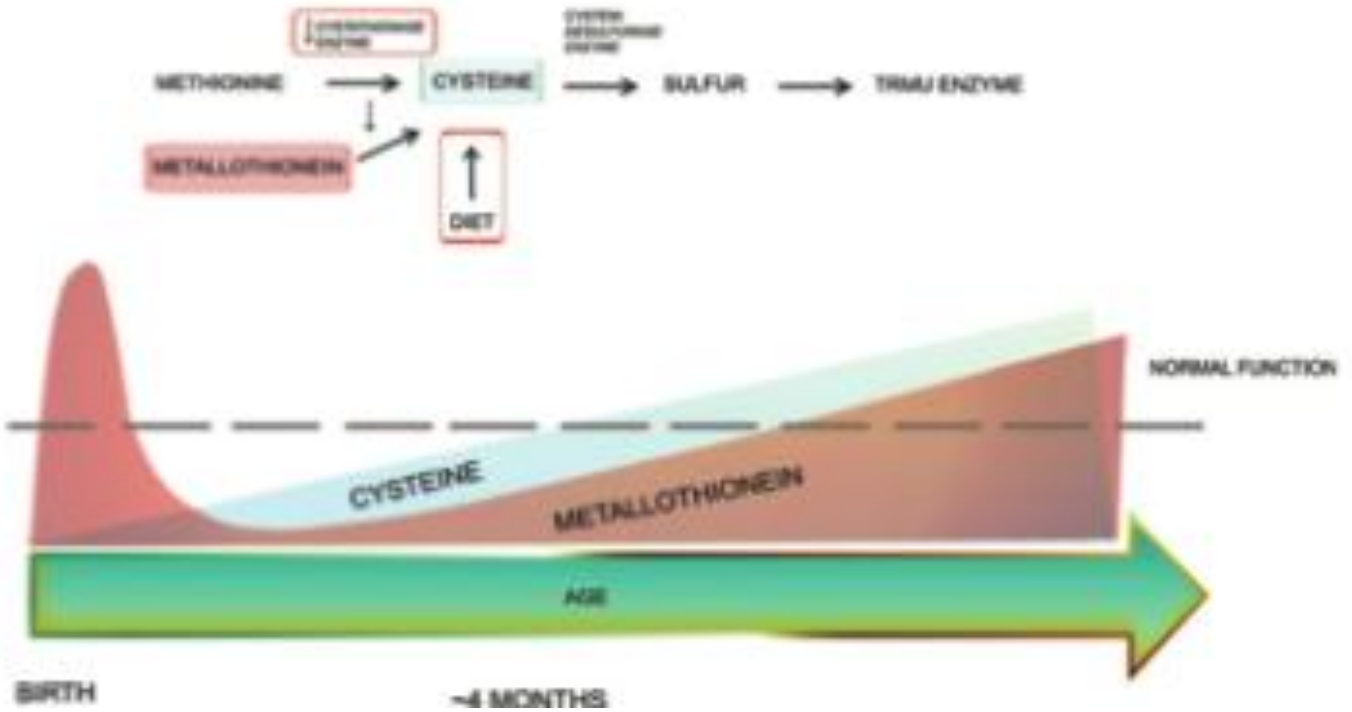
- TRMU role in modifcation of mitochondrial tRNA
- important for mitochondrial translation
- acute liver failure 1 day to 6 months
- onset later in infancy liver failure could be successfully treated, no relapse occurred later
- window of time (0- 4 months of age) with increased risk for liver failure withTRMU mutations
- postnatal decrease in concentration of cysteine, needed for TRMU protein activity
- respiratory chain deficit similar to MDS
- TRMU gene should be screened in newborns presenting with acute liver failure

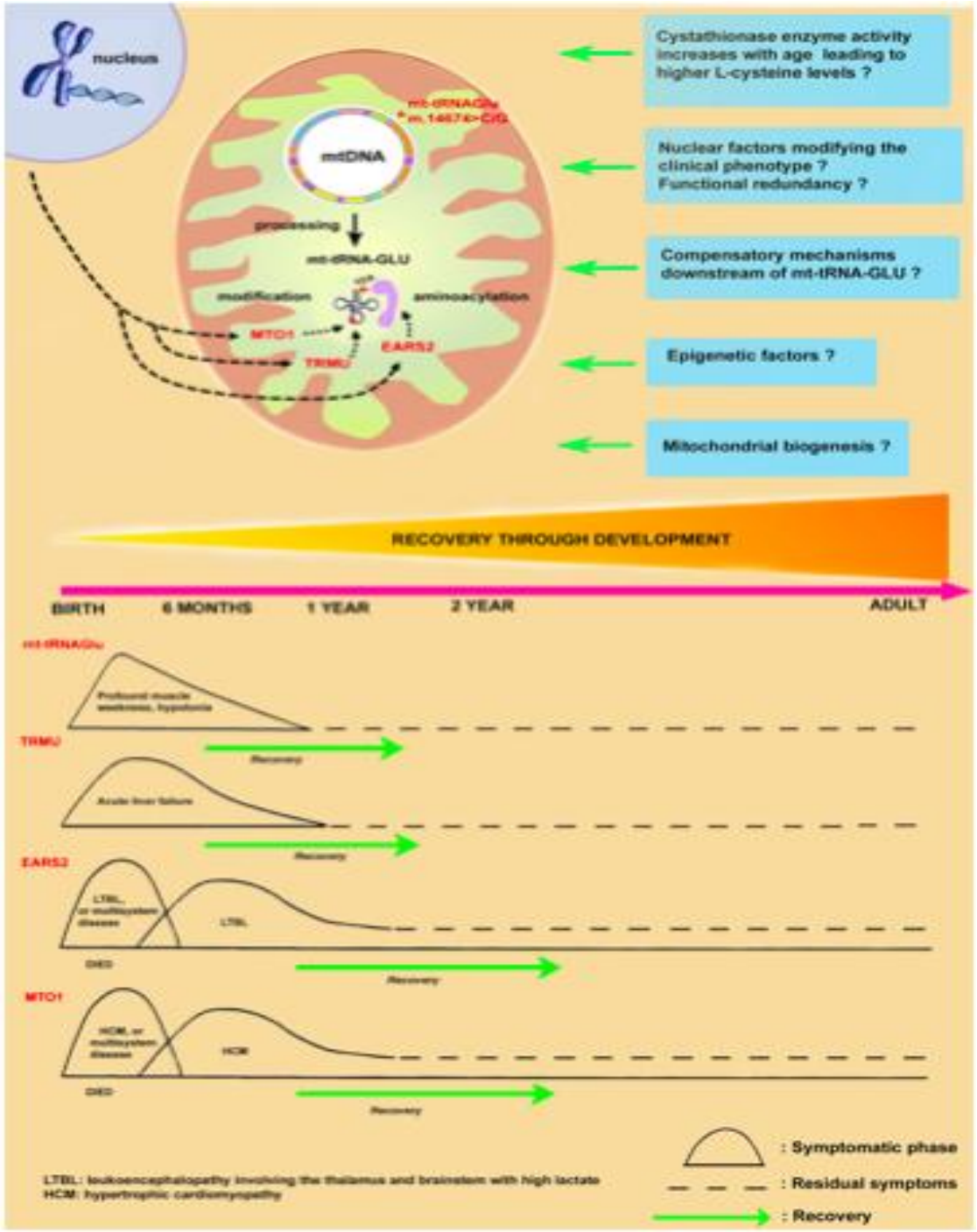
Reversible infantile mitochondrial diseases Veronila Boczonadi et al , 2014

- autosomal recessive mutations in TRMU gene cause severe infantile liver failure
- majority of patients complete spontaneous recovery if they survive first year of life
- several modification steps are required for efficient function of human mt-tRNAs, can affect some mt-tRNAs differently
  - → only mt-tRNAGlu, mt- tRNAGln and mt-tRNALys need to undergo 2-thiouridylation by tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU)
  - → this enzyme requires cysteine

Cysteine

- essential amino acid in first months of life, physiologically low activity of cystathionine gamma-lyase (cystathionase) in infants
- age-dependent, partially reversible clinical presentation and impairment of mt-tRNAGlu
- abnormal thiouridylation due to low dietary cysteine intake ?


METHIONINE
CYSTEINE
SULFUR
METALLOTHIONEIN
DIET
NORMAL FUNCTION
CYSTEINE
METALLOTHIONEIN
AGE
BIRTH
~4 MONTHS


nucleus
mt-tRNAGlu
m.14674>C/G
mtDNA
processing
mt-tRNA-GLU
modification
aminoacylation
MTO1
TRMU
EARS2
Cystathionase enzyme activity increases with age leading to higher L-cysteine levels ?
Nuclear factors modifying the clinical phenotype ?
Functional redundancy ?
Compensatory mechanisms downstream of mt-tRNA-GLU ?
Epigenetic factors ?
Mitochondrial biogenesis ?
RECOVERY THROUGH DEVELOPMENT
BIRTH
6 MONTHS
1 YEAR
2 YEAR
ADULT
mt-tRNAGlu
Profound muscle weakness, hypotonia
TRMU
Recovery
Acute liver failure
Recovery
EARS2
LTBL or multisystem disease
LTBL
DIED
Recovery
MTO1
HCM, or multisystem disease
HCM
DIED
Recovery
: Symptomatic phase
: Residual symptoms
: Recovery
LTBL: leukoencephalopathy involving the thalamus and brainstem with high lactate
HCM: hypertrophic cardiomyopathy

Cysteine

- supplementation of L-cysteine essential for normal TRMU activity, significantly improved most RC complex activities in TRMU deficient cells
- low dietary L-cysteine levels in infants could explain clinical manifestations
- increase in L-cysteine due to cystathionase activation through development would contribute to the recovery in these patients (Boczonadi et al 2013)

When to suspect?

- association of neuromuscular symptoms with liver dysfunction
- multisystem involvement in a patient with acute or chronic liver disease
- rapidly progressive course of liver disease,
- presence of lactic acidosis, hepatic steatosis, or ketonemia

Human Molecular Genetics, 2012, Vol. 21, No. 20 **4521–4529**
doi:10.1093/hmg/dds294
Advance Access published on July 23, 2012

Mitochondrial phenylalanyl-tRNA synthetase mutations underlie fatal infantile Alpers encephalopathy

Jenni M. Elo[1,†], Srujana S. Yadavalli[5,†], Liliya Euro[1], Pirjo Isohanni[1,6], Alexandra Götz[1], Christopher J. Carroll[1], Leena Valanne[7], Fowzan S. Alkuraya[8,10,11], Johanna Uusimaa[12], Anders Paetau[2] Eric M. Caruso[5], Helena Pihko[6], Michael Ibba[5], Henna Tyynismaa[1,3,*,†] and Anu Suomalainen[1,4,8,†]

Mutation in mitochondrial ribosomal protein S7 (MRPS7) causes congenital sensorineural deafness, progressive hepatic and renal failure and lactic acidemia.

Menezes MJ1 et al Hum Mol Genet. 2015 Apr 15;24(8):2297-307

Official Journal of the American Association for the Study of Liver Diseases

Valproic Acid-Induced Hepatotoxicity in Alpers Syndrome Is Associated With Mitochondrial Permeability Transition Pore Opening Dependent Apoptotic Sensitivity in an Induced Pluripotent Stem Cell Model

Shenghiao Li,[1,2] Jingyi Guo,[1,2*] Zhongfu Ying,[1*] Shen Chen,[1*] Liang Yang,[1] Keshi Chen,[1] Qi Long,[1] Dajiang Qin,[1] Duanqing Pei,[1] and Xingguo Liu[1]

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- AHS patient-derived hepatocytes are more sensitive to VPA-induced apoptosis through a POLG-involved, mitochondrial pathway
- findings suggest prospective testing of genetic markers or early signals for mitochondrial- dependent apoptosis pathways will identify individuals at high risk of potentially fatal VPA-induced liver toxicity

Valproic acid triggers increased mitochondrial biogenesis in POLG-deficient fibroblasts

Kamil S. Sitarz [a], Hannah R. Elliott [a,b], Betül S. Karaman [a,c], Caroline Relton [a,b], Patrick F. Chinnery [a], Rita Horvath [a,*]

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Valproate toxicity

- 1/3 patients with Alpers –Huttenlocher develop liver failure within 3 months of exposure
- different hypotheses
- direct inhibition of N-acetylglutamate by forming valproyl-CoA
- lysosomal membrane leakness with reactive oxygenspecies formation and decline on mitochondrial membrane potential
- increase of mitochondrial biogenesis

Valproic acid triggers increased mitochondrial biogenesis in POLG-deficient fibroblasts

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Conclusion

- more genes to come
- think mitochondrial
- think benign form
- investigate the right tissue
- gene panels and whole exome