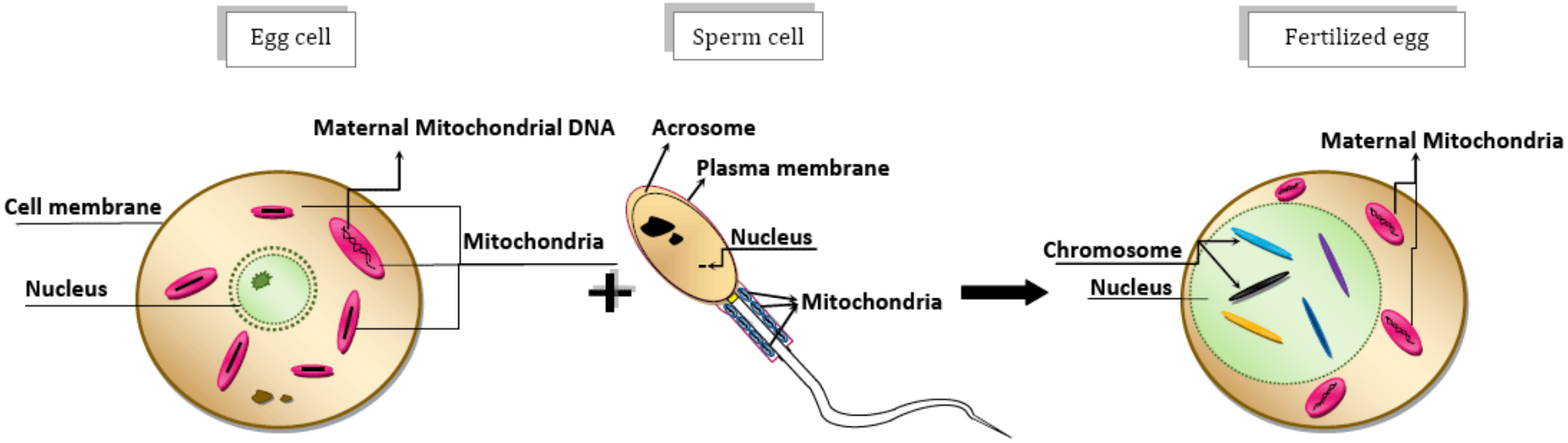
Lecture 4.
Mitochondrial genome and human diseases


Egg cell
Sperm cell
Fertilized egg
Maternal Mitochondrial DNA
Cell membrane
Mitochondria
Nucleus
Acrosome
Plasma membrane
Nucleus
Mitochondria
Maternal Mitochondria
Chromosome
Nucleus

Mitochondria in the cell, mitochondrial structure

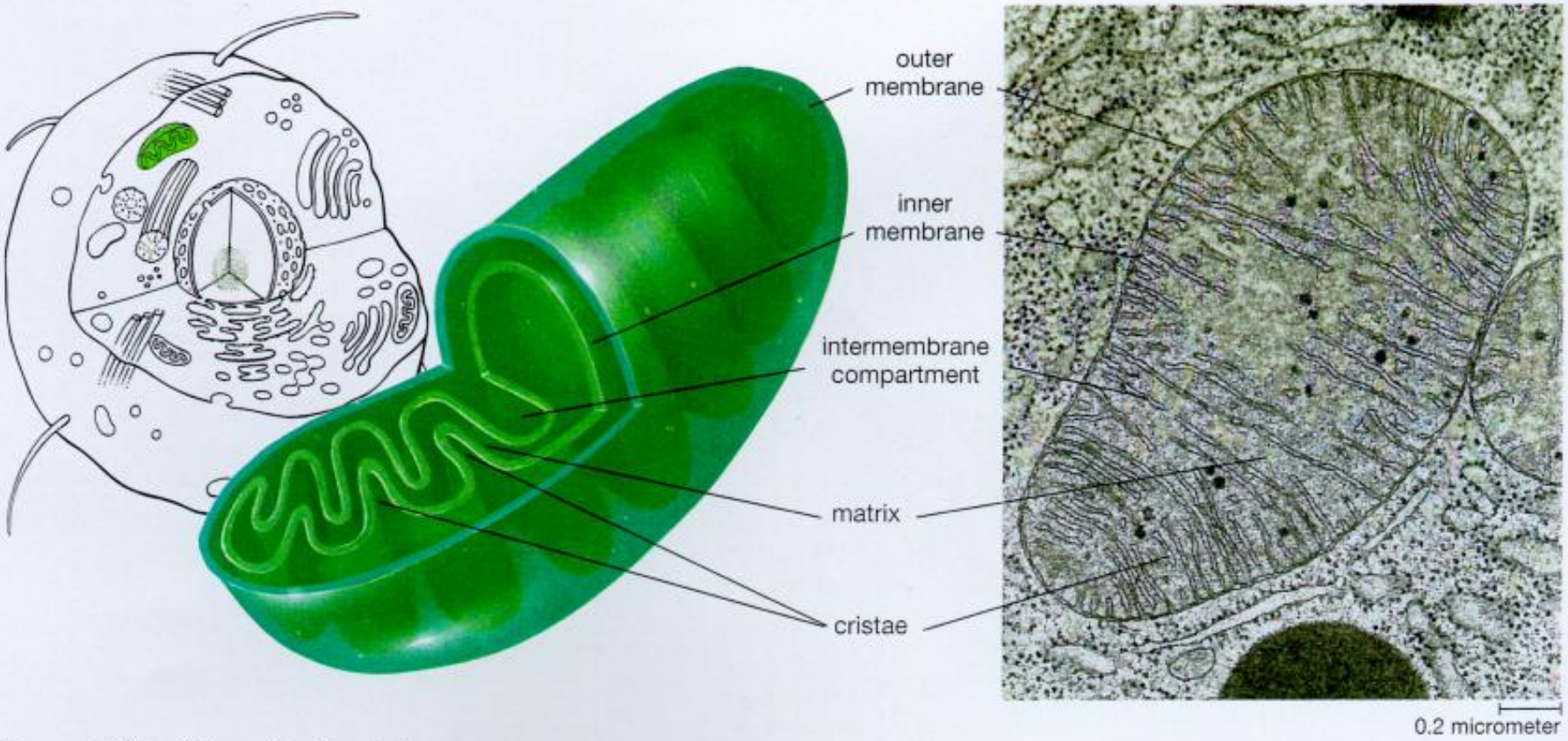


Figure 5-17 The mitochondrion

Mitochondria consist of a pair of membranes enclosing two fluid compartments, the intermembrane compartment between the outer and inner membranes and the matrix within the inner membrane. The outer membrane is smooth, but the inner membrane loops back and forth to form deep folds called cristae. Mitochondria are the site of aerobic metabolism.

Functions of mitochondria

- Synthesis of ATP - the "energy center" of the cell (95% is synthesized in mitochondria)
- Participation in the metabolism of amino acids, lipids, cholesterol, steroids, nucleotides
- Initiation of apoptosis processes (programmed cell death)
- Regulation of nuclear genome expression
- Participation in own reproduction

The human mitochondrial genome is 37 genes, 16. 569 nucleotide pairs

- 2 genes of ribosomal RNA
- 22 transport RNA gene
- 13 protein-coding genes

The DNA molecule is closed in the ring

Genes are located very tightly, on both DNA strings, sometimes overlap

reproduction of mitochondria is carried out according to the principle:
omnis mitochondrion e mitochondrion

The first mitochondrial diseases were described earlier than the discovered DNA in the mitochondria

1958 — Cairns-Seir Syndrome

1962 — Luft's disease: non-tyroidal hypermetabolism

(only 2 cases in 40 years)

1963 — DNA is discovered in the mitochondria
1981 – decoded human mitochondrial genome (Anderson et al)

1988 – the first pathogenic mutations of mtDNA were identified (Holt et al., Wallace et al).

Features of mitochondrial heredity

- Maternal inheritance
- The multicopy of genomes (hundreds of organelles, thousands of DNA molecules)
- Heteroplasmia
- Mitotic segregation
- Threshold effect

Inheritance is not according to Mendel

Fabrics with a low threshold of mutant DNA :

brain

a heart

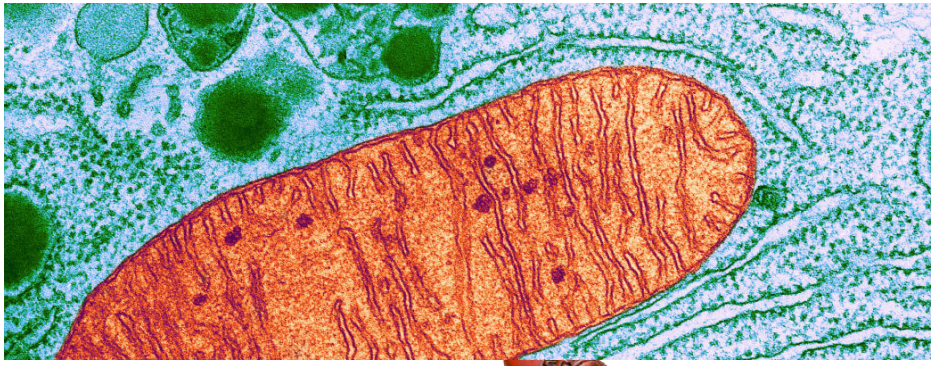
skeletal musculature

retina

renal tubules

endocrine glands

The cells of these tissues are the most metabolically active, energetically dependent



In the nucleus ~ 70,000 genes in two copies each

In the mitochondria 37 genes in thousands of copies each

Genes make up less than 1% of all nuclear DNA

Genes make up more than 92% of all mitochondrial DNA

Mutations in mitochondrial DNA people occur five times more often, than in nuclear,

because the

Mitochondria absorb more than 90% of cellular oxygen;

A large number of DNA-damaging free radicals is formed.

Wherein

- ✓ the mitochondrial genome is not protected by histone proteins;
- ✓ reparation processes in mitochondria less perfect than in the core

Described:

- ✓ **more than 190 pathogenic point mutations of mitochondrial DNA**
- ✓ **about 200 deletions, insertions and other structural reorganizations of mtDNA**

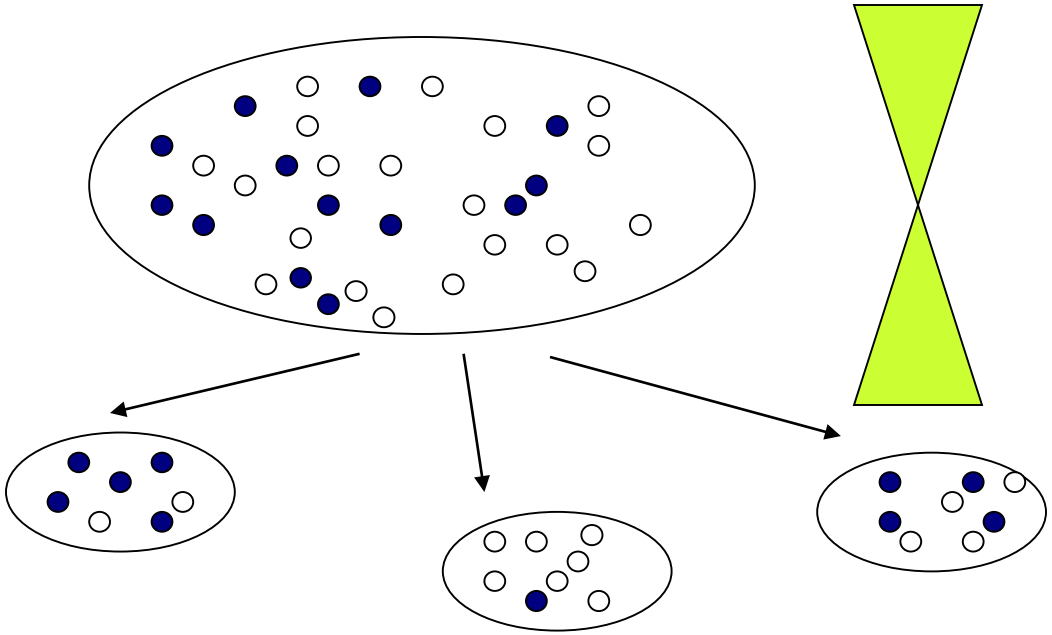
Sources of mitochondrial pathologies :

- **Changes in the genes of nuclear coding**
- **(more than 1000 genes encode mt proteins)**
- **Changes in the genes of mitochondrial coding (37 genes)**
- **The deposition of mtDNA plots (deletions, multiple deletions)**
- **Depletion of the mitochondrial DNA pool**

Features of mitochondrial gene mutations

One of the most important features is the clinical diversity of siblings.

This is a reflection of the "bottle neck effect". How many mutant molecules will enter the oocyte at an early stage of oogenesis is the case:



Features of mitochondrial gene mutations

Mutations can affect:

specific proteins - with point mutations and small deletions of structural genes,

as well as the mitochondrial genome as a whole:

- large deletions;
- mutations in the tRNA genes;
- mutations in rRNA genes.

As a result of mutations observed:

- Reduction of ATP synthesis
- Violation of the calcium balance of the cell
- Increasing the amount of ROS (reactive oxygen species)

Some mutations of nuclear DNA can lead to mutations of mitochondrial DNA:

gamma DNA polymerase gene
(carries out the synthesis of mtDNA);
thymidine phosphorylase gene
(disrupts thymidine metabolism);
the Twinkle gene (participates in maintaining integrity
mitochondrial genome).

Features of mutation of mitochondrial genes

Strict communication between the mutation site and the clinical phenotype is often absent:

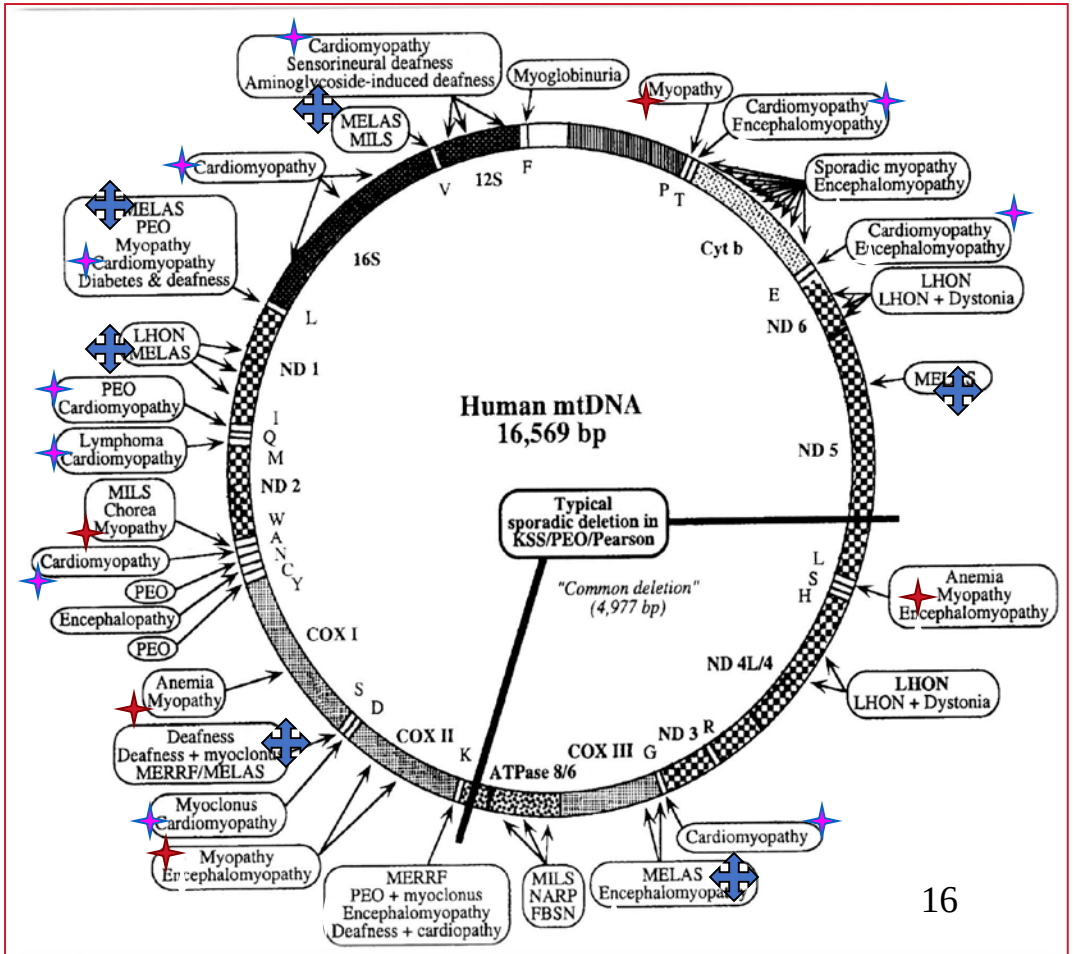
The same mutation can cause different symptoms.

One and the same clinical phenotype can form different mutations

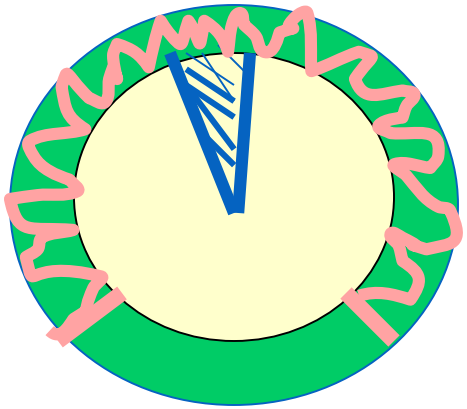
Exceptions:

MERRF syndrome - mutations are always in the tRNAlys gene;

LHON - mutations in the ND genes



2/3 known point mutations of mtDNA are concentrated in the tRNA genes (9% of the genome)



Most of all mutant points revealed in leucine tRNA

According to the etiological principle, 3 groups of hereditary diseases are distinguished.

Mitochondrial diseases due to gene mutations of nuclear DNA:

defects in transport substrates;

defects in recycling substrates;

defects of the enzymes of the Krebs cycle;

disturbance of oxidative phosphorylation;

disturbances in the respiratory chain;

defects in the import of proteins.

Mitochondrial diseases, which are based on mutations of mitochondrial DNA:

sporadic mutations;

point mutations of structural genes;

point mutations of synthetic genes.

Mitochondrial diseases associated with violation of intergenomic signal effects:

multiple deletions of mitochondrial DNA, but inherited by autosomal dominant type;

deletion (decrease in the number) of mitochondrial DNA, inherited by autosomal recessive type.

By pathogenesis, there are 3 main groups of mitochondrial diseases:

- ✓ Diseases of oxidative phosphorylation processes.
- ✓ Diseases of beta-oxidation of fatty acids.
- ✓ Defects in the metabolism of pyruvate and the Krebs cycle.

From the point of view of the leading biochemical defect mitochondrial diseases are divided into the following groups.

Transport substrate defects.

Deficiency of monocarboxylic translocase.

Disturbance of carnitine-acylcarnitine transport (primary carnitine insufficiency, carnitine system insufficiency, mixed forms of carnitine deficiency, secondary carnitine insufficiency, deficiency of carnithalmitoyltransferase 1 and 2, combined carnitine and carnitine-palmitoyltransferase deficiency).

Defects in the utilization of substrates.

Defects of oxidation of pyruvate:

- ✓ insufficiency of pyruvate decarboxylase;
- ✓ insufficiency of dihydrolipoyltransetylase;
- ✓ insufficiency of dihydrolipoyldehydrogenase;
- ✓ insufficiency of pyruvate dehydrogenase;
- ✓ insufficiency of pyruvate carboxylase;
- ✓ deficiency of carnitine-acetyltransferase.

From the point of view of the leading biochemical defect mitochondrial diseases are divided into the following groups.
Deficiency of metabolism of free fatty acids:
defects of beta-oxidation of fatty acids.
Defects in the respiratory chain.
Defects of NADH: KoQ-reductase complex (with normal carnitine and carnitine insufficiency).
Defects of KoQ cytochrome b, cl-reductase complex (KoQ-10 insufficiency, insufficiency of Fe-S proteins, cytochrome b deficiency, combined deficiency of cytochromes b and cl).
Insufficiency of cytochrome a, a3.
Insufficiency of cytochrome a, a3 and b.
Defects in the accumulation and transmission of energy.
Disturbances of oxidative phosphorylation with hypermetabolism (Luft's disease).
Disorders of oxidative phosphorylation without hypermetabolism.
Lack of mitochondrial ATPase.
Insufficient adenine nucleotide translocase.

Homoplasmy and heteroplasmy of the mitochondrial chromosome

The second unique characteristic of the genetics of mitDNA arises from the fact that most cells contain many copies of mitDNA molecules. When a mutation occurs in mitDNA, it is first present only in one of the molecules in the mitochondria. In the course of replicative segregation, the mitochondria, containing the mutant mitDNA, produces numerous copies of the mutant molecule.

In cell division, a cell containing a mixture of normal and mutant mitochondrial DNA can transmit very different proportions of mutant and wild mitochondrial DNA to daughter cells. One daughter cell can randomly receive mitochondria containing a pure population of normal or pure population of mutant mitochondrial DNA (a situation known as homoplasmy). In addition, the daughter cell can receive a mixture of mitochondria with and without mutation (heteroplasmy).

Since the phenotypic expression of the mutation in mitDNA depends on the relative proportions of normal and mutant mitDNA in cells that form different tissues, incomplete penetrance, variable expressiveness and pleiotropy are typical characteristics of mitochondrial diseases.

Leber's Syndrome: LHON (1871 г.)

- the inherited maternal vision loss occurs in people 20-30 years due to

- atrophy of the optic nerve and

- degeneration of the ganglionic layer of retina cells

The disease is associated with a mitochondrial DNA mutation transmitted from the mother in one of the ND genes (complex I).

In 70% of cases this is G11778A (ND4), and in Japan it is 90%

in 13% of cases, G3460A (ND1);

in 14% of cases, T14484C (ND6)

The mutation is in the homoplasmic state

Mysteries of Leber's Syndrome :

?? In 80-85% of cases, men are affected

(Does the X chromosome carry any locus of sensitivity?)

?? Only 50% of men and 10% of women carriers of pathogenic mutations of complex I actually have vision loss

?? Most often mutations leading to Leber's syndrome are found in the mtDNA of haplogroup J; this group is borne by about 15% of Europeans

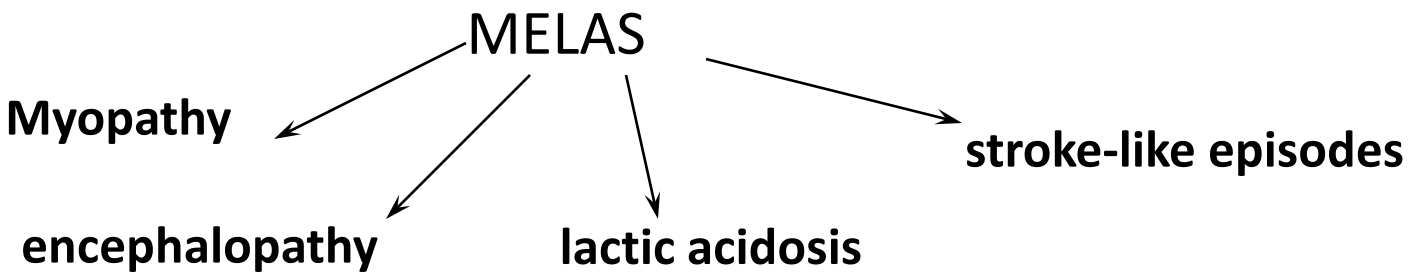
In the formation of the disease involved some additional factors (???)

Mutations of transport RNA genes

The most common point mutation:

A3243G in leucine tRNA

It was found in the majority of patients with the syndrome



The mutation occurs exclusively in the heteroplasmic state

??? **In some families A3243G causes predominantly cardiomyopathy, in others - diabetes and deafness, in the third PEO, in the fourth - encephalopathy**

Syndrome MELAS

***M**itochondrial **E**ncephalomyopathy, **L**actic **A**cidosis, and **S**troke-like episodes*

Mom: a phenotypically healthy woman of very small size

I marriage

II marriage

1st child
1988-2000
Cardiopathy, ZPR, ZFR.
Died suddenly after an injury

2nd child
1991-2007
Meningoencephalitis
Died of ischemic infarction of both hemispheres of the cerebellum

3rd child was born in 1998 Progressing myopathy, myocardial dystrophy

A mutation of MELAS in a son (80% of the mutant molecules in the blood) in the mother (40%)

Mutations of transport RNA genes (continued)

Mutation of A8344G in the gene of lysine tRNA at the level of mutant molecules> 85% leads to the syndrome MERRF:

Myoclonus-epilepsy;

"Torn" red muscle fibers;

mental retardation;

ataxia; muscle atrophy, etc.

Mutation dramatically reduces the efficiency of translation in mt and thus provokes a deficit in the respiratory chain

Mothers of patients are usually phenotypically healthy or have mild symptoms

Mutations of ribosomal RNA genes

The most common mutation is the gene 12S rRNA A1555G

↓

Causes non-syndromic hearing loss due to sensitivity of the mutation carriers to ototoxic aminoglycosides

Other mutations of the 12S and 16S genes cause cardiomyopathy, ataxia, MELAS, diabetes mellitus, sensory-neural loss of hearing

NARP (neuropathy ataxia and retinitis pigmentosa)

Mutation in the ATPase6 gene - transversion of T-G

in nucleotide 8993 (70-90% of the mutant DNA)

T8993G: leucine is substituted for arginine in ATPase6,

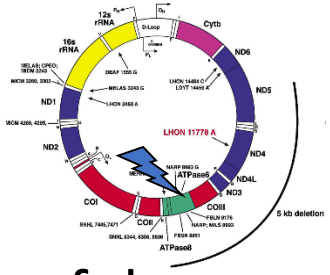
which leads to a violation of the synthesis of ATP

If the fraction of mtDNA is greater than 90%

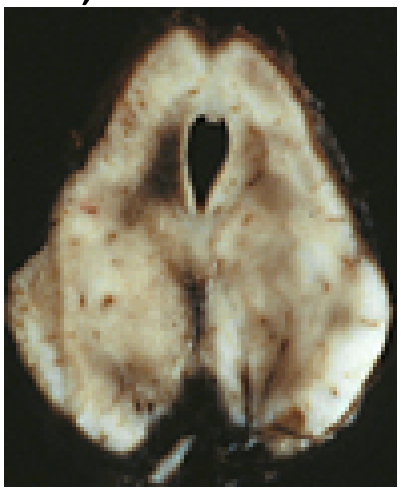
clinical manifestation is observed earlier and

symptoms are more severe: subacute necrotizing encephalopathy with features of the Leia syndrome (LS)

Lei's syndrome is a severe neurodegenerative disease :



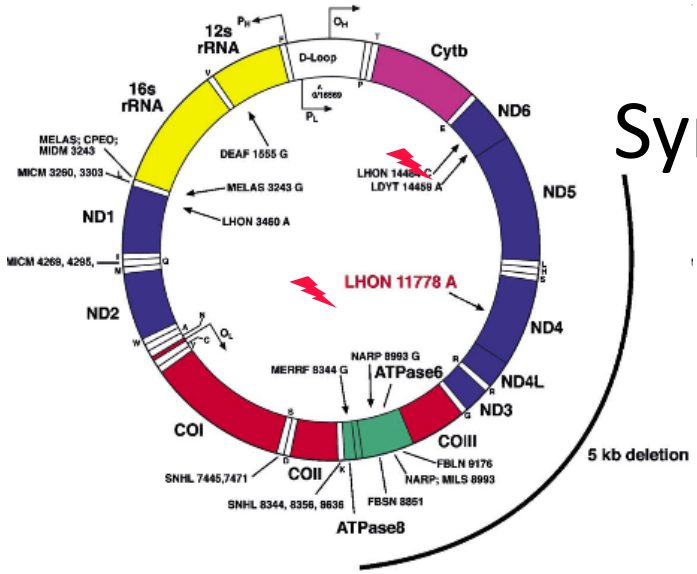
- symmetric necrotic lesions in the subcortical areas of the central nervous system - basal ganglia, thalamus, brainstem, spinal cord;
- demyelination, vascular proliferation and "gliosis";
- motor and mental regression, ataxia, dystonia, abnormal respiration

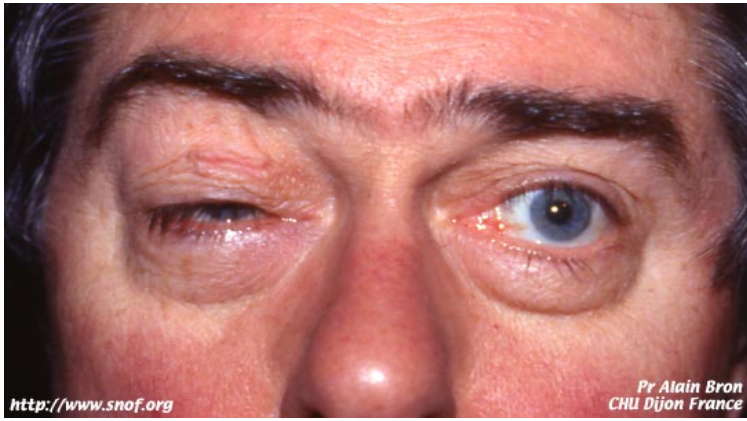


The disease begins in early childhood, rarely in the adult state;

Death usually occurs two years after the onset of the disease

Cairns-Seir Syndrome (KSS)



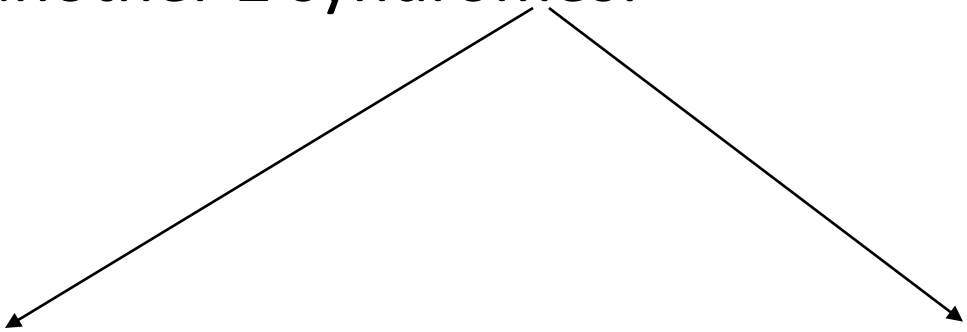


the cause is a major deletion of 5 kb.
5 genes of tRNA and 5 protein genes are lost

KSS – fatal multisystem pathology, manifests itself at the age of 4-18 years: CPEO, retinitis pigmentosa, ataxia, deafness, endocrine dysfunction, atrioventricular heart block, increased protein level in cerebrospinal fluid above 100 mg / dL, "ripped" fibers in skeletal muscles

Deletion is not inherited

The same 5 kd deletion. causes another 2 syndromes:



Syndrome PEO -

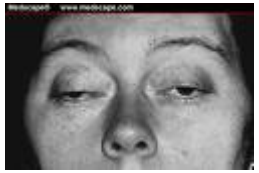
Progressive external ophthalmoplegia

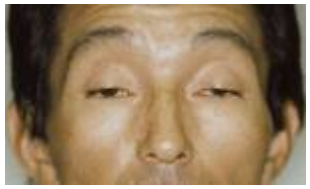
Pearson's Syndrome - PS

Hypoplastic anemia, impaired exocrine pancreatic function

All three syndromes are sporadic, formed depending on the segregation of mutant mtDNA with accumulation in different tissues

In the case of the same deletion of 5 thousand bp.
Instead of a fatal KSS, PEO can be observed





Pathology is associated with paralysis of external oculomotor muscles

Progressive external ophthalmoplegia, ptosis

The percentage of mutant molecules in this case is less than in the case of KSS syndrome, the syndrome is not associated with a threat to the life of the patient

Biochemically, the muscles detect respiratory chain enzyme defects, especially cytochrome oxidase

Mitochondrial deletion syndrome - MDS

In cells, 1 to 30% of the normal amount of mtDNA remains

The syndrome manifests itself in the first weeks after birth:
fatal hepatopathy;
myopathy with generalized hypotension;
Cardiomyopathy with convulsions (syndrome de Toni-Debre-Fanconi);
atrophy of proximal muscle groups;
loss of tendon reflexes.

Death occurs in severe cases in the first year of life

Pathologies caused by changes in the genes of the respiratory chain

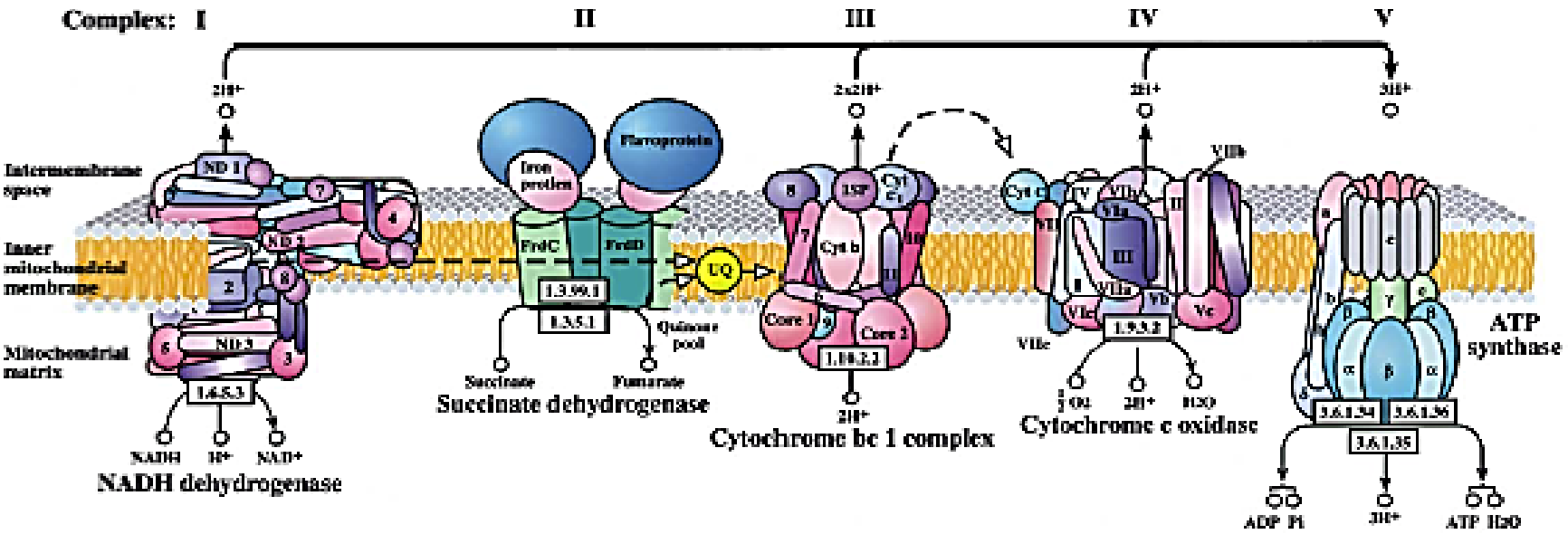
M

LHON
LHON+ dystonia
Sporadic myopathy

Sporadic myopathy

Sporadic myopathy
Encephalomyopathy

NARP
MILS
FBSN



Я

Subunits: >41/7 | 4/0 | 10/1 | 10/3 | 14/2

Leia's syndrome
Leukodystrophy

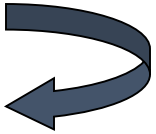
Leia's syndrome
Paraganglioma

Leia's syndrome
Cardioencephalopathy
Leukodystrophy / tubulopathy

How to diagnose a mitochondrial anomaly?

With clear symptoms - isolate blood from the vein and make a PCR analysis for point mutations or deletions

If the result of the blood test is negative, this does not mean the absence of the disease (heteroplasmy!)



You need to take a biopsy: a muscle or skin test

in adults

in children

For non-invasive testing use sediment of urine, scraping of the inner surface of the cheek, less often hair follicles

How to diagnose a mitochondrial anomaly?

The fresh muscle is analyzed histologically and histochemically

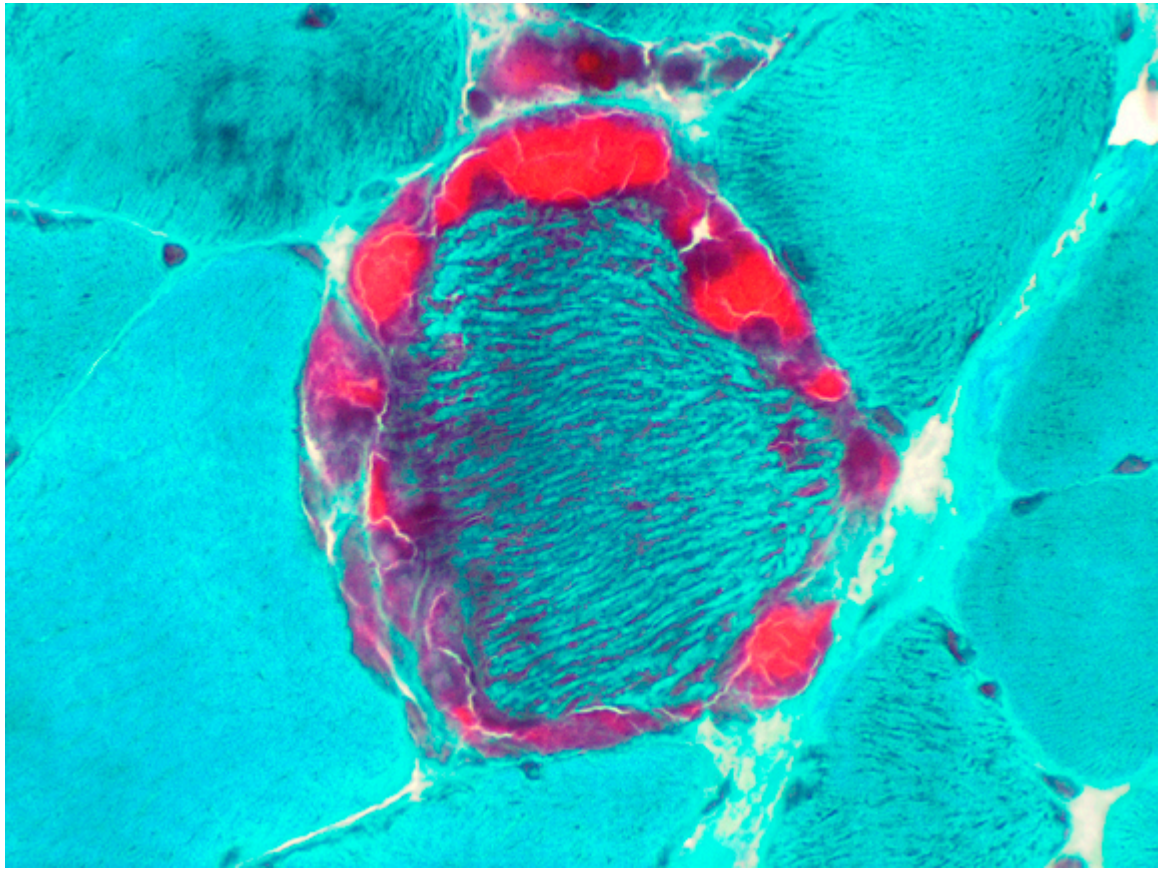
"Ragged" muscle fibers are detected when staining for succinate dehydrogenase activity or with the help of Homori "trichrome stain"

Measurements of the activity of individual components of the respiratory chain

fresh muscle

fibroblast culture

If a defect is found in one link, this indicates a mutation of the corresponding subunit (i or m), if the defects are multiple - a defect of mt tRNA or nuclear genes participating in mitochondrial work



"Ragged" muscle fibers

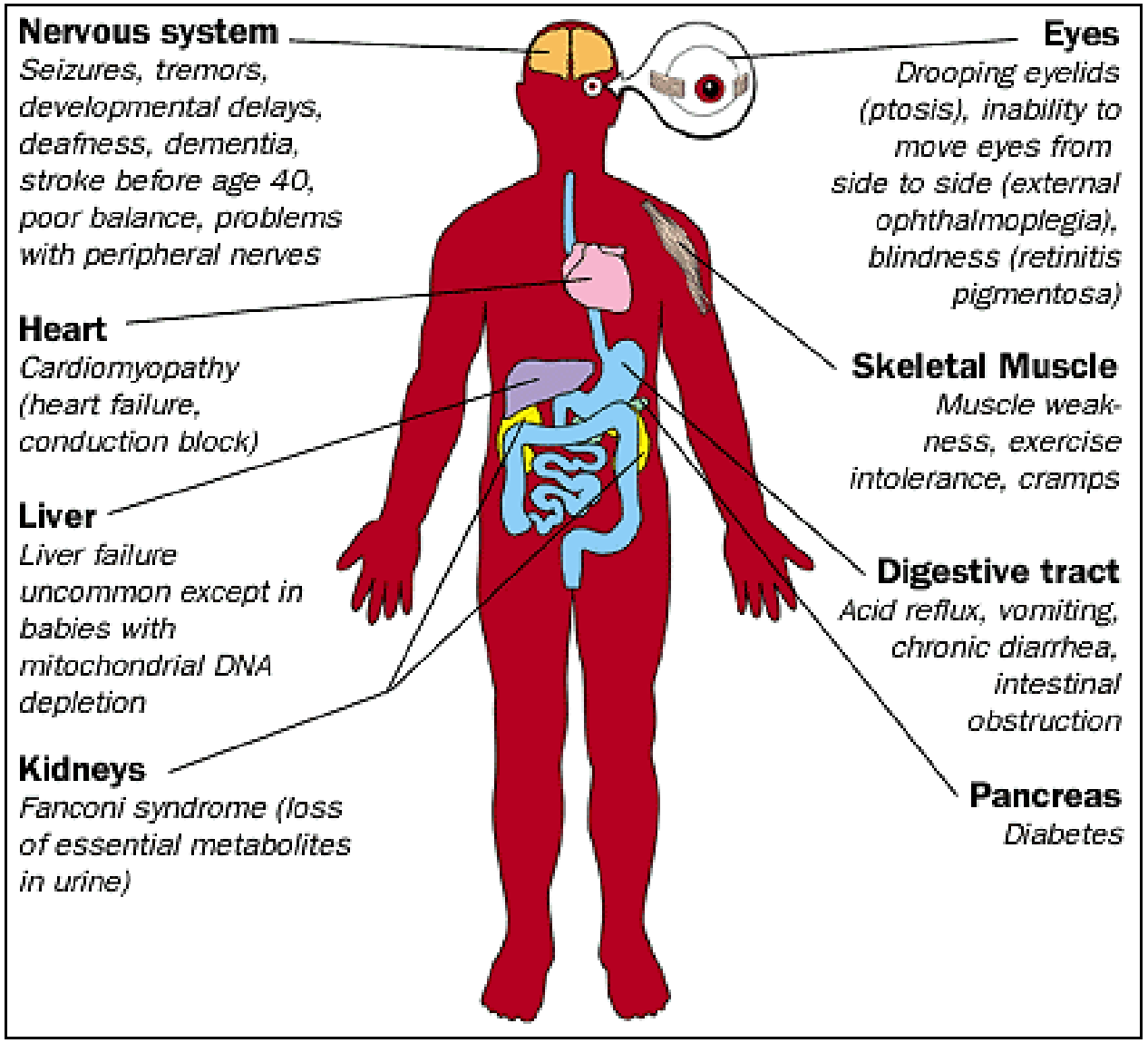
How to diagnose a mitochondrial anomaly?

Sometimes the defect manifests itself under load (NARP syndrome with mutation of the ATPase6 gene – clinical testing is needed:

physical activity with lactate measurements, magnetic resonance or infrared spectroscopy

Finally, in the case of not yet described, rare "private" mutations, direct mtDNA sequencing is performed

Typical for mitochondrial diseases
involvement of different organs and simultaneous manifestation of externally unrelated anomalies



If the patient has mitochondriopathy, then

After the transferred infectious diseases his condition can sharply worsen

also burdens the state

stress, starvation, hypothermia, prolon
immobility, sedation

Carefully apply local and general anesthesia!

Treatment of mitochondrial diseases - it is so real?

Pharmacological approach

Vitamins, cofactors, "free radical scavengers" - to prevent damage to the respiratory chain

The most successful example is dichloroacetate, used to reduce lactic acidosis in patients with MELAS

Success is partial and temporary, often therapy is ineffective

Treatment of mitochondrial diseases

Another approach is to reduce the mutant ratio: normal mtDNA

I. Increase the number of unmutated molecules by

«shift of genes»

In some patients with myopathy,% mutant mtDNA in satellite cells is lower than in skeletal muscle

Typically, satellite cells proliferate and merge with skeletal myofibrils in response to stress or exercise

The proliferation of satellite cells in skeletal muscles is induced

The proportion of normal mtDNA molecules in the muscle increased, the defect was corrected

Treatment of mitochondrial diseases

II. Reduce the number of mutant mtDNA molecules

Development of synthetic molecules that selectively bind to mutated DNA and block their replication

Introduction to the mitochondria enzyme restriction enzyme, selectively destroying the mutant DNA

Success has been achieved so far only *in vitro*

Treatment of mitochondrial diseases

«Molecular-intracellular reconstruction»

Import from the cytoplasm of normal tRNA instead of defective mitochondrial

The replacement of the defective complex of the respiratory chain by a normal one, obtained from another organism (yeast)

Transplantation of the egg nucleus from the mutant cytoplasm to normal

All these approaches are in the stage of experimental development

Treatment of mitochondrial diseases

Cure for mitochondrial disease is impossible today

Symptomatic treatment is used:

Physical → **Physiotherapy, aerobic gymnastics, moderate and light loads**

Pharmacological → **Anti-epileptic drugs, hormones, vitamins, metabolites, cofactors**

Surgical → **Blepharoplasty, cohlear implantation, heart, kidney, liver transplantation, subcutaneous endoscopic gastrotomy, cricopharyngeal myotomy**

Therapy of mitochondropathies

- Metabolic therapy (carnitine, sodium bicarbonate, dichloroacetate in order to block the inactivation of PDH and decrease the level of lactate (with the development of thiamine deficiency or polyneuropathy with prolonged use)
- In critical cases - methylene blue in a single dose of 2 mg / kg

Therapy of mitochondropathies

- adequate use of fluid and electrolytes
- avoid prolonged fasting and multi-carbohydrate foods
- ketogenic diet + succinic acid
- avoid prolonged heavy physical exertion
- timely and effective treatment of fever

Therapy of mitochondropathies

- effective treatment of epilepsy
- coenzyme Q10 twice daily for 4-5 mg / kg / day,
- Riboflavin 3-20 mg / kg / day in 4 divided doses
- vitamin K3 (menadione) at 1 mg / kg or 0.4 mg / kg / day, phylokvinone (K1) together with vit C50-60 mg / kg
- Sodium succinate 6 mg / kg

Therapy of mitochondropathies

- thiamin at 25-30 mg / kg - with insufficient PDH
- alpha-lipoic acid 5-50 mg
- tocopherol (vit E) 100-1000 mg / kg
- Creatine at 4-10 g per day for mitochondrial myopathies
- **corticosteroids do not apply**

A number of drugs provoke mitochondrial diseases or aggravate their course

Valproat: increases the frequency of seizures with MELAS, hepatotoxic

Aspirin, phenobarbital

Corticosteroids

Tetracycline, chloramphenicol

Aminoglycosides streptomycin, gentamicin, amikacin, neomycin, kanamycin are ototoxic

Etambutol (provokes the manifestation of LHON)

Statin (provokes the manifestation of MELAS)

Antiretroviral drugs: AZT - zidovudine, doxorubicin cause mtDNA deletion

The list is far from complete!