Late onset mitochondrial encephaloneuromyopathies

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Disclosure slide

• The speaker has no conflict of interest to disclose
Learning objectives

• To increase ability to recognise mitochondrial cytopathies
• To know the basic steps in diagnostic work out in the suspicion of mitochondrial cytopathy
• To understand the place of molecular genetic testing in mitochondrial cytopathies
• To know the basics of management in patients with mitochondrial cytopathies
Case report

- 50 years old woman
- Anxiety with panic attacks
- Premonitions
- Burnout syndrome
  - Tremor
  - Aphasia
  - Depression
  - Improvement with citalopram
- Overreacting
- Muscle weakness and pain since childhood
- Fatigue
- Influence of weather
- Gastrointestinal problems
  - Increased after stress
- Improvement after hysterectomy
- No diagnosis

- Present complains
  - fluctuating tremor
  - panic attacks
  - Worsening of the muscle symptoms
  - Exercise intolerance
  - myalgia, weakness
- Neurological investigations
  - Dystonia fingers right
  - Sensory deficits right
Case report

- 50 years old woman
- Anxiety with panic attacks
- Premonitions
- Burnout syndrome
  - Tremor
  - Aphasia
  - Depression
  - Improvement
- Overreacting
- Muscle weakness and pain since childhood
- Fatigue
- Influence of weather
- Gastrointestinal problems
  - Increased after stress
- Improvement after hysterectomy
- No diagnosis

- Present complains
- Fear of Parkinson, like in her father
- Improvement after citalopram
- Dystonia fingers right
- Sensory deficits right
- Muscle symptoms increased after stress
- Neural investigations
The choice of a genetic test

Analyse information

- **Phenotype**
  - Age at onset
  - Course
  - Symptoms
  - Signs
  - Additional investigations

- **Family history**
  - Pedigree
  - Phenotype

- **Prevalence**
  - Disorder
  - Gene mutation
  - Regional – Ethnic differences

- **Test resources**
  - Cost
  - Availability
Late onset mitochondrial encephaloneuromyopathies

- Introduction
- Biological background
- Mitochondrial cytopathies: presentation
- Mitochondrial cytopathies: diagnostic pathway
- Molecular diagnosis
- Treatment
Respiratory chain

www.nsf.gov

Intermembrane space

NADH → NAD⁺ → Fumarate → Succinate → Matrix

H⁺

CoQ

CytC

O₂ → H₂O → ADP + Pi → ATP

ATP synthase
Genetic information
mitochondrial DNA
nuclear DNA
Genetic background mitochondrial DNA

- Maternally inherited
- Circular DNA 16596 base pairs
- Encodes
  - 13 respiratory chain proteins
  - 2 rRNA and 22 tRNA
- Respiratory chain complexes I-V
Genetic background nuclear DNA

- All other genes
- About 1500 proteins
  - targeted to mitochondria
  - Involved in mtDNA maintenance (replication, maintenance, and translation)
  - Coenzym Q synthesis
  - Solute transport proteins
  - Mitochondrial fission and fusion
  - Mitochondrial biogenesis
  - Structural, assembly proteins
  - Regulation and stability elements
  - Intergenomic communication
Mitochondria in PD

- Synuclein
- PINK Mutations
- DJ 1 Mutations
- O Stress
- Cell death
- Tau
Mitochondrial cytopathies

- Disorders of oxidative phosphorylation, energy production
- Multisystem disorders
- Variety of symptoms
- Onset: childhood-adult
- Course: benign-severe
Late onset mitochondrial encephaloneuromyopathies

- Introduction
- Biological background
- Mitochondrial cytopathies: presentation
- Mitochondrial cytopathies: diagnostic pathway
- Molecular diagnosis
- Treatment
Stroke, ataxia, epilepsy, encephalopathy and migraines

Optic neuropathy, retinopathy and external ophalmoplegia

Deafness

Cardiomyopathy and conduction defects

Liver failure

Anaemia

Diabetes mellitus

Renal failure

Intestinal pseudo-obstruction and diarrhoea

Muscle weakness, exercise intolerance, cramps, atrophy, and hypotonia

Peripheral neuropathy
Mitochondrial disease should be included in the differential diagnosis for almost any neurologic symptom (Amy Goldstein 2012). Suspicion increased when 3 or more organs involved without a known etiology.
The proteiform presentation of mitochondrial cytopathy: a multisystem disorder: CNS

- Stroke
- Movement disorders
  - Ataxia
  - Myoclonus
  - dystonia
  - tremor
  - Bradykinesia
- Paroxysmal disorders
  - Epilepsy
  - Migraine

- Psychiatric symptoms
  - Psychosis
  - Depression
  - Panic attacks
  - ...
- Chronic encephalopathy
  - mental retardation
  - dementia
The proteiform presentation of mitochondrial cytopathy: a multisystem disorder: other neurological manifestations

- **Eyes, ears, and cranial nerves**
  - Retinopathy
  - optic neuropathy
  - glaucoma, cataract
  - Sensorineural deafness
  - Labyrinth

- **Peripheral nerves**
  - Polyneuropathy, sensory, motor

- **Muscle**
  - Weakness, atrophy
  - exercise intolerance
  - Fatigue
  - cramps, myotonia
  - external ophtalmoplegia
The proteiform presentation of mitochondrial cytopathy: a multisystem disorder

| **Heart**       | Cardiomyopathy, conduction defects |
| **Kidney**      | Renal failure, tubulopathy, cysts  |
| **Gastrointestinal** | pseudo-obstruction and diarrhoea, dysphagia, vomiting, anorexia, malabsorption |
| **Liver**       | hepatic failure, transaminase increase |
| **Blood**       | sideroblastic anaemia               |
| **Endocrine**   | Diabetes mellitus, hypothyrosis infertility, delayed puberty, thyroid dysfunction, hypogonadism, short stature, hypoglycaemia, osteoporosis, amenorrhoea |
Prevalence often underestimated

- 9.2/100,000 manifest mitochondrial cytopathy due to mitochondrial mutation in Northern England (Schaefer 2008)

- 1/400 prevalence of pathogenic mitochondrial mutations (Manwaring 2007)
Think about mitochondrial disorder

- **In stroke**
  - Non territorial distribution
  - Diffusion imaging: mixture of hypo- and hyperintense lesions

- **In epilepsy**
  - Epilepsia partialis continua
  - Myoculous
  - Status epilepticus
  - Worsening with valproate treatment (with hepatopathy)

- **In ataxia**
  - When associated with epilepsy
  - Cerebellar atrophy and white lesions

- **With ocular signs**
  - Retinopathy, ptosis and eye movement disorder

- **In sensorineural hearing loss**
  - Young onset
  - Accompanied by other system

- **Basal ganglia lesion**
  - Bilateral symmetric

- **Encephalopathy with hepatopathy**
  - Precipitation by drugs
Further suggestive features

- Recurrent
- Progressive, improvement
- precipitation
  - infection
  - fasting
  - Surgery
  - Medication toxic to mitochondria
- Neuro
  - Presenting
  - Part of a syndrome
  - Early, later
Example: CPEO, chronic progressive external ophthalmoplegia

- Bilateral, often asymmetric ptosis
- Progressive paresis of external eye muscle
- Additional symptoms
  - Muscles
    - Muscular exercise intolerance
    - Fatigue
    - Proximal weakness
    - Weakness of facial and pharyngeal muscles (dysphagia)
  - Heart
    - rhythm disturbance
    - Cardiomyopathy
  - Endocrine
    - Hypogonadism, delayed puberty, small stature, diabetes mellitus
  - Polyneuropathy
  - Cognitive disorder
  - Retinopathy, optic nerve atrophy
  - Ataxia
  - Respiratory insufficiency
  - Similarities with Kearns-Sayre-Syndrome (which is more severe)
<table>
<thead>
<tr>
<th>Abr.</th>
<th>disorder</th>
<th>Main aspects</th>
<th>Additional features</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHON</td>
<td>Leber Hereditary Optic Neuropathy</td>
<td>Subacute visual loss</td>
<td>Wolff-Parkinson-White syndrome, Multiple sclerosis-type disease</td>
</tr>
<tr>
<td>MERRF</td>
<td>Myoclonic epilepsy and ragged red fibers</td>
<td>Myoclonus-epilepsy, Ataxia</td>
<td>Optic nerve atrophy, lipomatosis, feet deformity, Short stature</td>
</tr>
<tr>
<td>MELAS</td>
<td>Mitochondrial Encephalopathy, lactic acidosis/stroke-like episodes</td>
<td>Episodes of lactic acidosis, acute CNS impairment, not according to vascular territory, epilepsy, migraine, dementia</td>
<td>Hearing loss, dysmotility weight loss</td>
</tr>
<tr>
<td>CPEO</td>
<td>Chronic progressive external ophthalmoplegia</td>
<td>Ptose, Ophthalmoparesis</td>
<td>Cardiac rhythm disorders, myopathy, endocrine disorders</td>
</tr>
<tr>
<td>KSS</td>
<td>Kerns Sayres Syndrome</td>
<td>Ophthalmoparesis, Ptose, small stature, AV-Block III, Retinopathy,</td>
<td>Glaucoma, hearing loss, diabetes</td>
</tr>
<tr>
<td>NARP</td>
<td>Neuropathy ataxia retinitis pigmentosa</td>
<td>Cerebellar ataxia, polyneuropathy</td>
<td>Retinitis pigmentosa, dementia, epilepsy, hearing loss</td>
</tr>
<tr>
<td>LS</td>
<td>Leigh Syndrome</td>
<td>Psychomotor retardation cerebellar ataxia, epilepsy, hypotonia, polyneuropathy</td>
<td></td>
</tr>
</tbody>
</table>
Mitochondrial cytopathies

- More than 300 mtDNA mutations
  - Gene encoding structural and assembly proteins
  - Translation mtDNA
  - Maintenance of mtDNA

- Syndromes
- Many overlapping presentations
Mitochondrial gene mutations in Neurological disorders

- Mutations in mtDNA genes encoding for
  - respiratory chain proteins
  - tRNAs or rRNAs
- Disorders due to mutations in nDNA genes
  - Respiratory chain proteins
  - Proteins implicated in mitochondrial metabolism
  - Proteins implicated in mitochondrial dynamics
  - Proteins correlated to mitochondrial function

LHON
MELAS
MERRF
NARP
Leigh syndrome
KSS
Mitochondrial gene mutations in neurological disorders

- Mutations in mtDNA genes encoding for
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  - proteins implicated in mitochondrial dynamics
  - proteins correlated to mitochondrial metabolism

Leigh syndrome
Leukodystrophy
GRACILE syndrome
leukodystrophy and tubulopathy
Mitochondrial gene mutations in Neurological disorders

- Mutations in mtDNA genes encoding for
  - respiratory chain proteins
  - tRNAs or rRNAs

- Disorders due to mutations in nDNA genes encoding for
  - respiratory chain proteins
  - proteins implicated in mitochondrial metabolism
  - proteins implicated in mitochondrial dynamics
  - proteins correlated to mitochondrial

Leigh syndrome
Alpers syndrome
infant encephalopathy
MNGIE
SANDO
Wolfram syndrome
Mitochondrial gene mutations in Neurological disorders

<table>
<thead>
<tr>
<th>Mutations in mtDNA genes encoding for:</th>
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</tr>
<tr>
<td>– proteins implicated in mitochondrial dynamics</td>
</tr>
<tr>
<td>– proteins correlated to mitochondrial function</td>
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</tbody>
</table>

- ADOA
- CMT type 2A, 4A, and 6
- AD
- PD
- HD
- ALS
- Friedreich ataxia
- Hereditary spastic paraplegia
Mito Symptoms

Prevalence of Physical Symptoms/Conditions in the Sample (N=36)

<table>
<thead>
<tr>
<th>Physical Symptom</th>
<th>Prevalence, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weakness</td>
<td>33 (92)</td>
</tr>
<tr>
<td>Visual problems</td>
<td>32 (89)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>29 (81)</td>
</tr>
<tr>
<td>Headaches</td>
<td>29 (81)</td>
</tr>
<tr>
<td>Chronic fatigue</td>
<td>27 (75)</td>
</tr>
<tr>
<td>Muscle spasms</td>
<td>27 (75)</td>
</tr>
<tr>
<td>GERD</td>
<td>19 (53)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>17 (47)</td>
</tr>
<tr>
<td>Asthma</td>
<td>14 (39)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>14 (39)</td>
</tr>
</tbody>
</table>

GERD = gastroesophageal reflux disease.

Psychiatric Diagnoses in the Sample (N=36)

<table>
<thead>
<tr>
<th>Psychiatric Diagnoses</th>
<th>Prevalence n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime MDD</td>
<td>19 (54)</td>
</tr>
<tr>
<td>Current MDD</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Recurrent MDD</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Lifetime bipolar disorder</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Current dysthymia</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Past hypomanic episode</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Lifetime panic disorder</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Current generalized anxiety disorder</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Past manic episode</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Current social phobia</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Lifetime attention-deficit disorder</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Current obsessive-compulsive disorder</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Lifetime psychotic disorder</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Current manic episode</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current hypomanic episode</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current panic disorder</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current psychotic disorder</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fattal 2007
<table>
<thead>
<tr>
<th>Comorbidities of Psychiatric Disease in Subjects with Mitochondrial Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects with psychiatric diagnosis</strong> (n=25)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
</tr>
<tr>
<td>Never married</td>
</tr>
<tr>
<td>Married</td>
</tr>
<tr>
<td>Divorced</td>
</tr>
<tr>
<td><strong>Education</strong></td>
</tr>
<tr>
<td>Did not finish high school</td>
</tr>
<tr>
<td>Finished high school</td>
</tr>
<tr>
<td>Some college</td>
</tr>
<tr>
<td>Finished college</td>
</tr>
<tr>
<td>Post-graduate</td>
</tr>
<tr>
<td><strong>Positive Family History</strong></td>
</tr>
<tr>
<td>Psychiatric</td>
</tr>
<tr>
<td>Medical</td>
</tr>
<tr>
<td>Mitochondrial</td>
</tr>
<tr>
<td><strong>Age (mean, years)</strong></td>
</tr>
<tr>
<td><strong>Number of Medications for mitochondrial disease (mean)</strong></td>
</tr>
<tr>
<td><strong>Total medications (mean)</strong></td>
</tr>
<tr>
<td><strong>Number of hospital admissions (mean)</strong></td>
</tr>
<tr>
<td><strong>Number of medical conditions (mean)</strong></td>
</tr>
</tbody>
</table>

* Wilcoxon two sample.
† Total might not add up to 35 because of missing data.
‡ P<.05.
Psychiatric symptoms in mitochondrial Syndromes

<table>
<thead>
<tr>
<th>Psychiatric Presentation</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mutations (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major depressive disorder</td>
<td>22</td>
<td>POLG (7), MELAS (4), unknown (3), other (8)</td>
</tr>
<tr>
<td>With psychotic features</td>
<td>14</td>
<td>POLG (6), MELAS (1), unknown (3), other (4)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>2</td>
<td>KSS (1), ANT1 gene mutation (1)</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>19</td>
<td>MELAS (11), POLG (2), KSS (1), other (5)</td>
</tr>
<tr>
<td>Psychotic disorder</td>
<td>17</td>
<td>MELAS (15), KSS (1), C3256T mutation (1)</td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>6</td>
<td>MELAS (6)</td>
</tr>
<tr>
<td>Frontal lobe syndrome</td>
<td>4</td>
<td>MELAS (3), twinkle mutation (1)</td>
</tr>
<tr>
<td>Personality change</td>
<td>2</td>
<td>MELAS (2)</td>
</tr>
<tr>
<td>Psychosomatic disorder</td>
<td>1</td>
<td>KSS (1)</td>
</tr>
</tbody>
</table>

Anglin 2012
Psy in Mito mice

Kasahara 2006
Psy in Mito mice

- Increased startle
- Increased conditioned fear
- Disturbed circadian rhythm
- Improved with lithium

Kasahara 2006
<table>
<thead>
<tr>
<th>Pat.</th>
<th>mtDNS</th>
<th>Anteil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Größe in kbp</td>
<td>in %</td>
</tr>
<tr>
<td>818</td>
<td>7-12</td>
<td>16-54</td>
</tr>
<tr>
<td>1672</td>
<td>6-10</td>
<td>35</td>
</tr>
<tr>
<td>1861</td>
<td>3-6</td>
<td>24-50</td>
</tr>
<tr>
<td>2195</td>
<td>6-13</td>
<td>44-47</td>
</tr>
<tr>
<td>2478</td>
<td>10-13</td>
<td>35</td>
</tr>
<tr>
<td>2917</td>
<td>2-7</td>
<td>5-11</td>
</tr>
<tr>
<td>3362</td>
<td>8-12</td>
<td>92</td>
</tr>
<tr>
<td>3501</td>
<td>3.4-13.4</td>
<td>34-37</td>
</tr>
<tr>
<td>3796</td>
<td>4-10</td>
<td>7</td>
</tr>
<tr>
<td>3867</td>
<td>6-8</td>
<td>58-87</td>
</tr>
<tr>
<td>4530</td>
<td>3-13</td>
<td>38</td>
</tr>
<tr>
<td>4572</td>
<td>6-8</td>
<td>21</td>
</tr>
<tr>
<td>4652</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>5103</td>
<td>6.5-12</td>
<td>31-37</td>
</tr>
<tr>
<td>5116</td>
<td>5-11</td>
<td>19</td>
</tr>
<tr>
<td>5156</td>
<td>6-10</td>
<td>62</td>
</tr>
<tr>
<td>5425</td>
<td>6-7</td>
<td>31-91</td>
</tr>
<tr>
<td>5589</td>
<td>5-8</td>
<td>20-34</td>
</tr>
<tr>
<td>5638</td>
<td>8-10</td>
<td>33-54</td>
</tr>
</tbody>
</table>

Bua 2006
Muskelbeschwerden

<table>
<thead>
<tr>
<th>Beschwerde</th>
<th>Anzahl betroffene Patienten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schnelle Ermüdung</td>
<td>19</td>
</tr>
<tr>
<td>Muskelkater nach Belastung</td>
<td>17</td>
</tr>
<tr>
<td>Myalgien in Ruhe</td>
<td>14</td>
</tr>
<tr>
<td>Muskelkrämpfe</td>
<td>13</td>
</tr>
<tr>
<td>Muskelzuckungen</td>
<td>12</td>
</tr>
<tr>
<td>Muskelsteifigkeit</td>
<td>10</td>
</tr>
<tr>
<td>Muskelschwäche</td>
<td>18</td>
</tr>
</tbody>
</table>
Late onset mitochondrial encephaloneuromyopathies

- Introduction
- Biological background
- Mitochondrial cytopathies: presentation
- Mitochondrial cytopathies: diagnostic pathway
- Molecular diagnosis
- treatment
Investigations: first steps

**Clinical**
- Thorough systematic history, including family history
  - Myalgia, muscular exercise intolerance
  - Fatigue, pain
  - Ptosis, ophthalmoplegia, earing loss
  - Cognitive impairment, epilepsy, migraine
  - Diabetes mellitus, Vitamin deficiency
  - Gastrointestinal problems
  - Heart failure
- Thorough neurological and general examination

**Laboratory**
- Blood cell count
  - Low counts in metabolic diseases
- Glucose, Hemoglobin A1c
- Electrolytes
  - Anion gap
- Lactate
  - Release tourniquet
- Ketones
  - Absent during fasting?
- Plasma ammonia
  - Most useful in fasting
- Creatine kinase
- Urine analysis
  - High pH: renal tubular acidosis

**Apparative**
According to symptoms
- ECG
- Imaging (Brain MRI)
  - Bilateral symmetric lesions
  - Basal ganglia and brainstem
  - Leukoencephalopathy
  - Multifocal hyperintense signal
- Spectroscopy: Lactate peak
Investigations: second steps

**Neurophysiology**
- EEG
- EMG
- Neurographies

**Laboratory**
- Serum pyruvate
  - Rapid deproteinization of the sample
- Lactate/Pyruvate Ratio
  - >20: lactate acidosis in impaired oxidative phosphorylation
- Amino acids (blood, urine, CSF)
  - Elevated alanine (pyruvate precursor)
  - Aminoaciduria: proximal renal dysfunction indication mitochondrial dysfunction (but also other conditions)
- Organic acids (blood, urine, CSF)
- Carnitine (blood, urine, muscle tissue)
  - With metabolite profile (Gas chromatography-mass spectroscopy)

**Other**
- Cardiological investigations
- Exercise test
  - Lactate
- Muscle biopsy
  - Histology
  - Biochemical analyses
- Genetic testing
MRI

- Bilateral symmetric lesions
  - Basal ganglia
  - Brainstem
  - Calcification (CT)
- Leukoencephalopathy
- Multifocal hyperintense signal
- apparent diffusion coefficient map can show a mixture of hypointensity and hyperintensity, suggestive of both cyto-toxic and vasogenic edema
- Not within vascular territorial boundaries

- Spectroscopy
  - Lactate peak
Non territorial involvement
MRI in MELAS
Optic nerve enhancement in LHON

Furuki 2012
Leigh Disease

resourhttp://radiopaedia.org
d
Sofou 2013
Lactate peak

Sofou 2013
Muscle biopsy findings in mitochondrial cytopathies

• Morphological anomalies
  – Myopathic changes
  – Acute, chronic denervation
  – Abnormal and increased numbers of mitochondria

• Histochemical changes
  – Ragged red fibres in Gomori-Trichrom staining
  – Succinatatedehydrogenase (SDH) Cytochrome oxidase staining: COX-negative/SDH-positive fibres

• Biochemical analysis
  – Isolated activities of respiratory complex enzymes I-V
  – Pyruvate dehydrogenase complex
  – Citrasynthase
  – Coenzyme Q10 level

• Electron microscopy
  – Ultrastructural changes in mitochondria
Muscle biopsy findings

SDH

COX

neuropathology-web.org

Kollberg 2005
Late onset mitochondrial encephaloneuromyopathies

- Introduction
- Biological background
- Mitochondrial cytopathies: presentation
- Mitochondrial cytopathies: diagnostic pathway
- Molecular diagnosis
- treatment
The place of diagnostic testing

First steps of investigations

Characteristic clinical syndrome (MELAS, LHON, MERRF...)

- yes
  - Genetic testing of common mutations (blood)
  - no
    - Muscle biopsy
      - histology
      - biochemistry
      - Molecular genetics

- no
  - negative
Molecular testing

- According to clinical presentation
  - Search common mutation (blood, urine, muscle)
- mtDNA deletion
  - Southern blot, long-range-PCR in muscle tissue
- mtDNA depletion
  - Southern blot, real-time-PCR in muscle tissue
- Isolated myopathy due to point mutations
  - Selective sequencing in muscle tissue
- Whole mtDNA sequencing

Step 1: search for common mutations (Muscle, leukocytes, cells in urine sediment or mouth)
Step 2: sequencing further genes, or whole mtDNA
Step 3: in case of possible nuclear mutation (for example multiple mtDNA deletions in muscle): assessment of nuclear DNA
Mitochondrial mutation types

- Structural rearrangements
  - For example deletions
  - Heteroplasmic
  - Singular deletions mostly sporadic
  - Multiple deletions mostly due to nuclear gene mutations (dominant or recessive)

- Quantitative disorders
  - depletion

- Point mutations
  - Mostly maternal inheritance
  - Heteroplasmic (only rarely homoplasmic)
Example: CPEO, chronic progressive external ophthalmoplegia

- Bilateral, often asymmetric ptosis
- Progressive paresis of extraocular muscles
- Additional symptoms
  - Muscles
    - Muscular exercise intolerance
    - Fatigue
    - Proximal weakness
    - Weakness of facial and pharyngeal muscles (dysphagia)
  - Heart
    - Rhythm disturbance
    - Cardiomyopathy
  - Endocrine
    - Hypogonadism
  - Polyneuropathy
  - Cognitive disorder
  - Retinopathy, optic nerve atrophy
  - Ataxia
  - Respiratory insufficiency
  - Similarities with Kearns-Sayre-Syndrome (which is more severe)

- Genetics
  - In 50% sporadic due to singular mtDNA deletions, rarely duplications
  - Rare maternally inherited mtDNA point mutations (3243A>G most frequent)
  - Autosomal dominant, or autosomal recessive (rarer)
    - POLG1, POLG2, PEO1, RRM2B, SLC25A4, OPA1 genes

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Complex I deficiency</th>
<th>Complex IV deficiency</th>
<th>Complex V deficiency</th>
<th>Complex II deficiency</th>
<th>Coenzyme Q 10 deficiency</th>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>OPA3</td>
<td>AD/AR</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tbody>
</table>
Variable expression

- Same mutation with different presentation, even in the same family
- Similar clinical presentation with different mutations

Example: MERRF
most frequent mutation: 8344A>G in tRNA lysine
also found in Leigh disease and myopathy with truncal lipoma
MERRF may also be due to 8356T>C

Italian mitochondrial disease database (1086 patients)
42 patients with 8344A>G
age at onset 0-66 years old
ragged-red fibres in 96%
myoclonus more frequently associated with ataxia than with epilepsy
Neuromuscular involvement in 77%
CNS involvement in 56%
lipomatosis in 32%

(Mancuso 2013)
Genetic counselling in mitochondrial Cytopaties

- inheritance
  - autosomal dominant
  - autosomal recessive
  - maternal
- phenocopies
- Variable phenotype
- Variable Expression
EFNS guidelines on the molecular diagnosis of mitochondrial disorders

J. Finsterer\textsuperscript{a}, H. F. Harbo\textsuperscript{b}, J. Baets\textsuperscript{c,d,e}, C. Van Broeckhoven\textsuperscript{d,e}, S. Di Donato\textsuperscript{f}, B. Fontaine\textsuperscript{g}, P. De Jonghe\textsuperscript{c,d,e}, A. Lossos\textsuperscript{h}, T. Lynch\textsuperscript{i}, C. Mariotti\textsuperscript{j}, L. Schöls\textsuperscript{k}, A. Spinazzola\textsuperscript{l}, Z. Szolnoki\textsuperscript{m}, S. J. Tabrizi\textsuperscript{n}, C. M. E. Tallaksen\textsuperscript{o}, M. Zeviani\textsuperscript{l}, J.-M. Burgunder\textsuperscript{p} and T. Gasser\textsuperscript{q}
Case report

- 50 years old woman
- Anxiety with panic attacks
- Premonitions
- Burnout syndrome
  - Tremor
  - Aphasia
  - Depression
  - Improvement with citalopram
- Overreacting
- Muscle weakness and pain since childhood
- Fatigue
- Influence of weather
- Gastrointestinal problems
  - Increased after stress
- Improvement after hysterectomy
- No diagnosis

- Present complains
  - fluctuating tremor
  - panic attacks
  - Worsening of the muscle symptoms
  - Exercise intolerance
  - myalgia, weakness
- Neurological investigations
  - Dystonia fingers right
  - Sensory deficits right
Case report

- Family History
  - Parkinson
  - Tremor
  - Dementia
  - Sudden death due to cardiac arrhythmia
  - Epilepsy
  - Myalgia
  - Anxiety disorder
  - Depressions
Case report

- Genetics
- Mitochondrial genome
  - Uncle
    - 3-6 kpb deletion
    - 24-50%
  - Father
    - 5-10 kpb deletion
    - 78-95%
Late onset mitochondrial encephaloneuromyopathies

- Introduction
- Biological background
- Mitochondrial cytopathies: presentation
- Mitochondrial cytopathies: diagnostic pathway
- Molecular diagnosis
- treatment
Mitochondrial cytopathy: general measures

- Food intake
  - Balanced caloric regime, several small meals.
- Avoid smoking and alcohol
- Avoid extreme temperatures, high altitude
- Avoid risks of infection
- Systematic search for complications
- Avoid drugs which may impair mitochondrial function
  - Valproate, barbiturates, some antibiotics (oxazolidinones, aminoglycosides, chloramphenicol, tetracyclines), Ringer Lactate infusion,
- Exercise
- Supplements, vitamin
Mitochondrial cytopathy: symptomatic treatment

- **Antiepileptic drugs**
  - Avoid Valproate (mitochondrial toxic)

- **Migraine Therapy**
  - Acute (caution with triptans in stroke like episodes)

- **Hormone/Vitamins Substitution**
  - Thyroid, Vitamin D, Diabetes

- **Pain**
  - Musculoskeletal (non steroidal anti-inflammatory drugs, opioid drugs sometimes useful in small doses)
  - Neuropathic (gabapentin, pregabalin)
  - Central pain modulation (tricyclic antidepressant
  - (orthopedic, neuropathic, central)
Mitochondrial cytopathy: treatment

- **Esthetic operation**
  - Eyelid ptosis
- **Cardiac anomalies**
  - Arhythmia, Heart failure due to cardiomyopathy
- **Anemia** (Pearson Syndrome)
  - Blood transfusion
- **Gastrointestinal tract**
  - Pancreatic enzymes
  - Gastrokinetic drugs (dromperidone)
  - Bowel regulation
- **Corticosteroids** (prednisone 5-60 mg)
  - Some patients respond positively, caution with side effects on long term therapy
Mitochondrial cytopathy: exercise

- Endurance training
- Moderate resistance training
- Safe
- Improves muscular performance
Mitochondrial cytopathy treatment: Supplements

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td>5-20g</td>
</tr>
<tr>
<td>Thiamine (Vitamine B1)</td>
<td>100-800 mg</td>
</tr>
<tr>
<td>Riboflavin (Vitamine B2)</td>
<td>400 mg</td>
</tr>
<tr>
<td>Niacinamide (Vitamine B3)</td>
<td>100-500 mg</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>30-100 mg/kg</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>5-15 mg/kg</td>
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<tr>
<td>Folate</td>
<td>1-10 mg</td>
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<tr>
<td>Vitamine E</td>
<td>400-1200 IU</td>
</tr>
<tr>
<td>Selenium</td>
<td>25-50µg</td>
</tr>
<tr>
<td>Alpha Lipoic Acid</td>
<td>200-600 mg</td>
</tr>
</tbody>
</table>

**Step-wise procedure**

- one substance at a time
- Gradual increase
- Check tolerance
- add the next one

- Define clinical markers
- Establish cocktail on an individual basis
Follow-up assessments

- **According to phenotype**
  - Brain
    - MRI
  - Myopathy
    - Muscle power, functional scales, pain assessment
    - Laboratory: CK, Lactate
  - Cardiopathy
    - EKG
    - Exercise testing
  - Renal involvement
    - Creatinin, Clearance
    - Proteinuria,
  - Endocrine disorder
    - Thyroidea, blood sugar, parathormone, calcium, vitamine D.
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