Mitochondrial Cytopathies

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Prevalence of mitochondrial cytopathies

- Mitochondrial diseases can become clinically relevant in children as well as in adults
- Childhood-onset mitochondrial diseases are often severe
- Late onset often with nonspecific features (hypotonia, weakness, fatigue, exercise intolerance)

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Prevalence per 100,000 individuals (95% CI)</th>
<th>Region</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6</td>
<td>8.9 (5.3–14)</td>
<td>Western Sweden</td>
<td>189</td>
</tr>
<tr>
<td>0–10</td>
<td>15 (9.8–21)</td>
<td>Central region of Portugal</td>
<td>190</td>
</tr>
<tr>
<td>0–16</td>
<td>4.7 (2.8–7.6)</td>
<td>Western Sweden</td>
<td>189</td>
</tr>
<tr>
<td>0–16</td>
<td>6.2 (4.5–8.4)*</td>
<td>South eastern Australia</td>
<td>9</td>
</tr>
<tr>
<td>0–16</td>
<td>71 (32–136)*</td>
<td>South eastern Australia (families of Lebanese ancestry)</td>
<td>9</td>
</tr>
<tr>
<td>0–18</td>
<td>7.5 (5–10)</td>
<td>North West Spain</td>
<td>191</td>
</tr>
<tr>
<td>0–18</td>
<td>~12</td>
<td>Northern Finland</td>
<td>192</td>
</tr>
<tr>
<td>Childhood</td>
<td>~11</td>
<td>Ireland</td>
<td>10</td>
</tr>
<tr>
<td>Childhood</td>
<td>~10</td>
<td>Japan</td>
<td>193</td>
</tr>
<tr>
<td>Adults (&gt;16)</td>
<td>9.6 (8.3–11)</td>
<td>North East of England (mtDNA)</td>
<td>11</td>
</tr>
<tr>
<td>Adults (&gt;16)</td>
<td>2.9 (2.2–3.7)</td>
<td>North East of England (nDNA)</td>
<td>11</td>
</tr>
</tbody>
</table>

Mitochondrial cytopathies

- Heterogeneous group of diseases
- Impaired oxidative phosphorylation
- Can involve several organs or tissues
- Increasing number of tissues affected over time
- CNS almost consistently involved in late stages
The mitochondrial respiratory chain

NADH-CoQ reductase  succinate-CoQ reductase (SDH)  red-CoQ-cyt c reductase  cytochrome c oxidase (COX)  ATP synthetase
mtDNA and nDNA

Di Mauro 2003

Emma et al, Nat Rev Nephrol 2016
Type of mutations

- mtDNA genes mutations/deletions
  - de novo or maternal inheritance
  - heteroplasmy
    - threshold effect
    - proliferative segregation

- Nuclear DNA gene mutations
  - classic mendelian genetics
  - all mitochondria are affected to the same extent
    - structural mitochondrial genes
    - functional genes
      - mitochondrial assembly and dynamics
      - mDNA maintenance and replication

from www.mitoresearch.org
Diagnosis

- **Spectrophotometric studies**
  - enzyme activities: CI, CII, CIII, \([\text{CII}+\text{CIII}]\), CIV, CV

- **Polarographic studies**
  - oxygen consumption by isolated mitochondria or whole cells in the presence of different oxidative substrates (pyruvate, glutamate, succinate, etc...)

- **Genetic studies**
  - mtDNA sequencing
    - specific mutations (m.3243A>G)
    - (better in urinary sediment; may be missed in blood)
  - next generation sequencing
    - Small gene panels - large gene panels – exome sequencing
    - COQ genes should be included in SRNS panels
Diagnosis

- Also check plasma (and CSF?) lactate, pyruvate
- Acylcarnitines
- **Consider FGF21 and/or GDF15 plasma levels**
- Neuroimaging and hearing test
- Cardiovascular and endocrine evaluation

NB: often observed without hyperlactacidemia
Renal investigations

- SRNS
- Renal Fanconi syndrome
- RTA + glomerular proteinuria
- Renal Fanconi syndrome
Renal diseases in mitochondrial disorders

- generally Fanconi syndrome
- RTA
- isolated hyperaminoaciduria
- isolated hypomagnesemia
- Barrter-like


only a few case reports
# Renal abnormalities in mitochondrial diseases

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n=42 cases</th>
<th>Severe renal disease</th>
<th>Mild tubular disorder</th>
<th>Normal renal function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalomyopathy</td>
<td>17</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Leigh syndrome</td>
<td>7</td>
<td>–</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Isolate myopathy</td>
<td>6</td>
<td>–</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Neonatal multi-system</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Kearns-Sayre syndrome</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Pearson syndrome</td>
<td>3</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100%</strong></td>
<td><strong>20%</strong></td>
<td><strong>30%</strong></td>
<td><strong>50%</strong></td>
</tr>
</tbody>
</table>
Mitochondrial glomerular diseases

- Mostly FSGS
- Generally associated with other symptoms
- Two clearly defined genetic defects:
  - CoenzymeQ10 biosynthesis defects
  - tRNALeu mutations

A3243G tRNA$^{Leu}$ mutation

- **MELAS** (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes)
- **MIDD** (Maternally Inherited Diabetes and Deafness)

- Patients with a renal phenotype
  - Generally FSGS, but also cases of TIN and cystic kidney disease
  - Other symptoms:
    - hearing loss: 80-90% (not always severe)
    - diabetes mellitus: 20-30%
    - neuromuscular: 10-20%
    - cardiomyopathy, retinopathy
  - Age of diagnosis 10-30 years
  - Progression to CRF

Some cases are misdiagnosed as having Alport syndrome!

- **Proteinuria and deafness**
  - Alport syndrome
  - A3243G tRNA<sup>Leu</sup>
  - COQ6
  - MYH9
  - ARHGDIA
  - INF2

- **Systematic mtDNA screening of Alport syndrome cohorts:**
  - 1/63 misdiagnosis (Jansen et al, JASN 1997)
  - 1/23 misdiagnosis (Cheong et al, Pediatr Nephrol 1999)
UK urine proteomic study in 117 adult patients (18-75 years)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial DNA defects:</td>
<td></td>
</tr>
<tr>
<td>m.3243A&gt;G</td>
<td>75 (64)</td>
</tr>
<tr>
<td>m.8344A&gt;G</td>
<td>16 (14)</td>
</tr>
<tr>
<td>other mtDNA point mutations</td>
<td>11 (9)</td>
</tr>
<tr>
<td>single, large scale mtDNA deletion</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Nuclear DNA defects:</td>
<td></td>
</tr>
<tr>
<td>POLG</td>
<td>4 (3)</td>
</tr>
<tr>
<td>C10orf2/PEO1</td>
<td>1 (1)</td>
</tr>
<tr>
<td>COX10</td>
<td>1 (1)</td>
</tr>
<tr>
<td>multiple mtDNA deletions</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenotype of m.3243A&gt;G</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic patients (N=75)</td>
<td></td>
</tr>
<tr>
<td>MELAS</td>
<td>14 (19)</td>
</tr>
<tr>
<td>MIDD</td>
<td>33 (44)</td>
</tr>
<tr>
<td>Other</td>
<td>28 (37)</td>
</tr>
<tr>
<td>Symptomatic patients (N=75)</td>
<td></td>
</tr>
<tr>
<td>Elevated urinary RBP</td>
<td>29 (39)</td>
</tr>
<tr>
<td>Elevated urinary albumn</td>
<td>23 (31)</td>
</tr>
<tr>
<td>Non symptomatic patients (N=20)</td>
<td></td>
</tr>
<tr>
<td>Elevated urinary RBP</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Elevated urinary albumn</td>
<td>3 (15)</td>
</tr>
</tbody>
</table>
### Deafness > Proteinuria > Diabetes Mellitus

**Table 1.** Interval (yr) between onset of proteinuria and recognition of extrarenal manifestations in nine adult patients with A3243G mtDNA mutation and renal involvement\(^a\)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Deafness</th>
<th>Neuromuscular Manifestations</th>
<th>Diabetes Mellitus</th>
<th>Macular Dystrophy</th>
<th>Cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-22</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>-5</td>
<td>0</td>
<td>+5</td>
<td>+5</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-6</td>
<td>-5</td>
<td>+2</td>
<td>-</td>
<td>+4</td>
</tr>
<tr>
<td>4</td>
<td>-15</td>
<td>+14</td>
<td>+12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-3</td>
<td>-</td>
<td>+5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-5</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+5</td>
<td>+12</td>
<td>+12</td>
<td>+12</td>
<td>+12</td>
</tr>
<tr>
<td>8</td>
<td>-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+6</td>
</tr>
<tr>
<td>9</td>
<td>-16</td>
<td>-1</td>
<td>-2</td>
<td>+7</td>
<td>+6</td>
</tr>
</tbody>
</table>

\(^a\) — No involvement.
Renal manifestations can be isolated

% of heteroplasmy:
- **Bl**: Blood
- **HF**: Hair follicle
- **BS**: Buccal swab
- **US**: Urine sample
- **RB**: Renal biopsy

A3243G MELAS mutation

Died at 50yrs of cancer
“Renal problems”

Died at 38yrs in a car accident
Renal failure

48yrs
- **Glucose intol.**
- **Hearing Loss**
- **Proteinuria**

Bl 40%
HF 35%
BS 25%
US 55%

Died at 32yrs
Renal failure

Bl 15%
HF 10%
BS 5%
US 65%

16yrs
- **Diabe**
- **Hearing Loss**
- **Renal Failure**

Bl 45%
HF 30%
BS 40%
US 65%
RB 80%

Courtesy Leonardo Salviati
A3243G tRNA\textsuperscript{Leu} mutation

Table 3. Renal pathology in five patients with A3243G mutation: diagnoses and semiquantitative lesion scoring\textsuperscript{a}

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of Glomeruli</th>
<th>Global Sclerosis</th>
<th>Segmental Sclerosis</th>
<th>Location of Segmental Sclerosis</th>
<th>Podocyte Hypertrophy</th>
<th>Tubulointerstitial Changes\textsuperscript{b}</th>
<th>Arteriolar Hyaline Thickening\textsuperscript{b}</th>
<th>Arterial Fibrous Intimal Thickening\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6 (60%)</td>
<td>3 (30%)</td>
<td>Parahilar, peripheral</td>
<td>+</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>3 (43%)</td>
<td>0 (0%)</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>Parahilar</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>–</td>
<td>+</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>3 (75%)</td>
<td>0 (0%)</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Kidney specimens were analyzed by light microscopy, using histologic sections at 3 to 4 microns and staining with hematoxylin and eosin, and PAS or silver. Grading of severity of interstitial or vascular fibrosis relied on the Banff 97 working classification (18).

\textsuperscript{b} 0, No; 1, mild; 2, moderate; 3, severe.

Guery et al, Kidney Int 2003
Coenzyme Q10 (ubiquinone)

- Discovered in 1957 by Fred Crane
- Available in food, but most originates from de novo synthesis
- Electron transporter in the mitochondrial respiratory chain
- Anti-oxidant
- Other functions...

Coenzyme Q10 biosynthesis

Regulatory enzymes
Coenzyme Q10 biosynthesis defects: many phenotypes

- Encephalopathy
  - Leigh-like
  - MELAS-like
  - Multiple System Atrophy –Like
  - Cerebellar Ataxia
  - Ponto-cerebella Hypoplasia
  - Seizures
  - Cognitive Impairment

- Renal involvement
  - Steroid Resistant Nephrotic syndrome (SRNS)
  - Tubulopathy

- Myopathy
  - No specific features, often lipid accumulation

- Sensory
  - Peripheral neuropathy
  - Hearing loss
  - Retinopathy
  - Optic atrophy

- Heart
  - Hypertrophic cardiomyopathy

- Liver
  - Liver failure
# Coenzyme Q10 biosynthesis defects: common features

<table>
<thead>
<tr>
<th>Neurological Symptoms</th>
<th>PDSS1</th>
<th>PDSS2</th>
<th>COQ2</th>
<th>COQ4</th>
<th>COQ6</th>
<th>COQ7</th>
<th>COQ9</th>
<th>ADCK3</th>
<th>ADCK4</th>
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<tbody>
<tr>
<td>Encephalomyopathy</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ataxia</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Dystonia and/or spasticity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Severe mental retardation</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deafness</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal Symptoms</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid-resistant NS</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tubulopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

**Early onset SRNS**

**Onset during childhood**
First reports of a primary coenzyme Q10 deficiency

**A Mutation in Para-Hydroxybenzoate-Polyprenyl Transferase (COQ2) Causes Primary Coenzyme Q₁₀ Deficiency**

Catarina Quinzii,¹ Ali Naini,¹ Leonardo Salviati,² Eva Trevisson,² Plácido Navas,³ Salvatore DiMauro,¹ and Michio Hirano¹

¹Department of Neurology, Columbia University College of Physicians and Surgeons, New York; ²Servizio di Genetica Clinica ed Epidemiologica, Department of Pediatrics, University of Padova, Padova, Italy; and ³Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Seville, Spain

- Infantile encephalopathy with renal dysfunction

**Prenylidiphosphate synthase, subunit 1 (PDSS1) and OH-benzoate polypreynyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders**

Julie Mollet,¹ Irina Giurgius,¹ Dimitri Schlemmer,¹ Gustav Diellner,² Dominique Chretien,¹ Agnès Dulahodde,³ Delphine Basq,⁴ Pascale de Lonlay,⁵ Arnold Munrich,⁵ and Agnès Rélig¹

- PDSS1: encephalopathy / no renal disease
- COQ2: encephalopathy / congenital nephrotic syndrome

*Am J Hum Gen 2006*

*J Clin Invest, 2007*
### Renal disease in COQ2 mutations

**Diomedi-Camassei et al, JASN 2007**

<table>
<thead>
<tr>
<th>Age onset (months)</th>
<th>Extra-renal symptoms</th>
<th>Renal symptoms</th>
<th>Renal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>None</td>
<td>SRNS</td>
<td>FSGS Collapsing</td>
</tr>
<tr>
<td>birth</td>
<td>Encephalopathy</td>
<td>ARF</td>
<td>Crescentic GN</td>
</tr>
<tr>
<td></td>
<td>Seizures</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypotonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Encephalopathy</td>
<td>SRNS</td>
<td>FSGS NOS</td>
</tr>
<tr>
<td></td>
<td>Seizures</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Optic nerve atrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>None</td>
<td>SRNS</td>
<td>FSGS NOS</td>
</tr>
</tbody>
</table>
Renal disease in COQ6 mutations

- 6 different mutations in the monooxygenase 6 gene (COQ6) in 13 subjects from 7 families
- Early-onset SRNS with sensorineural deafness
- Lack of complementation in coq6-deficient yeast
- Knockdown of coq6 causes apoptosis that is partially reversed by coenzyme Q10 treatment (podocyte & zebrafish embryos)
- coq6 is expressed in podocytes and in the stria vascularis of the inner ear in rats

Heeringa et al, J Clin Invest, 2011
Renal disease in PDSS2 mutations

- Leigh syndrome
- Nephrotic syndrome
- CoQ₁₀ deficiency (muscle and fibroblasts)
Prenyl-diphosphate synthase (Pdss2\textsuperscript{kd/kd}) conditional KO mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>Albuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>KO in podocytes</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>KO in tubular epithelium</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

**Pdss2\textsuperscript{kd/kd}**

CoQ deficiency: diffuse
↑ROS: only in the kidneys

Coenzyme Q10 treatment prevents the renal phenotype

Peng et al, PLoS Genetics, 2010
Quinzii et al, FASEB J 2013
ADCK4 (now COQ8B) defects

**ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ_{10} biosynthesis disruption**

- 2% of SRNS
- Mutations in the aarF domain containing kinase 4 (ADCK4)
- Low CoQ_{10} levels and RC activity in cells from mutated individuals
- KO of adck4 in zebrafish and Drosophila recapitulates the disease
- Most patients have no other significant symptoms

Ashraf et al, JCI 2013
Two cousins with COQ2 mutations that developed SRNS **in adolescence** had only **mild neurological symptoms**

One infant with COQ6 mutation presenting with SRNS at 8 months but **no deafness** and **no encephalopathy**
The spectrum of COQ2 mutations

- Multisystem atrophy
- Retinitis pigmentosa (6-7th decade)
- Fatal Multiorgan Failure (Birth)
- Isolated steroid resistant nephrotic syndrome (1st-2nd decade)
- Nephrotic syndrome
- Encephalomyopathy (LS) (2-3 months)

Graph showing the relationship between ATP, ROS, and residual CoQ activity.

- ↓↓↓ RC (ATP)
- ↑↑↑ ROS (ATP)
The spectrum of COQ2 mutations

- **Mild CoQ deficiency**: ± normal ATP, Moderate ROS
- **Intermediate CoQ deficiency**: Mild ATP ↓, High ROS
- **Severe CoQ deficiency**: Low ATP, Low ROS

- **Multisystem atrophy**: Nephrotic syndrome ± encephalomyopathy
- **Severe multiorgan failure**: ± encephalomyopathy

![Graph showing CoQ6 content (pmol/mg wet weight)]

- WT
- Met128Val
- Asn244Ser
- Tyr297Cys
- Ser466Asn
- Met182Arg
- Asn401Ilefs14
- Arg197His
- Ala302Val

- Multisystem atrophy
- Nephrotic syndrome ± encephalomyopathy
- Severe multiorgan failure

Courtesy L. Salviati
Clinical response to CoQ₁₀ therapy

COQ2 mutation

COQ6 mutation

ADCK4 mutation

Montini et al, NEJM 2008

Lovric et al, Nephrol Dial Transpl 2016

Korkmaz et al, JASN 2015
Several glomerular and tubular disorders associated with mitochondrial cytopathies have been reported in the past decades.

Mitochondrial defects should be suspected in patients that present with renal symptoms and multi-organ involvement.

Absence of other symptoms does not rule out a mitochondrial disorder; proteinuria may be the initial symptom.

t-RNALEU mutations cause FSGS and are frequently associated with deafness and diabetes mellitus.

Defects in CoQ10 biosynthesis respond to oral CoQ10 supplementation and should be suspected if EM studies show proliferation of abnormal mitochondria in podocytes.
Thank you

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