Mitochondrial Fatty Acid Oxidation Deficiencies
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Menu
- Biochemistry of mitochondrial fatty acid metabolism
- Fatty acid β-oxidation deficiencies
- Diagnostic strategies
- Molecular genetics and pathogenesis
- Prevalent mutations and diagnosis
- Conclusion

Enzymes and metabolites in mitochondrial fatty acid oxidation

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Enzymes and metabolites in mitochondrial fatty acid oxidation

Cell membrane
- RCOCoA
- Carnitine

Cytosol
- FATP
- RCOOH
- ACP

Mitochondria
- VLCAD
- ETF
- ETF DH
- MCAD
- SCAD
- LCAD
- CPTII
- CPTI
- CPT

Enzyme deficiencies in mitochondrial fatty acid oxidation

Cytosol
- Acetyl-CoA

MCAD: medium chain acyl-CoA dehydrogenase

SCAD: short chain acyl-CoA dehydrogenase

LCAD: long chain acyl-CoA dehydrogenase

ETF: electron transfer flavoprotein

ETF DH: electron transfer flavoprotein dehydrogenase

Discovery rate of mitochondrial fatty acid oxidation deficiencies

Year
Number of deficiencies
0 5 10 15 20 25

MCAD
ETF/ETF DH
CPTII
CAT
OPTI

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Elucidation sequence for MCAD deficiency
- Pt with recurrent hypoglycaemic attacks and lethargy
- Biochemical investigation; Dicarboxylic acid in urine
- Enzyme investigation; Decreased MCAD activity in cultured fibroblasts
- Gene investigation; Mutation in the MCAD gene

Discovery rate of mitochondrial fatty acid oxidation deficiencies
- MCAD
- ETF/ETF DH
- CPTI
- SCAD
- CACT
- LCHAD
- VLCAD
- VLCAD
- SCHAD

Strategy of investigation
- Clinical symptoms
  - Hypoketotic hypoglycaemia, vomiting, drowsiness or coma are common symptoms
  - Cardiomyopathy, hepatomegaly or muscle weakness may be indicative
  - Development delay, failure to thrive and feeding difficulties are also seen
- Metabolic investigations
  - Urine organic acids
  - Blood acyl-carnitines

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Gas chromatographic – mass spectrometric analysis of organic acid in urine

MCAD deficiency indicated

Strategy of investigation

- Clinical symptoms
- Metabolic investigations
  - Urine organic acids
  - Blood acyl-carnitines
- Enzyme determination
  - Pathway flux
  - Specific enzyme activity
- Genotyping
- Neonatal screening

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Neonatal screening of fatty acid oxidation defects

- Sufficiently high frequency
  MCADD: 1/10,000 - 50,000 in Caucasians
- Treatable disease
  Frequent meals and fasting avoidance
- Rapid and reliable analysis
  Tandem mass spectrometry

Strategy of investigation

- Clinical symptoms
- Metabolic investigations
  - Urine organic acids
  - Blood acyl-carnitines
- Enzyme determination
  - Pathway flux
  - Specific enzyme activity
- Genotyping
- Neonatal screening

Fatty acid oxidation genes with defects

<table>
<thead>
<tr>
<th>Gene</th>
<th>Structure</th>
<th>Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnitine transporter (CAT)</td>
<td>OCTN2</td>
<td>10</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase 1 (CPT I)</td>
<td>CPTA</td>
<td>18</td>
</tr>
<tr>
<td>Carnitine/acyl-carnitine transferase (CCT)</td>
<td>CACT</td>
<td>9</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase 2 (CPT II)</td>
<td>CPT2</td>
<td>5</td>
</tr>
<tr>
<td>Very-long-chain acyl-CoA dehydrogenase (VLCAD)</td>
<td>ACADVL</td>
<td>20</td>
</tr>
<tr>
<td>Mitochondrial trifunctional protein (MTP) (Long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD))</td>
<td>14HADH</td>
<td>20</td>
</tr>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase (MCAD)</td>
<td>14HADH</td>
<td>16</td>
</tr>
<tr>
<td>Short-chain acyl-CoA dehydrogenase (SCAD)</td>
<td>ACADS</td>
<td>12</td>
</tr>
<tr>
<td>Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCD)</td>
<td>14HADH</td>
<td>8</td>
</tr>
<tr>
<td>Electron transfer flavoprotein (ETF)</td>
<td>ETFB</td>
<td>5</td>
</tr>
<tr>
<td>Electron transfer flavoprotein ubiquinone oxidoreductase (ETF-UCO)</td>
<td>ETFQH</td>
<td>13</td>
</tr>
</tbody>
</table>

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Expression of MCAD variant proteins

MCAD cDNA containing plasmid

Expression of MCAD variant proteins

E. coli cell

The transformed E. coli cells were cultivated for 3 hr at 31°C and MCAD activity determined in extracts

In addition, the activity was determined after co-over-expression of GroESL (Hsp60/10)

GroESL (Hsp60/10) cDNA containing plasmid


Expression of MCAD activity in variant MCAD proteins without and with GroE

Folding mutations

Severe mutations

985A > G


Picture done by Peter Bross, Research Unit for Molecular Medicine
Expression of variant proteins

- Missense variant proteins may fold more or less efficiently, depending on the cellular conditions, such as chaperone efficiency.
- It is not possible to predict with any certainty the effect of a given missense gene variation, because the folding pathway is not yet predictable.

Disease-associated gene variations

- VLCAD
- MCAD
- SCAD

Missense variation
- In-frame ins/del
- Out-of-frame ins/del
- Stop codon
- Splice error

Prevalence of MCAD 985A>G

<table>
<thead>
<tr>
<th></th>
<th>G/G</th>
<th>G/non-G</th>
<th>Non-G/non-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>64</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>USA</td>
<td>74</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>18%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Prevalence of 985A>G alleles: 90%
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Frequency of MCAD 985A>G


Disease frequency: 1/29000

Diagnostic strategy for suspected MCAD deficiency

Patient
Clinical symptoms
Urine/blood abnormalities

985A>G assay

985G/985G

Heterozygous 985G or non 985G

MCAD gene sequencing

β-ox assay

985G/X or X/Y

Low MCAD

Diagnosis confirmed

Family study

Fatty acid oxidation genes with defects

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<td>MTPA4</td>
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<td>Medium-chain acyl-CoA dehydrogenase (MCAD)</td>
<td>ACADM</td>
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<td>Short-chain acyl-CoA dehydrogenase (SCAD)</td>
<td>ACADS</td>
</tr>
<tr>
<td>Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCSAD)</td>
<td>ACADH</td>
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<td>Electron transferring flavoprotein: ubiquinone oxidoreductase (ETF-UQ)</td>
<td>ETFUDH</td>
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Conclusion

- Fatty acid oxidation deficiencies (FAOD) are some of the most common metabolic disorders.
- FAOD may be severe, early onset, with life-threatening liver/heart symptoms, or they may be milder, later onset, with neuromuscular symptoms.
- FAOD are included in neonatal screening programs in many countries, especially because of the 'high' frequency of MCAD deficiency.
- Many different types of gene variations have been identified, some resulting in no protein, and many resulting in missense variant proteins, the amount of which are dependent on the cellular conditions.
- Prevalent mutations in some of the genes make molecular genetic diagnosis easy.