Confirmation the diagnosis by Reduced Very-long –chain Acyl-coA Dehydrogenase Activity in Newborns Identified by Newborn Screening.

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Neonatal Mass Screening in Flanders from 01/01/2012

Ministry of Health of the Flemish Community

PCMA vzw  VCBMA

27,000-40,000 newborns screened/lab/year
## Comparison: Core panel USA-Flanders

<table>
<thead>
<tr>
<th>OA</th>
<th>FAO</th>
<th>AA</th>
<th>Hb</th>
<th>other</th>
<th>OA</th>
<th>FAO</th>
<th>AA</th>
<th>Hb</th>
<th>other</th>
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</thead>
<tbody>
<tr>
<td>IVA</td>
<td>MCAD</td>
<td>PKU</td>
<td>HbSS</td>
<td>CH</td>
<td>IVA</td>
<td>MCAD</td>
<td>PKU</td>
<td>HbSS</td>
<td>CH</td>
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<tr>
<td>GA1</td>
<td>VLCAD</td>
<td>MSUD</td>
<td>HbS/b</td>
<td>CAH</td>
<td>GA1</td>
<td>VLCAD</td>
<td>MSUD</td>
<td>HbS/b</td>
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<td>HMG</td>
<td>LCHAD</td>
<td>HCY</td>
<td>HbS/C</td>
<td>BIOT</td>
<td>HMG</td>
<td>LCHAD</td>
<td>HCY</td>
<td>HbS/C</td>
<td>BIOT</td>
</tr>
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<td>TFP</td>
<td>CIT1</td>
<td>GALT</td>
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<td>TFP</td>
<td>GALT</td>
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<tr>
<td>MMA</td>
<td>CUD</td>
<td>ASA</td>
<td>HEAR</td>
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<td>MMA</td>
<td>CUD?</td>
<td>HEAR</td>
<td></td>
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<tr>
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<td>TYR1</td>
<td>CF</td>
<td></td>
<td></td>
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<td>TYR1</td>
<td>CF</td>
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<tr>
<td>BKT</td>
<td>MADD</td>
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<td></td>
<td>BKT</td>
<td>MADD</td>
<td></td>
<td></td>
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</tbody>
</table>

**Mandatory screening panel**
Mitochondrial fatty acid β-oxidation

Mitochondrial fatty acid \( \beta \)-oxidation

- Provides energy in the postabsorptive and fasted state
- Important energy source for the heart
- Important during exercise in skeletal muscle

Mitochondrial fatty acid β-oxidation
Mitochondrial fatty acid β-oxidation

Provides energy in the postabsorptive and fasted state

Hypoketotic hypoglycemia

Important energy source for the heart

Cardiac disease

Important during exercise in skeletal muscle

Hypotonia;

Myopathy and

Rhabdomyolysis
Substrate specificity of the different beta-oxidation enzymes

R. Wanders et al. J Inherit Metab Dis (2010); 33: 479-494
Acylcarnitines in fatty acid oxidation disorders

- **VLCAD deficiency**
  - C14:2, C14:1, C14 (C14:1/C16)

- **MCAD deficiency**
  - C6, C8, C10:1, C10

- **LCHAD / MTP deficiency**
  - C16:1OH, C16OH, C18:1OH, C18OH

Used by Permission by S. Houten, Metabolics.be 2012
Incidence of MCADD 1:14,000 ~PKU

High incidence of unexpected mutation frequencies found by neonatal screening:

- Pennsylvania, USA
- Sydney, Australia

B. Wilcken: "It is not yet clear which patients (MCADD “variants”) with disorders diagnosed by such screening would have become symptomatic if screening had not been performed".
Screening Algorithm VLCADD
Methods: MS/MS Xevo (Waters); Neobase kits (Non-derivatized method; PerkinElmer).
Screening parameter C14:1 acylcarnitine (cutoff: <0.39 µmol/L).

Results:
- The bloodsampling takes place between 3-4 days postpartum.
- The recall rate is 0.07%. The false positives have a C14:1 median concentration of 0.52 µmol/L (range 0.43-0.87).
- Specificity: 99.93%.
- In 2012 the two first patients affected by VLCADD were found in our population. Two newborn girls were found with a C14:1 acylcarnitine concentration of 1.33 and 3.59 µmol/L, resp. The C14:1/C2 ratio was elevated in both patients (normal range <0.023).
## Screening Results The Netherlands 2007-2012

### VLCAD-patiënts 2007 - 06-2012 (EHP: 1.012.408)

<table>
<thead>
<tr>
<th>Year</th>
<th>C14:1</th>
<th>Confirmed</th>
<th>Missed</th>
<th>Extra det.</th>
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<tr>
<td>2007</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2008</td>
<td>3+1(^1) = 4</td>
<td>3+1(^1) = 4</td>
<td>0</td>
<td>3 - 1 = 2 (^1)</td>
</tr>
<tr>
<td>2009</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2010</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2011</td>
<td>3</td>
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<td>onbekend</td>
</tr>
<tr>
<td>06-2012</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>onbekend</td>
</tr>
<tr>
<td>Totaal</td>
<td>16</td>
<td>9</td>
<td>0</td>
<td>4 (^1)</td>
</tr>
</tbody>
</table>

\(^1\) één kind met C14:1=0.70; zou met huidige afkappgrens ook gevonden zijn.

13 VLCAD

Used by Permission of P. Schiele, RIVM; BeNeLux 2013
Revision of Screening Strategy

VLCAD deficiency
- C14:1, C14:2, C14, C16
- C14:1/C16 Ratio

VLCAD deficiency
- **C14:1**
- **C14:1/C2 Ratio**
- C14:2, C14, C16
- (C12, C12:1)
- C14:1/C16 Ratio
Revision of Screening Strategy

VLCAD deficiency
• C14:1, C14:2, C14, C16
• C14:1/C16 Ratio

VLCAD deficiency
• **C14:1**
• **C14:1/C2 Ratio**

No prediction of being at risk to develop clinical disease
A functional enzyme activity assay is the only reliable method to predict the clinical course in patients with VLCADD detected by newborn screening: patients showing a <10% residual enzyme activity are at risk to develop clinical disease (*).


(* U. Spiekerkoetter, Duesseldorf, Germany, 2009)
Confirmation of diagnosis

- The diagnosis was confirmed by enzyme activity measurement in lymphocytes (AMC, Amsterdam, The Netherlands).
- The residual enzyme activity of VLCADD was 0.61 and 0.24 nmol/min/mg protein, resp (controls: 1.84-4.80 nmol/min/mg protein; 10% enzyme activity = 0.66).
Treatment; Follow-up
Clinical outcome

• Both patients are asymptomatic, including normal cardiac findings, at the age of diagnosis (4-6 weeks) and follow-up during 12 months;
• The patient with the lowest enzyme activity was put on a strict diet:
  – Normal muscle tone;
  – Normal motor development;
  – 1 hospitalisation during an episode of vomiting
  • **AVOID CATABOLISM**
• The other patient is carefully followed up in time under no dietary restriction of long-chain fatty acids
• No free L-carnitine deficiency

Conclusions

• VLCADD has a wide clinical spectrum ranging from cardiomyopathy in infants to episodic rhabdomyolysis and exercise intolerance in adolescents;

• Neonatal screening has shown that VLCAD deficiency is the second commonest fatty acid oxidation disorder in Europe and the USA, with a prevalence between 1:50,000 and 1:100,000. This is much higher than what was detected clinically;

• Newborn screening of VLCADD is performed by MS/MS: parameters C14/1 and/or C14:1/C2 ratio;
Conclusions Ctd

• Confirmation of disease: enzymatic assay of VLCADD in lymphocytes (DNA analysis);
• The severely affected infants are treated with a formula enriched with Medium Chain fatty acids (MCT) and breast feeding is avoided. The mildly affected patients do not need a special diet;
• Early dietary intervention improves the outcome of severely affected patients.
Acknowledgements

• *PCMA vzw team*
• *Local Government of the province of Antwerp*
• *Minister of Health of the Flemish Community and Administration*
• *All colleagues from The Newborn Screening centers of Belgium, The Netherlands, (Peter Schiele, RIVM) , Luxembourg, Vienna, Washington and CDC*
• *Ron Wanders, AMC, Amsterdam, The Netherlands*
• *PerkinElmer, Waters*
THANK YOU