# Neonatal Screening: Possibilities and Problems

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Neugeborenen Screening
Dépistage Néonatal
Screening Neonatale
Screening dal Novnaschi

CHILDREN'S RESEARCH CENTER (CRC)

University of Zurich[UZH]

# Presentation Outline

- What is Neonatal Screening (NBS)
- What can / should be screened
- NBS challenges

# Neonatal Screening

- Neonatal Screening is a combination of procedures, laboratory tests, diagnostics and therapeutic measures which aim at a presymptomatic recognition of individuals affected by a frequent, severe but usually treatable disease.
- *Neonatal Screening is a public health activity.*

# Neonatal Screening

**CAVE**

Newborn Screening is NOT a diagnostic procedure!!

A pathological screening result must always be confirmed by standard diagnostic procedures.

# Neonatal Screening

- An efficient NBS is operated as a partnership between different professionals:
  - *Sample collection*
    - Midwives, Nurses and MD's in Hospitals and / or at home
  - *Analysis / Result interpretation / First intervention*
    - Screening Center and Laboratory
  - *Diagnosis Confirmation / Treatment / Follow-up*
    - Medical specialist for the disease screened

# Neonatal Screening

- Some of the disorders most frequently included in NBS panels

*Biochemical Tests*

- PKU
- CH
- Biotinidase Deficiency
- Galactosaemia
- CAH
- G6PD
- CF

# Expansion of Neonatal Screening

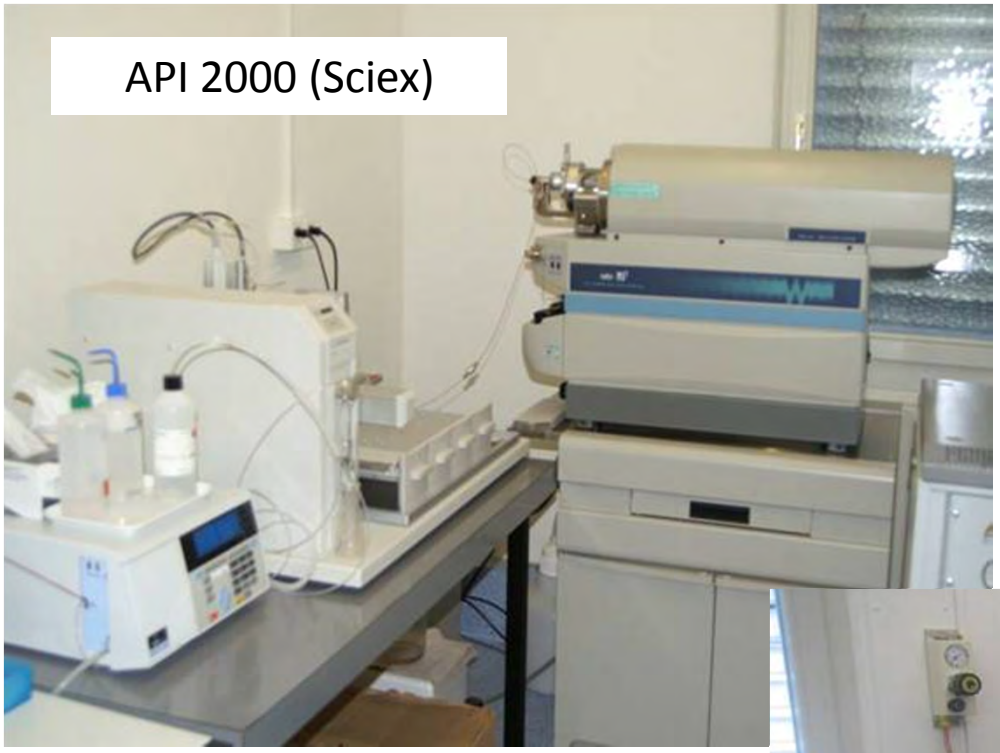
- Under expansion of Neonatal Screening we usually understand the addition of MS/MS technology to an established NBS program with the aim of detecting a wide range of metabolic disorders…but
- It is not excluded that in future other technologies will be used to “expand” NBS

# Why expand

- New treatment possibilities are being introduced
- New diagnostic procedures are being developed
- New analytical methodologies are available

***More possibilities to help neonates with severe conditions***

# MS/MS instruments




# Neonatal Screening

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*Biochemical Tests*

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*MSMS Tests*

- PKU
- MCAD
- GA-I
- MSUD

# Neonatal Screening and MS/MS

- Applicability to dried blood samples first described in 1990 by Millington et. al (JIMD 1990 13:321-324)
- Method further developed by Rashed in 1994
- End of 1990's start of first regional and/or national programs for "Expanded Neonatal Screening"

# Neonatal Screening and MS/MS

- MS/MS is fast becoming integrated routinely into Neonatal Screening
- As a consequence many disorders that were previously inaccessible have been included in NBS programs
- MS/MS is the first multiplex technology leading to expansion and to the inclusion of many potentially treatable condition in current Neonatal Screening programs

# What do we get with MS/MS?

- Multiplex assay:
  - Amino acids and Acylcarnitines measured simultaneously
- High specificity and sensitivity
- Good precision and reproducibility
- Large number of analytical results
  - Data management becomes indispensable in order to avoid errors

# What is different

- Change from single analyte to multi-analyte assays
  - Advantages:
    - Multiple parameters available at the same time for assessment
    - Time and cost savings
    - Also very rare diseases can be included in NBS
    - Decision making *should* become simpler

# What is different

- Change from single analyte to multi-analyte assays
  - Disadvantage:
    - Instrumentation expensive
    - Reagents (Internal Standards) expensive
    - If “Home Brew” assays are used it is necessary to have a MS/MS specialist in the team

# How to expand

- Clinical aspects
  - Disorders to be included

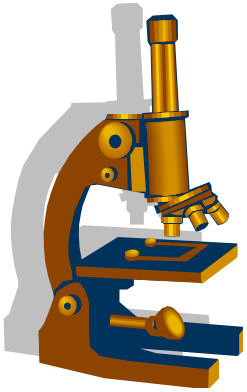
# Disorder Selection

**Should be Evidence Based not technology driven!**

# Disorder Selection

- Selection of diseases to be included:
  - How many?
    - Quality or Quantity?
  - Care should be taken to avoid choosing non-diseases
  - What about non-treatable conditions?
  - Disorder relevant for the population screened

# Interpretation of Results



Multiplex Assay:

Several Results per Disease

# MS/MS Data Management

- The introduction of MS/MS technology has resulted in a quantum leap in the ability of early detection of Inborn Errors in neonates
- The “flood” of data generated by MS/MS must be carefully managed in order to obtain the positive effects and to reduce errors and misinterpretation of results

# Lessons Learnt

- There is no diagnostic significance to isolated mild elevation of single analytes like…..
  - C6, C10, C12, C12:1, C14, C18:1 or C18:2

# Lessons Learnt

- But the strength of the multi-parameter analysis should be used:
  - Use all possible parameter
  - Group them per disorder
  - Be careful when looking at one single parameter
- If necessary perform second tier tests to confirm the first results

# Second Tier Assays

- As a general rule the availability of more than one measurement increases in most cases the reliability of a screening program
- But there are consequences that have to be taken into account
  - Delay in reporting results, Logistics, Costs…….

# Second Tier Testing

## MSUD

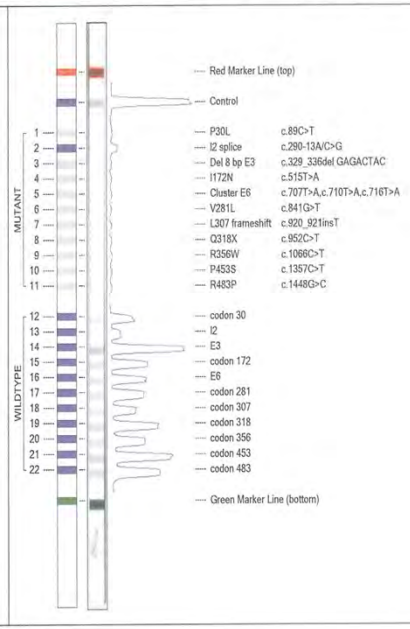
### Second-Tier Test for Quantification of Alloisoleucine and Branched-Chain Amino Acids in Dried Blood Spots to Improve Newborn Screening for Maple Syrup Urine Disease (MSUD)

**Devin Oglesbee[1,2], Karen A. Sanders[1], Jean M. Lacey[1], Mark J. Magera[1], Bruno Casetta[4], Kevin A. Strauss[5], Silvia Tortorelli[1,2], Piero Rinaldo[1,2,3] and Dietrich Matern[1,2,3,a]**

Departments of[1] Laboratory Medicine and Pathology,[2] Medical Genetics, and[3] Pediatric and Adolescent Medicine, Mayo Clinic College of Medicine, Rochester, MN;[4] Applied Biosystems, Monza, Italy;[5] The Clinic for Special Children, Strasburg, PA.

[a]Address correspondence to this author at Biochemical Genetics Laboratory, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Fax 507-266-2888; e-mail matern@mayo.edu.

# Second Tier DNA Testing for CAH

| | |
|---|---|
| Assay Type | CAH StripAssay<br>Catalog No. 4-380, Revision 05/2011<br>Assay for the molecular analysis of mutations associated with congenital adrenal hyperplasia (CAH) |
| Lot Number | - |
| Assay ID | 2013-01-31 14:19 |
| Assay Date | Thursday, January 31, 2013 - 2:19:41 PM |
| Operator | - |
| Sample ID | **Strip 1** |
| Patient | A837 |
| Result | **I2 splice heterozygous** |
| Details | CYP21A2:<br>P30L normal, I2 splice heterozygous, Del 8bp E3 normal, I172N normal, Cluster E6 normal, V281L normal, L307 frameshift normal, Q318X normal, R356W normal, P453S normal, R483P normal |
| Strip |  |



| | |
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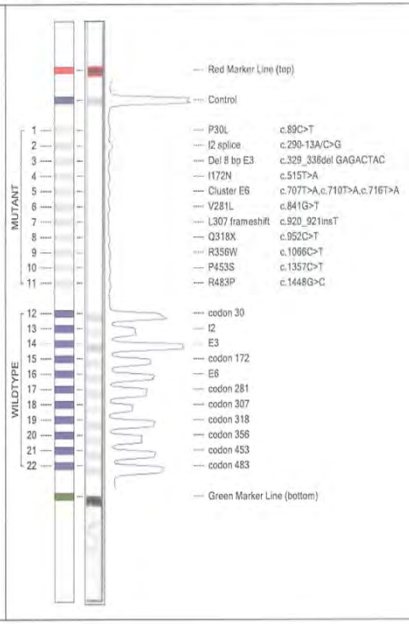
| Lot Number | - |
| Assay ID | 2013-01-31 14:19 |
| Assay Date | Thursday, January 31, 2013 - 2:19:41 PM |
| Operator | - |
| Sample ID | **Strip 5** |
| Patient | A844 |
| Result | **normal** |
| Details | CYP21A2:<br>P30L normal, I2 splice normal, Del 8bp E3 normal, I172N normal, Cluster E6 normal, V281L normal, L307 frameshift normal, Q318X normal, R356W normal, P453S normal, R483P normal |
| Strip |  |

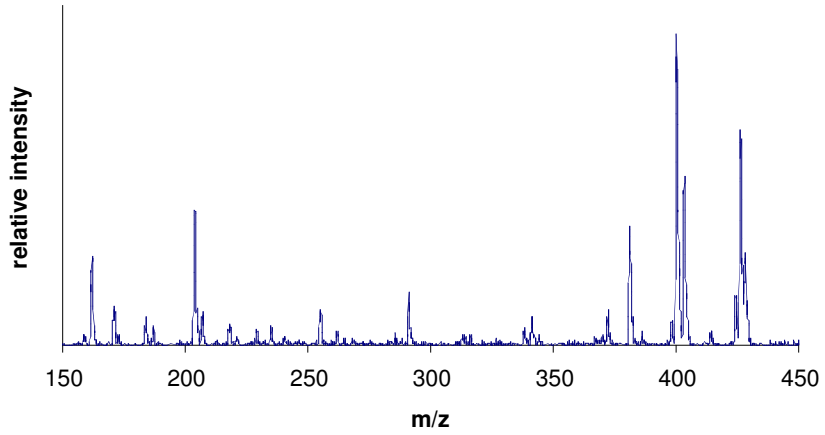
# Expanded NBS: advantages and challenges

- The daily practical experiences in NBS present with many more big and small problems
- Not everything is "Black or White"
- It is the gray area that poses most of the challenges

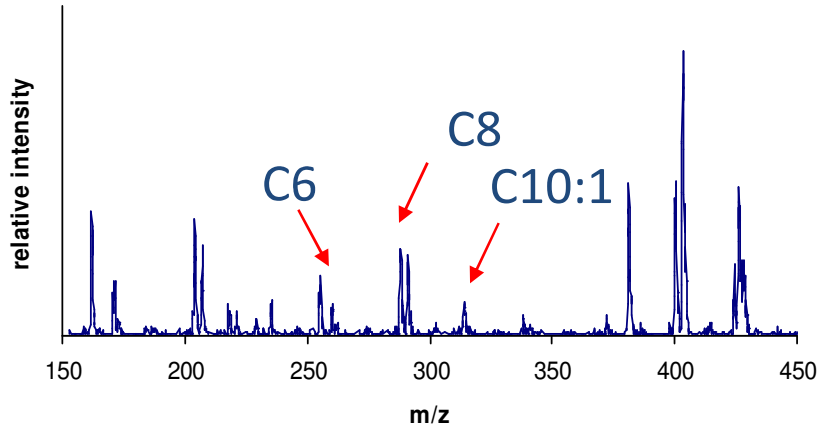
# Case A: Boy, 7 Months

- Born before MS/MS NBS implemented
  - Presentation: Vomiting, light fever
    - Pediatrician: enterogastritis → Paracetamol
  - Boy found dead in bed the next morning
  - PM: fatty liver and other organs

→ FAO Defect?




Normal




Patient
(Original NBS specimen)

| | |
|---|---|
| C6 | Hexanoylcarnitin |
| C8 | Octanoylcarnitin |
| C10:1 | Decenoylcarnitin |

**→ typical profile of MCAD-deficiency**

(**M**edium **C**hain **A**cyl-CoA **D**ehydrogenase)

# MCAD - Deficiency

## Symptoms

- Catabolism → Accumulation of medium chain fatty acids
- Lethargy, hypokethotic hypoglycemia, coma
- Usually appears in the first 3 years of life.
- Neurological late damage after the first presentation

## Therapy

- Avoiding prolonged fasting periods
- Meals at regular intervals
- Carnitine per os (50mg/kg/d, doubled if necessary)

# Case B1: Boy

- At 40h: Cardiac arrest
  $\rightarrow$ Cardiopulmonary resuscitation
- At 4: Weeks discharged

- At 2 Months
  – vomiting + diarrhea
  – hypotonia, lethargy, hepatomegaly

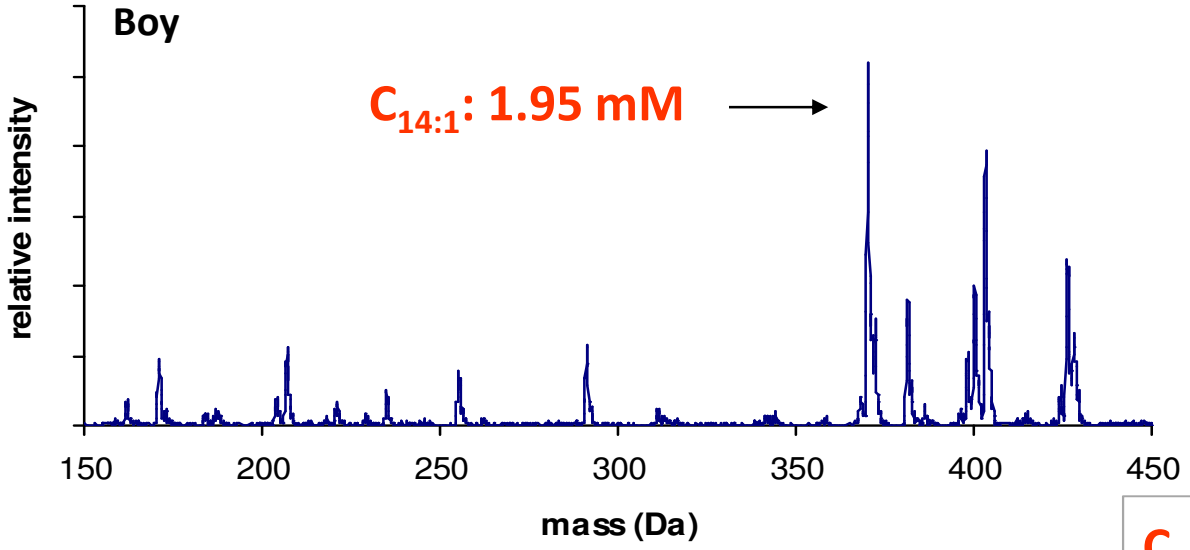
# Case B1: Laboratory findings

- Severe carnitine deficiency
- Abnormal organic acids
  - Urine: $C_6$, $C_8$, $C_{10}$
  - Plasma: $C_{12}$, $C_{14}$, $C_{16}$
- Dd: defect in longchain fatty acids metabolism?
  VLCAD?
- Outcome:
  - Delayed psychomotor development

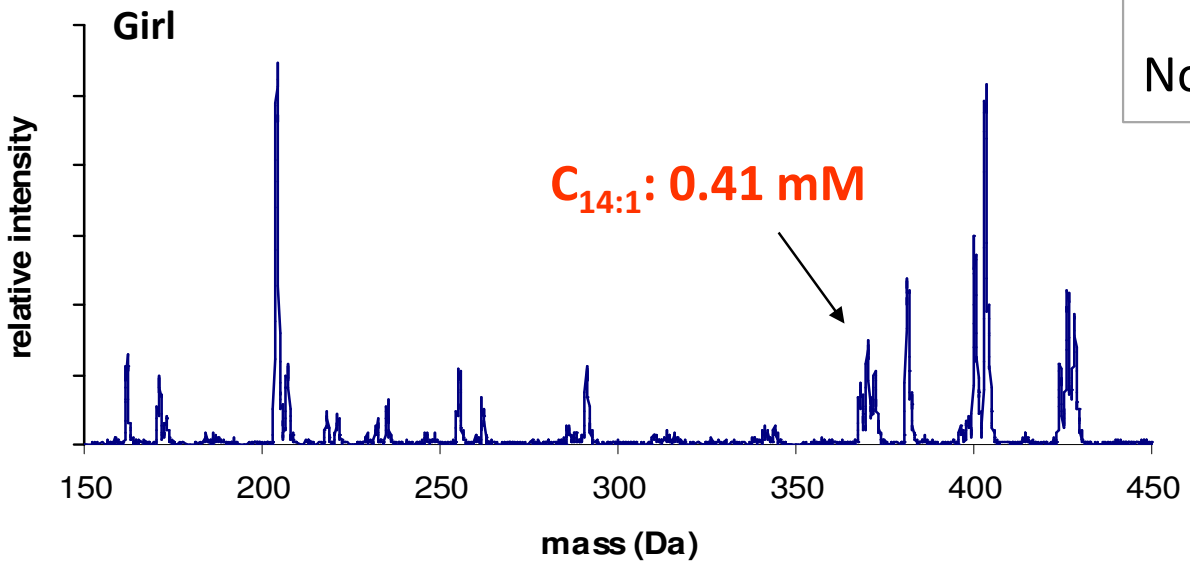
# Case B2: Girl

- Presentation:
  - Perinatal Asphyxia, APGAR 3/5/5
  - CPK day 2 ↑↑ / Acylcarnitine-Profile: C14:1 ↑
- Dd: VLCAD-Deficiency
  (Very Long Chain Acyl-CoA Dehydrogenase)
- Outcome:
  - Normal psychomotor development

# VLCAD - Deficiency




"severe"




"mild"

$C_{14:1}$

Normal: ~ 0.04 mM

# VLCAD-Deficiency: NBS limits

- Boy
  - Symptoms present at birth (before NBS)
  - Frequently hospitalized due to metabolic crisis
  - Psychomotoric development delayed
  - Outlook unclear

- Girl
  - Incidental finding
  - Outlook good, therapy necessary?

# Neonatal Screening (NBS)

- NBS is a very effective tool for identifying infants affected by severe and sometimes life-threatening disorders
- The use of advanced analytical tools like MS/MS is not only a great advantage but also a challenge
- Only a careful use of these technique will avoid harm and generate great benefit

# Children´s Hospital Zürich

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