Expanding Newborn Screening
Fiction and Facts

Early information of parents and organisation of the postanalytical phase are crucial for successful implementation.
De organisatie met terreinwerkzaamheden voor de uitvoering van het Vlaams bevolkingsonderzoek naar aangeboren aandoeningen bij pasgeboren via een bloedstaal zijn:

Provinciaal Centrum voor opsporing van metabole aandoeningen (PCMA)
Deurnestraat 201
2670 Wilrijk
Tel. 03 765 90 20
info@pcma.provint.be

Vlaamse Centrum Brussel voor opsporing van aangeboren metabole aandoeningen (VCMA)
UZ Brussel, Kinderteknikkliniek
Laarbeeklaan 201
1000 Brussel
Tel: 02 474 6260 of 02 474 5511
kinderheelvaart@uz.brussels

Contact:
Koning Albert I-laan 95, box 33
1050 Brussels
Tel. 02 622 18 99
proevenlegen@pcma.provint.be

Vlaams bevolkingsonderzoek naar aangeboren aandoeningen bij pasgeboren via een bloedstaal

F. Eyskens, MD, PhD
1,2 PCMA vzw, Antwerp, Belgium
Neonatal Mass Screening in Flanders from 01/01/2012

Ministry of Health of the Flemish Community

PCMA vzw  VCBMA

40,000-50,000 newborns screened/lab/year
Proposed mass screening program

• Phenylketonuria/Hyperphenylalaninemia
• Congenital Hypothyroidism
• Congenital adrenal hyperplasia
• Biotinidase deficiency
• MCADD, MADD, MMA, PA, GA1, IVA, MSUD

Proposed selective screening
• Galactosemia

New pilot screening programs:
• Lysosomal Storage Diseases
• Duchenne Muscular Dystrophy
• Cystic Fibrosis

* Pilot study from 1 may 2002-30 april 2004, implemented in Flanders
Screening Program from January 2007

IRT (stop: 2003)
IRT screening: Results

- Total number screened (1987-1999): 372,674
- CF patients found: 92 (prevalence: 1/4000)
- False positives: 2,445 (0.7-1.25%)
- False negatives: 8 (Delfia: 6)
- Sensitivity (%): 92
- Specificity (%): 99.3
- Positive predictive value: 3.6 %
- Negative predictive value: 99.99%
CRITERIA for Screening Programs (WHO: Wilson & Jungner)

- Important health problem
- Asymptomatic stage in natural evolution
- Accepted treatment for patients
  - *Secondary prevention by genetic counseling and prenatal diagnosis*
- Facilities for further diagnosis
- Suitable test available: simple, reasonably priced, repeatable, sensitive, specific, acceptable
- Natural history of the disease is well known
- Screening should be a continuous process
Recycling activity of the vitamin biotin
MULTIPLE CARBOXYLASE DEFICIENCY

Valine
Isoleucine
Methionine
Threonine
Odd Chain FFA
Cholesterol

Glucose

Leucine

3-hydroxyisovaleric, C5-OH

3-methylcrotonylcoA

3-methylglutaconylcoA

Fatty acids

Pyruvate

Krebs Cycle

3-methylcrotonylcoA

AcetylcoA

MalonylcoA

Ketone bodies, C2

PropionylcoA, C3

PCC

Lactate

SuccinylcoA

MethylmalonylcoA

Methylcitric acid
Propionylglycine
3-Hydroxy-n-valeric acid
Tiglylglycine, C5:1
Screening: BIOTINIDASE DEFICIENCY

Not for public use
Neonatal mass screening for biotinidase deficiency in the province of Antwerp.

1988-2013 (included in screening program of Flanders since 2007).

- False-positive rate (prematures): 0.01%
- False-negative results: none reported
- Sensitivity (%): 100
- Specificity (%): 99.99
- Positive predictive value (%): 39
- Prevalence of profound deficiency: 1:38,000
One Test-One Analyte - One Disease

One Test-Multiple Analytes - Several Diseases
Neonatal mass screening by multiplex technology

FIA-tandem MS,
LC-MS-MS
Newborn Screening: Toward a Uniform Screening Panel and System—Executive Summary

Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, R. Rodney Howell, MD, American College of Medical Genetics Newborn Screening Expert Group
### Comparison: Core panel USA-Flanders

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MS/MS Broad screening, Brussels 1999-2004 versus well-defined screening program, Antwerp 2002-2008
120,000-185,000 newborns screened

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State Newborn Screening in the Tandem Mass Spectrometry Era: More Tests, More False-Positive Results

Beth A. Tarini, MDab, Dimitri A. Christakis, MD, MPHabc, H. Gilbert Welch, MD, MPHde

abRobert Wood Johnson Clinical Scholars Program, bDepartment of Pediatrics, and cChild Health Institute, University of Washington, Seattle, Washington; dVeterans Affairs Outcomes Group, White River Junction, Vermont; eCenter for the Evaluative Clinical Sciences, Dartmouth Medical School, Hanover, New Hampshire
Mitochondrial fatty acid β-oxidation

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

Substrate specificity of the different beta-oxidation enzymes

A. Acyl-CoA dehydrogenases

B. Enoyl-CoA hydratases

C. 3-Hydroxyacyl-CoA dehydrogenases

D. 3-Ketoacyl-CoA thiolases

R. Wanders et al. J Inherit Metab Dis (2010); 33: 479-494
Normal vs MCAD deficiency

- C₂ carnitine
- C₃ carnitine
- C₈ carnitine
- C₁₀:₁ carnitine
- C₁₆ carnitine

Normal vs MCAD deficiency
Molecular genetic analysis: confirming diagnosis

The most common mutation found in MCADD before screening

Detection of the G985 mutation by PCR

63 bp
43 bp

Control
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".
- E.M. Maier, Germany, EMG 2009: novel missense mutations and protein misfolding
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

Control

Patient

Used by Permission by S. Houten, Metabolics.be 2012
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
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)
The diagnosis was confirmed by enzyme activity measurement in lymphocytes (AMC, Amsterdam, The Netherlands).

The residual enzyme activity of VLCADD was 0.61, 0.24 and <0.17 nmol/min/mg protein, resp.

(controls: 1.84-4.80 nmol/min/mg protein; 10% enzyme activity = 0.66).
CONFIRMATION OF DIAGNOSIS & Follow-up

• MS/MS is screening

• The diagnosis is made by:
  ▪ Organic acid analysis by GC-MS
  ▪ Amino acid analysis by liquid chromatography
  ▪ Enzymatic tests in tissues
  ▪ Molecular genetic analysis

• DO NOT SCREEN IF YOU ARE UNABLE TO PERFORM FURTHER INVESTIGATIONS AND FOLLOW-UP OF PATIENTS AND PARENTS
Confirmatory analysis: MS/MS is doing it by itself

Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: The Mayo Clinic experience (2004–2007)

D. Matern · S. Tortorelli · D. Oglesbee · D. Gavrilov · P. Rinaldo
Europe plays catch-up on neonatal screening as US skips ahead

David Holmes
EQUITY

- Demand
- Need

Information
Epidemiology
Registries

Disease confirmation
Clinical utility
Treatment
Expert Centers

Technology
Training

Politics/Ethics
Cost-effectiveness
Quality assurance

SUSTAINABILITY
Scoring according to test availability

newborn screening expert group of the American College of Medical Genetics
SHOULD WE SCREEN FOR (TREATABLE) LSDs?

MPSI, II, IVA and VI

Pompe
Infantile-Onset Pompe Disease

Head Lag
Infantile-Onset Pompe Disease

Cardiac Manifestations
FIGURE 4 Survival and motor outcomes for patients whose disease was detected by newborn screening compared with those whose disease was diagnosed clinically

Screening improves outcome

Disease Progression: Severe MPS I

10 months

12 months

22 months

34 months

39 months
short stature (122.4 cm)  
underweight (BMI 17.7)

coarse facial features  
bossing forehead  
protrusion of the eyes  
glue ears & SNHL  
full cheeks & thick lips  
underdeveloped secondary sexual characteristics  
thoracic kyphosis  
hepatomegaly  
dysostosis multiplex  
cervical kyphosis  
elbow flexion contracture  
bilateral limited abduction hips  
camptobrachydactyly (claw hands)  
flexed-knees  

motoric decline: e.g. reduced walking endurance  
shortened Achilles tendon  
mild bilateral macular degenerative changes  
hypertrophied mucous membranes nose/mouth  
severe OSA  
bilateral limited abduction shoulder joint  
mild valvular disease (thickening aortic, mitral)  
severe restrictive and obstructive lung disease (VC 39% pred. value)  

SNHL: sensorineural hearing loss  
OSA: obstructive sleep apnea  
VC: vital capacity
Presented at Department of Radiology with knee trauma

-- > conclusion:

✓ no fracture
✓ suspicion of skeletal dysplasia

= indication for a genetic referral

c.937C>G [p.P313A]

① Homozygous missense mutation in the ARSB gene

② Urine GAG analysis (DMB test)

24.5 mg GAG/mmol creatinine (reference values 0.6–2.6)

③ Separation of the GAG by electrophoresis

presence of dermatan sulphate
absence of heparan sulphate

④ Leucocyte arylsulfatase B (ASB) activity

<0.5 nmol/mg/min (reference values 2.2–18.6)
Short Stature (MPS VI)

Patients With Maroteaux-Lamy Syndrome (MPS VI). This inherited disease is estimated to affect about 1,100 people worldwide.²

The growth chart of a patient with MPS VI (open circles). Enzyme-replacement therapy was started at 7 years of age (arrow).

Morquio A: Musculoskeletal manifestations

- Skeletal dysplasia
  - Spinal abnormalities
  - Pectus carinatum
  - Hip dysplasia
  - Genu valgum
  - Ankle valgus
  - Hand abnormalities
  - Flat facial features
  - Mandibular protrusion
- Short stature
- Joint subluxation
- Joint instability
- Joint degeneration
- Abnormal gait
- Weak hand grip

Management includes regular imaging studies and corrective surgery, physical therapy, pain management as needed, and walker/wheelchair use

Left image: Kalteis et al, *Arthroscopy*, 2005

LSD incidence:

Current view of LSD incidence underestimated:

- Incidence of Fabry in Italy: 1/3100 births (Spada et al, 2006, Am J Hum Genet)
- Incidence of Fabry in Taiwan: 1/1250 (Lin et al, 2010, J Inherit Metab Dis)
- Incidence of Pompe in Taiwan: 1/41000 (Chien et al, 2009, Pediatrics)
- Incidence of 1 per 2315 births (3 LSD) (Mechtler et al, 2012, Lancet)
  - Gaucher: 1/17000
  - Pompe: 1/8700
  - Fabry: 1/3900
- Incidence of Fabry, Pompe, and MPS-I is estimated at 1/7500 births (3 LSD) (Scott et al, 2013, J Pediatr)
  - Fabry: 1/7800
  - Pompe: 1/27800
  - MPS-I: 1/35500
Available Techniques

- **Chamoles method (2001):**
  - Fluorescence/enzymatic assay
  - Single assay-Single disease model

- **Meikle et al (Hopwood)(2004-2006):**
  - Multiplexed immune quantification
  - Specific antibodies-two-tier approach
  - Low sensitivity for detection of Pompe & Gaucher

- **Gelb/Li et al (2004); Genzyme (Zhang et al)(2008)**
  - ESI-MS/MS
  - Analytically multiplex screening
  - MPSI,II,VI, Pompe, Fabry, Gaucher, Niemann-Pick, Krabbe: specific substrates and Internal Standards
  - ExtQC-CDC

- **Millington**
  - Digital microfluidics platform
  - =multiplex platform of Chamoles method
  - MPSI, VII, Pompe, Fabry
NBS for LSD worldwide:

- USA (Spacil 2013 et al, Clin Chem)
  - Washington: pilot for Pompe, Fabry, Gaucher, Krabbe, Niemann-Pick A/B, MPS-I, MPS-II, MPS-IVA and MPS-VI
  - New York – Krabbe screening
  - several states passed legislation to include LSD screening

- Taiwan – Pompe screening (Chien et al, 2009, Pediatrics)

- Austria – Vienna: anonymous study for Gaucher, Pompe, Fabry and Niemann-Pick A/B (Mechtler et al, 2012, Lancet)
<table>
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<th>Fluorescence Enzymatic assay</th>
<th>ESI-MS/MS Enzymatic assay</th>
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<td>10,279</td>
</tr>
<tr>
<td>Recall rate %</td>
<td>0.82</td>
<td>0.039</td>
</tr>
<tr>
<td>False positives</td>
<td>117</td>
<td>4</td>
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Technological milestones:

- Mechtler et al. (J of Chromatogr B, 2011)
  - Abolish liquid-liquid and solid phase extraction
  - Short incubation (3h)
  - Simultaneous quantification of five LSDs
    - DBS punch and buffer 1: Gaucher, Niemann-Pick A/B, Pompe and Fabry diseases
    - DBS punch and buffer 2: MPS-I
  - Turboflow – UHPLC-MS/MS (no solid phase extraction and substrate interference)
  - Cost (without MPS-I) = 1 euro/sample

→ Disadvantages for PCMA
  - Some LSDs of interest are not included
  - 2 DBS and buffers required
How to proceed:

• According to protocol Spacil et al. (Clin Chem, 2013):
  • Assay of 7 enzymes in 1 or 2 buffers, with 1 or 2 punches:
  • 4+3 plex assay: 2 DBS punches
    • 1 for Pompe, Fabry, Gaucher, MPS-I in 4-plex buffer
    • 1 for MPS-II, MPS-IVA, MPS-VI in 3-plex buffer
  • 7 plex assay: all the above on 1 DBS punch in 1 assay possible with MS/MS (XEVO)
  • Pre-analytical steps in 96 well plates
    • Assay cocktail added to DBS
    • 16h incubation
    • Acetonitril added to quench reaction and precipitate proteins
    • Centrifugation
    • Supernatant transferred to other 96 well plate for analysis
  • Injection on dual column (UPLC)
  • MS/MS (Xevo) quantification of 7 enzyme product and internal standards
Pompe Screening by MS/MS
Discrimination: infantile versus late-onset

Activity (µmol/h/L)

Infantile  Late-onset
Validation tests:

- Quality Control Assessment - Centers for Disease Control (CDC), Atlanta (De Jesus 2009 et al, Clin Chem)
  - Instrument calibration curve
  - Linearity
  - Precision and accuracy (CV)
  - Detection limit
  - Carry-over and column contamination
  - Evaluation of incubation time
  - Evaluation 4+3-plex compared to 7-plex

- Case study
- Stability study
- (Establish reliable reference range)
• 132,538 newborns screened
  ▪ 1:1239 genotyped: 2/3 pathogenic mutations; 1/3 pseudodeficiency
  ▪ Good differentiation by lymphocyte enzymatic GAA activity: low false positive rate
  ▪ However: no discrimination between infantile and late-onset disease!!

• Two-tier screening strategy is indicated, WHAT?

• In the near future it will be also possible to perform a genetic and mutational scan across the whole genome of the fetus in a non-invasive manner by analyzing cell-free fetal DNA in maternal blood as early as the 5th week of gestational age. These high-throughput methods applied to neonatal and non-invasive prenatal screening of genetic diseases, including inborn errors of metabolism, are raising further technical, political and ethical issues.
Ethics?

It is not about how we will screen, but What and Why should we screen?
CONCLUSIONS

- Mass screening of newborns should stay centralised in those screening labs that have the most experience and the best performance; only these labs can implement new techniques and new screening programs.
- Multiplex technology MS/MS have changed screening strategies.
- Neonatal mass screening programs:
  - differ depending on the population screened
  - are not determined by the available technology
- Screening is a dynamic rather than a static process.
THANK YOU

- PCMA vzw
- Local Government of the province of Antwerp
- Minister of Health of the Flemish Community and Administration
- All colleagues from Belgium, The Netherlands, Luxembourg, Austria, USA (CDC, Washington), SGS-Ewacs
- PerkinElmer, Waters, Biomarin, Shire, Genzyme