The RUSP and Beyond

Michele Caggana Sc.D., FACMG
April 25, 2016
Molecular Workshop
Centers for Disease Control and Prevention
Where Were We in 2004 – Beginning of MS/MS

Programs with **Mandated and ACTIVE** MS/MS Component

From NNSGRC (updated 9/8/04)

13/51 States (25%), 41% of US births
Newborn Screening in the US - 2004

Panels

1. Toxo
2. Tyro
3. HIV
4. CF
5. G6PD

Map showing the distribution of newborn screening panels across the United States.
Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children

January 2005 Meeting Notice

Federal Register: December 15, 2004 (Volume 69, Number 240)

In accordance with section 10(a)(2) of the Federal Advisory Committee Act (Public Law 92-463), notice is hereby given of the following meeting:

**Name:** Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children (ACHDGDNC).

**Dates and Times:** January 13, 2005, 9 a.m. to 5 p.m. January 14, 2005, 9 a.m. to 5 p.m.

**Place:** Ronald Reagan Building and International Trade Center, 1300 Pennsylvania Avenue, NW, Washington, DC 20004.

**Status:** The meeting will be open to the public with attendance limited to space availability.

**Purpose:** The Advisory Committee provides advice and recommendations concerning the grants and projects authorized under the Heritable Disorders Program and technical information to develop policies and priorities for this program that will enhance the ability of State and local health agencies to provide for newborn.
Establishes the “RUSP”

Executive Summary

Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, and R. Rodney Howell, MD, editors

The Maternal and Child Health Bureau commissioned the American College of Medical Genetics to outline a process for the standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance. The process of identification is described, and recommendations are provided. Genet Med 2006;8(5, Supplement):
15-11S.

Key Words: Newborn screening, genetics, public health, congenital, metabolic disease

Introduction

In the United States, newborn screening is a highly visible and important state-based public health program that began over 40 years ago. States and territories mandate newborn screening of all infants born within their jurisdiction for certain disorders that may not otherwise be detected before developmental disability or death occurs. Newborns with these disorders typically appear normal at birth. Appropriate compliance with the medical management prescribed can allow most affected newborns to develop normally. As the model for public health-based population genetic screening, newborn screening is nationally recognized as an essential program that aims to ensure the best outcome for the nation’s newborn population.

<table>
<thead>
<tr>
<th></th>
<th>MS/MS</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylcarnitines</td>
<td>(9) OA</td>
<td>(5) FAO</td>
<td>(6) AA</td>
<td>(4) Hematology</td>
<td>(6) Others</td>
</tr>
<tr>
<td>IVA</td>
<td>MCAD</td>
<td>PKU</td>
<td>SCA</td>
<td>HYPOTh</td>
<td></td>
</tr>
<tr>
<td>GA-I</td>
<td>VLCAD</td>
<td>MSUD</td>
<td>Hb S/ Th</td>
<td>BIOT</td>
<td></td>
</tr>
<tr>
<td>HMG</td>
<td>LCHAD</td>
<td>HCY</td>
<td>Hb S/C</td>
<td>CAH</td>
<td></td>
</tr>
<tr>
<td>MCD</td>
<td>TFP</td>
<td>TYR I</td>
<td></td>
<td>GALT</td>
<td></td>
</tr>
<tr>
<td>MUT</td>
<td>CUD</td>
<td>ASA</td>
<td></td>
<td>HEAR</td>
<td></td>
</tr>
<tr>
<td>Cbl A,B</td>
<td></td>
<td>CIT</td>
<td></td>
<td>CF</td>
<td></td>
</tr>
<tr>
<td>3MCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EVERYTHING IS A COMPETITION WITH THAT GUY.
Newborn Screening Tests by U.S. States, 2006

Source: March of Dimes. Data reported from NNSGRC as of June 1, 2006.
©2006 March of Dimes Birth Defects Foundation. All rights reserved.
Newborn Screening Tests by U.S. States, 2007

Source: March of Dimes.
Data reported from NNSGRC as of June 1, 2007.
©2007 March of Dimes Foundation. All rights reserved.
Sec 1: NBS Saves Lives Act (2007) 
Public Law 110-204; 110th Congress

- Section 2: Improved NB and child screening for heritable
- Section 3: $$ “Evaluating the effectiveness…”
- Section 4: ACHDNC
- Section 5: Information Clearinghouse (Baby’s First Test)
- Section 6: Lab quality and surveillance (our friends at CDC) and IAC (CDC, AHRQ, NIH)
- Section 7: Contingency planning “CONPLAN”; Hunter Kelly Research Program

Reauthorized in 2014; signed by President Obama; now famous Section 12
Advisory Committee

- Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children
- SACHDNC -- (SACK’ - DUNK)

- Discretionary Status when NBS SLA expired, 4/23/2013
- DACHDNC -- (DACK’ - DUNK)

- NBS SLA Reauthorization signed 12/8/2014
- ACHDNC -- (ACK’ – DUNK)
Chartered in February, 2003
First meeting June 7, 2004
Newborn Screening Saves Lives Act, 2007
Section 4 reads:

“(3) make systematic evidence-based and peer-reviewed recommendations that include the heritable disorders that have the potential to significantly impact public health for which all newborns should be screened, including secondary conditions that may be identified as a result of the laboratory methods used for screening”
SACHDNC

- Provides guidance to the Secretary, HHS, about the conditions that should be included in newborn screening
- If endorsed by the Secretary, the conditions become part of the RUSP
- Although newborn screening programs are operated at the state level, many strive to follow the RUSP
Composition of SACHDNC

- Members approved by HHS
- 1/10 Committee members is from a State NBS Program
- Liaisons from AAP, ASTHO, AAFP, ACMG, ACOG, AMCHP, APHL, Genetic Alliance, MOD, NSGC, SIMD, even DOD
- Ex Officio members: AHRQ, CDC, FDA, HRSA, NIH, designated federal official
Nominate a Condition

The RUSP is a list of disorders that are screened at birth and recommended by the Secretary of the Department of Health and Human Services (HHS) for states to screen as part of their state universal newborn screening (NBS) programs. Disorders on the RUSP are chosen based on evidence that supports the potential net benefit of screening, the ability of states to screen for the disorder, and the availability of effective treatments. It is recommended that every newborn be screened for all disorders on the RUSP. Most states screen for the majority of disorders on the RUSP; newer conditions are still in process of adoption. Some states also screen for additional disorders. Although states ultimately determine what disorders their NBS program will screen for, the RUSP establishes a standardized list of disorders that have been supported by the Committee and the Secretary of HHS.

Conditions for consideration by the Committee for the Recommended Uniform Screening Panel (RUSP) must be nominated.

The Committee encourages individuals and organizations to form multi-disciplinary teams to submit nominations for conditions to be considered for inclusion on the RUSP. Teams should include researchers and/or clinicians with expertise on the condition being nominated, advocacy and/or professional organizations with knowledge of issues relevant to newborn screening, and interested consumers/individuals.

To apply, the lead nominator or proponent should submit a Nomination Package that includes:

- Cover letter by the lead nominator that identifies all multi-disciplinary team members and their organizational affiliation(s), if applicable;
Nomination Process


- Condition, Treatment, NBS information

- Confirmatory testing information

- Pilot data

- References

Generally advocacy, clinicians, and scientists work together
Condition Review Process

• Based on 3 reports:
  
  • Systematic evidence review
  
  • Assessment of the bounds of benefit and harm
  
  • Evaluation of the capability of states to implement comprehensive screening – “Public Health Impact”
Principles for Making Recommendations

- Evidence based
- Health benefit to screened individual is the chief outcome that matters
- Account for feasibility and readiness of State Programs for screening
- Recommendations not impacted or modified based on insurance, medico-legal liability or legislation
## Evaluation of Magnitude and Certainty of Net Benefit; State Capability

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>There is high certainty that adoption of screening for the targeted condition would lead to a significant net benefit.</td>
</tr>
<tr>
<td>B</td>
<td>There is moderate certainty that adoption of screening for the targeted condition would lead to a significant benefit.</td>
</tr>
<tr>
<td>C</td>
<td>There is high or moderate certainty that adoption of screening for the targeted condition would lead to a small to zero net benefit.</td>
</tr>
<tr>
<td>D</td>
<td>There is high or moderate certainty that adoption of screening for the targeted condition would lead to a negative net benefit.</td>
</tr>
<tr>
<td>L</td>
<td>There is low certainty regarding the net benefit from screening.</td>
</tr>
</tbody>
</table>
## SACHDNC Decision Matrix

<table>
<thead>
<tr>
<th>NET BENEFIT</th>
<th>FEASIBILITY</th>
<th>READINESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ready</td>
</tr>
<tr>
<td>Significant Benefit</td>
<td>High Certainty</td>
<td>High or Moderate Feasibility</td>
</tr>
<tr>
<td></td>
<td>Low Feasibility</td>
<td></td>
</tr>
<tr>
<td>Moderate Certainty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero to Small Benefit</td>
<td>High or Moderate</td>
<td></td>
</tr>
<tr>
<td>Negative Benefit</td>
<td>Low Certainty</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Does Molecular Testing Add Value??

- Increase in sensitivity of a primary test, effect on specificity?
- Identification of carriers; teaching moments
- Predictions regarding phenotype
- Clinicians’ perception, diagnostic tool
When / Why Use a Molecular Test?

- To increase sensitivity without compromising specificity
  - Lower IRT cutoff to avoid missing CF cases
  - Example of Krabbe disease; LSDs?

- To increase specificity of a complex assay
  - Allow differentiation of hemoglobinopathies & thalassemias (e.g. Hb S/b-thalassemia)
When / Why Use a Molecular Test?

- When the primary analyte is transient
  - The primary analyte is present for only a limited time after birth and analysis of a second specimen could result in a false negative. (e.g. VLCAD / CPT2)

- To speed diagnosis in order to avoid serious medical consequences
  - GALT enzyme activity is decreased by heat & humidity, increase in false positive screens
  - Genotyping helps sort out the true positives for faster diagnosis.
When / Why Use a Molecular Test?

- When there are significant founder mutations in a population

  - Due to high frequency (1 in 176 live births) of MSUD in Mennonite population in WI, mutation analysis for p.Y438N serves as primary screen for MSUD for Mennonites.

  - CPT1a in Alaskan Inuit (p.P479L) & Hutterite populations (p.G710E)
When / Why Use a Molecular Test?

- When diagnostic testing is slow and/or invasive
  -- Traditional confirmatory testing for VLCAD & CPT1a involves skin biopsy (invasive to collect and slow to grow)

- When no other test exists for the analyte
  - Severe combined immunodeficiency – screen is the TREC assay, not the genomic DNA; 2010 added to the RUSP – up to ~40 states screening for this condition
Molecular Analysis in Newborn Screening
A Staged Approach

- Genotyping Panel of Mutations -- Single Gene
- Sequencing Single Gene
- Sequencing Panel of Genes
- Sequencing of NBS Genes
- Genome Exome

- Ongoing in routine NBS
- Experimental in NBS
- Offered clinically and research outside NBS

S. Cordovado, Ph.D.
CFTR2 panel of disease causing mutations

First Level

Galactosemia

5-9 mutations commonly tested
Entire coding sequence of an entire gene

KRABBE DISEASE
emergent results

- VOUS
- Phenotype predictions
- Timeliness
- 41.3% reduction in referrals

Other LSDs? -- pseudodeficiency
Next Gen Sequencing and Cystic Fibrosis Newborn Screening

94% of referred CF screens are false positives in NYS

- Screen positive – ↑IRT and at least 1 CF causing mutation
  - Most assays detect a panel of mutations that cause CF
  - >2000 known mutations/variants in CFTR gene

- Not all CFTR mutations cause classic CF
  - Will identify CF related metabolic syndrome or unknown variants
  - Can limit sequence detection to known mutations but will miss cases?
  - How many missed cases can we live with?
  - Can’t we do better?

Hughes EE et al., Hum Mutat, 37:201-208
Next Gen Sequencing and SCID Newborn Screening

Issue: SCID is a spectrum of disorders that can only be differentiated by identifying causative mutations

- Many genes involved in SCID
- Immunologists can provide better care when SCID causative mutations are known quickly
- Screening labs can provide timely mutation analysis
- When public health provides mutational analysis, ensures health equality
Entire coding sequence of all known genes catalogued as disease-causing

**Current NBS for severe combined immunodeficiency:**

- Measure T-cell receptor excision circles
- <125 TRECṣ constitutes a referral
- Immunologists order CBC, flow, mitogen studies
- Molecular tests order by candidacy, multi-gene panel(s), insurance issues, available labs
- Becomes iterative, slow, stressful process
Entire coding sequence of all known genes in a given biochemical pathway

- Modifiers
- Phenotype predictions
- Infantile, juvenile, late
Entire coding sequence of all known NBS genes

- Complete
- Only looking at NBS
- Can turn off analysis
- Easily modifiable
- Similar information
- Economy of scale
- Still ‘manageable’

- Under consideration in NY
- Establishment of NBS core
Whole exome or whole genome analyses

- Complete
- All disease / onset
- VOUS
- Screening v. diagnostic
- No phenotype yet
- Consent
- No longer ‘manageable’ currently
What Else is Being Discussed for Addition on the RUSP?

- Spinal muscular atrophy – deletion of exon 7 in SMN1 gene (pilot in NY hospitals)
- Fragile X syndrome – CGG repeat (>200); AGG
- Duchenne muscular dystrophy – creatine kinase followed by deletion/duplication; DNA sequence – specificity; other conditions
- Other LSDs (Gaucher disease/Niemann-Pick disease, Fabry disease – mild/severe mutations, frequency issues)
- Guanidinoacetate methyltransferase deficiency
Spinal Muscular Atrophy

- 1 in 6,000 frequency
- Four types, type I is most severe
- Deletion (most often) of SMN1
- #copies of SMN2 important
- No treatment, but early diagnosis useful; some data on efficacy in affected children
- Dr. Tom Prior developed a molecular assay
LUMINEX TECHNOLOGY – SPINAL MUSCULAR ATROPHY

We are using 7900s Moving to QuantStudios Multiplexing??

Depiction of assay developed by Dr. Tom Prior

Wadsworth Center
FRAGILE X SYNDROME

5' UTR CGGCGGCGG repeat

- 5-44 normal
- 45-54 grey zone
- 55-200 premutation
- >200 full mutation
ACMG-Standards and Guidelines For Fragile X Testing  2006

PCR and Capillary Southern Electrophoresis and RNA

1. NF
2. AM
3. F 28&52 repeats
4. F 26&52 repeats
5. F 18& ~80 repeats
6. NM
7. NM, underloaded
8. NF
9. NM
10. NM
11. AF, underloaded
12. NTM
13. F 20&70 repeats
14. F 27&42 repeats
15. NF
16. NF
17. NM
Will reduce Southern blots—hard if not impossible to do from a DBS.

How would that fit in a screening laboratory workflow?
Fragile X-Tremor Associated Syndrome

- Described in 2001
- Tremor, PD, brain atrophy
- Affects NTMs
- Same test as FRAX
- Came to attention via grandsons
- FRAX? No FXTAS!
- Excess mRNA
- ~20-30% of NTMs
- Brain inclusions

Paul and Randi Hagerman
UCDavis
Duchenne Muscular Dystrophy

- Parental attitudes for screening (6-12 month olds)

- CK levels; dystrophin mutations (2nd tier)

- Cohort identified for treatment / trials -- no efficacy yet proven – early steroids?

- CDC working on quality assurance
NBS programs existed during two decades in at least 8 countries (UK, Germany, France, Belgium, USA, Australia, New Zealand, Canada), Wales had a false negative rate that was unacceptable 66 affected and 15 false negatives

Pilot program models all involved a single-tier analysis using Creatine Kinase in 2 steps: creatine kinase (CK) activity testing from DBS, confirmation from serum sample + DNA mutation analysis from samples with high CK concentrations

CDC-Mendell collaboration/pilot study in US 2008-2013

- Two-tier system of analysis for newborn screening - initial biochemical CK from DBS sample -> direct mutation analysis from same DBS
- Low false positive rate (< 0.2%)
- No data on false negative rate
Newborn screening for DMD

- Mutation spectrum known
- No common mutations - required to screen full gene
- New therapies – somelinal clinical trials are on going, earlier treatment beneficial??

Madhuri Hegde, Ph.D.
Deletions (65%)
Point Mutations (30%)
Duplications (5%)

Mutation Spectrum

Madhuri Hegde, Ph.D.
Gene Mutation Spectrum

Duchenne Muscular Dystrophy (DMD)
- 30% small mutations
- 65-70% deletions/duplications

LGMD genes
- >90% small mutations
- <10% deletions/duplications

Pompe disease
- Common mutations- c.-45T>C
- Exon 18 deletion

Small mutations
(nucleotide substitutions, small indels)

Larger mutations
(exon and multi-exon dels and dups)

Madhuri Hegde, Ph.D.
CNV Analysis

Madhuri Hegde, Ph.D.
Dried Blood spot card

Punched Blood spots

Initial CK level testing

Elevated CK levels

NGS for DMD/LGMD/CMD mutation detection

positive

Establish Diagnosis

negative

Advise clinical follow-up/ Repeat CK level testing
More than 25 LGMD types are linked to specific gene loci

Madhuri Hegde, Ph.D.
DOGMA IS CHANGING

- Need for Information
- Technology
- Rapid Clinical Advances

atccggacttaatcgatcctttgatac........
Thank You!!