Improved Specificity of Newborn Screening for Congenital Adrenal Hyperplasia (CAH) by Second Tier Steroid Profiling using Tandem Mass Spectrometry (MS/MS)

Piero Rinaldo, Si Houn Hahn, Dietrich Matern

Biochemical Genetics Laboratory
Mayo Clinic College of Medicine
Rochester, MN
Congenital Adrenal Hyperplasia (CAH)

- Caused (>90%) by 21-hydroxylase deficiency
- Deficient synthesis of cortisol
- Accumulation of precursor steroids (17-OH P)
- US incidence (1990-99): \(~1:19,000\) births
- Different clinical phenotypes
- Proven benefit of early diagnosis by NBS
- Limitations of existing methods
  - Stress, prematurity
  - Antibody cross reactivity
CAH Screening in the USA
(NNSGRC, Updated March 30, 2004)
(http://genes-r-us.uthscsa.edu)

Screened
Not screened

15/51 37/51
23% 77%

US Incidence: 1:19,000
~50 cases/year undiagnosed at birth
CAH Screening in MN (2003)

- No. of births: 71,147
- True positives: 5
- Incidence: 1:14,229
- False positives: 340
- False (+) rate: 0.48%
- Cost F/U (Mayo): $847
- Annual cost of F/U: $287,980

(Annual cost in CA: >$2,000,000)
Steroid Profiling by MS/MS

- Analysis in DBS of cortisol, 17-OH progesterone (d₈ IS), and androstenedione
- For analytical parameters and validation see method in *Clinical Chemistry* 50:621-625 (March 2004)

---

**Improved Specificity of Newborn Screening for Congenital Adrenal Hyperplasia by Second-Tier Steroid Profiling Using Tandem Mass Spectrometry**

Jean M. Lacey, Carla Z. Minutti, Mark J. Magera, Angela L. Tauscher, Bruno Casetta, Mark McCann, James Lymp, Si Houn Hahn, Piero Rinaldo, and Dietrich Matern
1 Cortisol
2 Androsten.
3 $d_8$ 17-OHP
4 17-OHP

Normal Control

Classic CAH

False Positive
Steroid Profiling by MS/MS

- Analysis in DBS of cortisol, 17-OH progesterone ($d_8$ IS), and androstenedione
- For analytical parameters and validation see method in *Clin Chem* 50:621-625 (March 2004)
- Clinical validation by analysis of 1222 blinded DBS from six centers (France, Germany, Korea, USA)
  - 1001 Normal newborns (screening negative)
    - Mean BW 3183 g (range: 409 - 5740 g)
  - 31 Confirmed CAH patients
    - Mean BW 3501 g (range: 2278 - 4336 g)
  - 190 False positive cases
    - Mean BW 1994 g (range: 422 – 4400 g)
[17-OHP] in DBS by FIA in 190 False Positive Cases

Birth Weight (g)

17-OHP (ng/mL)
[17-OHP] in DBS by FIA and by MS/MS in 190 False Positive Cases (Blinded)
[17-OHP] in DBS by FIA and by MS/MS in 190 False Positive Cases

What about these?

Normal results
Improved Specificity of Newborn Screening for Congenital Adrenal Hyperplasia by Second-Tier Steroid Profiling Using Tandem Mass Spectrometry

Jean M. Lacey, Carla Z. Minutti, Mark J. Magera, Angela L. Tauscher, Bruno Casetta, Mark McCann, James Lym, Si Houn Hahn, Piero Rinaldo, and Dietrich Matern


Strategy for Multicomponent CAH Screening

17-OHP < 12.5

Y

Screening Negative

N

Ratio < 3.5

Y

Screening Negative

N

Screening Positive

* Ratio = Androstenedione + 17-OHP

Cortisol

A + B

C
Reduction of CAH False Positives by 2\textsuperscript{nd} Tier MS/MS Testing (MN Study)
Reduction of CAH False Positives by 2nd Tier MS/MS Testing (MN Study)
Reduction of CAH False Positives by 2nd Tier MS/MS Testing (MN Study)

17-OHP plus Ratio
Sensitivity 100% (95% CI: 76.8-100%)
Specificity 98% (95% CI: 96.9-98.9%)
### Reduction of % False Positives (N=190)

<table>
<thead>
<tr>
<th>Sorted by</th>
<th>FIA</th>
<th>MS/MS</th>
<th>Delta (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day of Collection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>111</td>
<td>18</td>
<td>- 84</td>
</tr>
<tr>
<td>Day 3</td>
<td>60</td>
<td>2</td>
<td>- 97</td>
</tr>
<tr>
<td>Day 5</td>
<td>19</td>
<td>1</td>
<td>- 95</td>
</tr>
<tr>
<td><strong>Birth weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1500</td>
<td>66</td>
<td>8</td>
<td>- 88</td>
</tr>
<tr>
<td>1501-2000</td>
<td>27</td>
<td>3</td>
<td>- 89</td>
</tr>
<tr>
<td>2001-2500</td>
<td>35</td>
<td>5</td>
<td>- 86</td>
</tr>
<tr>
<td>&gt;2500</td>
<td>62</td>
<td>5</td>
<td>- 92</td>
</tr>
</tbody>
</table>

Minutti et al. Steroid profiling by MS/MS improves the positive predictive value of newborn screening for congenital adrenal hyperplasia. JCEM (2004), in press.
Joint Newborn Screening Program in Minnesota (Spring 2004)

## Established Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Screening</th>
<th>Follow up</th>
<th>2nd Tier Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cong. Hypothyroidism</td>
<td>S, FU</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Galactosemia (GALT)</td>
<td>S, FU</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Sickle Cell Anemia</td>
<td>S, FU</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>21-Hydroxylase Def (CAH)</td>
<td>S, FU</td>
<td>2T</td>
<td></td>
</tr>
<tr>
<td>MS/MS (AA, OA, FAO)</td>
<td>FU</td>
<td>S, C</td>
<td></td>
</tr>
</tbody>
</table>

- **S**: Screening
- **FU**: Follow up
- **C**: Confirmatory (UoM)
- **2T**: 2nd tier test in DBS
The 2004 Minnesota NBS Card

COMPLETELY FILL ALL CIRCLES WITH BLOOD FROM REVERSE SIDE. ALLOW TO AIR DRY (4 HRS.). DO NOT HEAT.
Birthing Place

MDH Laboratory

Newborn Screening
- CH
- GALT
- CAH
- SCA

Mayo BGL

MS/MS Screening
### The good NEW days?

<table>
<thead>
<tr>
<th></th>
<th>2003</th>
<th>2004-05</th>
</tr>
</thead>
<tbody>
<tr>
<td>False positives</td>
<td>340</td>
<td>51</td>
</tr>
<tr>
<td>False (+) rate</td>
<td>0.48%</td>
<td>0.07%</td>
</tr>
<tr>
<td>Cost clinical F/U</td>
<td>$287,980</td>
<td>$43,197</td>
</tr>
<tr>
<td>Cost 2(^{nd}) tier test</td>
<td>$0</td>
<td>$10,115</td>
</tr>
<tr>
<td>Total F/U cost</td>
<td>$287,980</td>
<td>$53,312</td>
</tr>
<tr>
<td>Cost difference (savings)</td>
<td>(81%)</td>
<td></td>
</tr>
</tbody>
</table>

- Cost clinical F/U: $847 per case
- Cost 2\(^{nd}\) tier test: $35 per test
Conclusions

- 2nd Tier testing of the original DBS by MS/MS can significantly reduce
  - False positive rate of CAH screening
    - 169/190 blinded cases ruled negative
  - TAT of results (90% less repeats)
  - Cost of clinical follow up
- Other 2nd tier tests under development
  - Succinylacetone (SSIEM, September 2004)
  - Homocysteine (Clin Chem 45:1517, 1999)
Acknowledgements

Jean M. Lacey
Mark J. Magera
Angela L. Tauscher
James F. Lymp
Mark McCann
Donald H. Chace
Donald Zimmerman

Carla Z. Minutti
Bruno Casetta
David Cheillan
Claude Dorche
Andreas Schulze

Thank You for Your Attention