CYP21A2 Mutations Found in Congenital Adrenal Hyperplasia Patients in the California Population

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21 Hydroxylase Deficiency

- **Classic CAH – Salt Wasting**
  - Severe to complete loss of 21-OH activity
    - Loss of electrolyte homeostasis
    - Adrenal crises and risk of death

- **Classic CAH - Simple Virilizing**
  - Partial 21-OH activity
    - Normal sodium balance
    - Elevated androgen production

- **Non-classical CAH**
  - Usually asymptomatic until puberty
Primary CAH Newborn Screen

- Primary Screen by Immunoassay for 17-α OHP
- High false-positive rate
  - 17-α OHP levels are high in premature and/or stressed babies
    - Stratification by birth weight or gestational age for 17OHP cut-offs
  - Lack of specificity with immunoassay
    - Cross-reaction with other steroids
    - Matrix effects
Second-Tier CAH Screens

- **CAH Steroid Profiling by LC MS/MS**
  - \( \frac{([17-OHP] + [4-androstenedione])}{[cortisol]} \)

- **CAH Molecular Screening of CYP21A2 mutations**
  - Gene rearrangements
    - PCR or Multiple Ligation Probe Amplification (MLPA)
  - CYP21A2 mutation analysis
    - Multiplex mutation panel genotyping
    - Complete gene sequencing
Collaboration with California NBS

- California has been screening for CAH since 2005
  - Primary 17OHP screen with FIA - four birth weight cutoffs
  - 2\textsuperscript{nd} tier MS/MS for steroid panel for slightly elevated 17OHP

- Collaboration to characterize newborn specimens of CAH cases
  - Mixture of 128 of Classic and Non-classic CAH and screen negatives
  - 50 normal controls, blinded to analysts

- Goal: Determine if genotype analysis of CYP21A2 could increase the specificity of CAH screening for California NBS
Challenges for CAH Molecular Screening

- CAH is a multi-gene disorder
  - 90-95% due to 21OH deficiency – CYP21A2
  - 5% due to 11β-hydroxylase – CYP11B1
  - 17α-hydroxylase, 3β-hydroxysteroid dehydrogenase, lipoid CAH

- Chromosomal region is complex
  - RCCX gene module repeats
  - CYP21A1P pseudogene sequence 98% identical to CYP21A2

- Not known if common mutation panel adequately covers the California population
Common CYP21A2 Mutation Panel

Gene deletions (30kb Δ and intragenic Δ) plus gene conversions account for ~30% of CAH-causing mutations
CYP21A2 Genomic Region

HLA Class I

HLA-B

510 kb

RLP2

CYP21A2

RCCX Module 1

RCCX Module 2

30 kb

HLA Class III

RCCX

HLA Class II

HLA-DR

300 kb

Chr 6p

C4A

C4B

TNXA

RP2

CYP21A1P

TNXB

RP1

CYP21A2
PCR-Based Detection of Chromosome Deletion and Gene Conversion Alleles

Most-common chromosome arrangement

30Kb Deletion

Gene Conversion
CYP21A2 and CYP21A1P PCR

CYP21A2
A2-F

CYP21A1P
A1P-F

E1  E2  E3  E4  E5  E6  E7  E8  E9  E10

150bp del

A2-F + TNXB-R
5.6 kb

30kb Deletion
A1P-F + TNXB-R
6.1 kb

Gene Conversion
A2-F + TNXA-R
5.5 kb
Genotyping Approach

- Long-range PCR profile to detect 30 kb deletions and gene conversions
- Perform complete gene sequence of CYP21A2 and the 30 kb deletion and gene conversion PCR amplicons
- Evaluate gene copy number by MLPA for 30 kb deletions, gene conversions, and possible hemizygous CYP21A2
Results of CYP21A2 Genotyping

- 128 from NBS screen positive and screen negative CAH cases
  - 114 samples with CYP21A2 mutations – 89% of cases
  - 9.6% of 228 chromosomes with multiple mutations

- 50 normal population controls
  - 1 carrier for Salt Wasting allele (M239K)
  - 1 carrier for a gene conversion
  - 4 carriers for likely tri-allelic RCCX repeat with Q318X in cis
  - 2 carriers for Non-Classic alleles, V281L and c.*13A>G
### CYP21A2 Panel Mutations

<table>
<thead>
<tr>
<th>CYP21A2 Mutations</th>
<th>Phenotype</th>
<th>Count</th>
<th>%</th>
<th>US Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P30L</td>
<td>Non-Classical</td>
<td>1</td>
<td>0.4</td>
<td>0.8</td>
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<tr>
<td>IVS2G</td>
<td>Salt Wasting/S. Virilizing</td>
<td>59</td>
<td>25.7</td>
<td>23.4</td>
</tr>
<tr>
<td>IVS2G + Other Mutations</td>
<td></td>
<td>12</td>
<td>4.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Exon 3 8bp deletion</td>
<td>Salt Wasting</td>
<td>8</td>
<td>3.5</td>
<td>0.5</td>
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<tr>
<td>I172N</td>
<td>Simple Virilizing</td>
<td>13</td>
<td>5.7</td>
<td>12.6</td>
</tr>
<tr>
<td>I172N + Other Mutations</td>
<td></td>
<td>4</td>
<td>1.7</td>
<td>---</td>
</tr>
<tr>
<td>I236N/V237E/M239K</td>
<td>Salt Wasting</td>
<td>8</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>V281L</td>
<td>Non-Classical</td>
<td>4</td>
<td>1.7</td>
<td>12.6</td>
</tr>
<tr>
<td>F306+1</td>
<td>Salt Wasting</td>
<td>3</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Q318X</td>
<td>Salt Wasting</td>
<td>15</td>
<td>6.5</td>
<td>3.3</td>
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<tr>
<td>Q318X + Other Mutations</td>
<td></td>
<td>7</td>
<td>3.0</td>
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</tr>
<tr>
<td>R356W</td>
<td>Salt Wasting</td>
<td>18</td>
<td>7.8</td>
<td>3.6</td>
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<tr>
<td>P453S</td>
<td>Non-Classical</td>
<td>0</td>
<td>---</td>
<td>0.5</td>
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</table>

<table>
<thead>
<tr>
<th>CYP21A2 Gene Recombinants</th>
<th>Phenotype</th>
<th>Count</th>
<th>%</th>
<th>US Frequency (%)*</th>
</tr>
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<tbody>
<tr>
<td>30 KB Deletion</td>
<td>Salt Wasting</td>
<td>47</td>
<td>20.4</td>
<td>30.5 - Combined</td>
</tr>
<tr>
<td>A2 Deletion - non 30 KB del PCR</td>
<td>Salt Wasting</td>
<td>12</td>
<td>5.2</td>
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<tr>
<td>Large Scale Gene Conversion</td>
<td>Salt Wasting</td>
<td>4</td>
<td>1.7</td>
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CYP21A2 Mutations not on Panel

<table>
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<tr>
<th>Additional Mutations</th>
<th>Phenotype</th>
<th>Count</th>
<th>%</th>
<th>US Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.-4C&gt;T, c.738+74T</td>
<td>Undetermined</td>
<td>1</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>T201A</td>
<td>Predicted Benign</td>
<td>1</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>I291N</td>
<td>Predicted Damaging</td>
<td>1</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>R316X</td>
<td>Salt Wasting</td>
<td>1</td>
<td>0.43</td>
<td></td>
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<tr>
<td>H366Y</td>
<td>Salt Wasting</td>
<td>3</td>
<td>1.30</td>
<td>0.8</td>
</tr>
<tr>
<td>H366Y, c.*13A&gt;G</td>
<td>Salt Wasting</td>
<td>1</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>R427C</td>
<td>Salt Wasting/S. Virilizing</td>
<td>1</td>
<td>0.43</td>
<td>0.3</td>
</tr>
<tr>
<td>R483A1nt</td>
<td>Salt Wasting</td>
<td>5</td>
<td>2.17</td>
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<tr>
<td>R483W, c.*13A&gt;G</td>
<td>Salt Wasting</td>
<td>1</td>
<td>0.43</td>
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<tr>
<td>c.*13A&gt;G</td>
<td>Non-Classical</td>
<td>1</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

3 specimens detected by PCR or Common Panel
- A2 Deletion / I291N
- A2 Deletion / H366Y
- A2 Deletion / c.-4C>T, C.738+74T

*Finkielstain et al. (2011)
Highlights of California CAH Cases

Out of 128 CAH screen-positive specimens

- 114 with mutations for both copies of CYP21A2
- 26 specimens with >2 mutations in cis in an allele – phase determined for all but one sample
- Overall CYP21A2 mutation profile similar to large US family study
  - 9 mutations not on common panel
  - 111/114 specimens with at least 1 mutation from panel
Questions Going Forward

- **CYP21A2 mutation panels**
  - Classic CAH vs Non-Classic mutations
  - What is minimal frequency for inclusion

- **Samples with no CYP21A2 mutations detected**
  - Fail-safe 17OHP cutoffs?
  - Additional gene analysis
    - CYP11B for 11β-OH, CYP17A for 17α-OH

- **Screening appropriate procedure**
  - Rapid and cost effective targeted genotyping from DBS
  - Interpretation of results – gene rearrangements and phasing
Acknowledgments

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.