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

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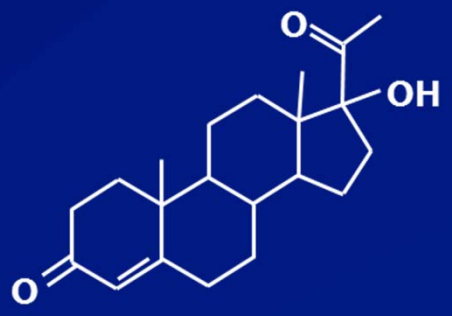
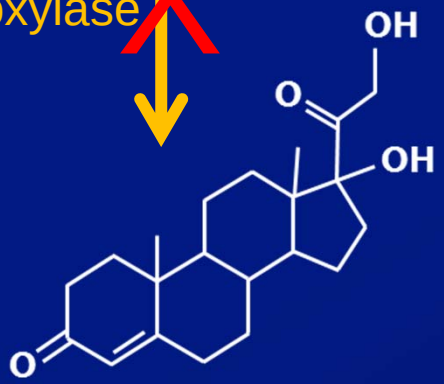
# 21 Hydroxylase Deficiency

- **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




*17-α Hydroxyprogesterone*

21-Hydroxylase




*11-Deoxycortisone*

# 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**
  - ([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

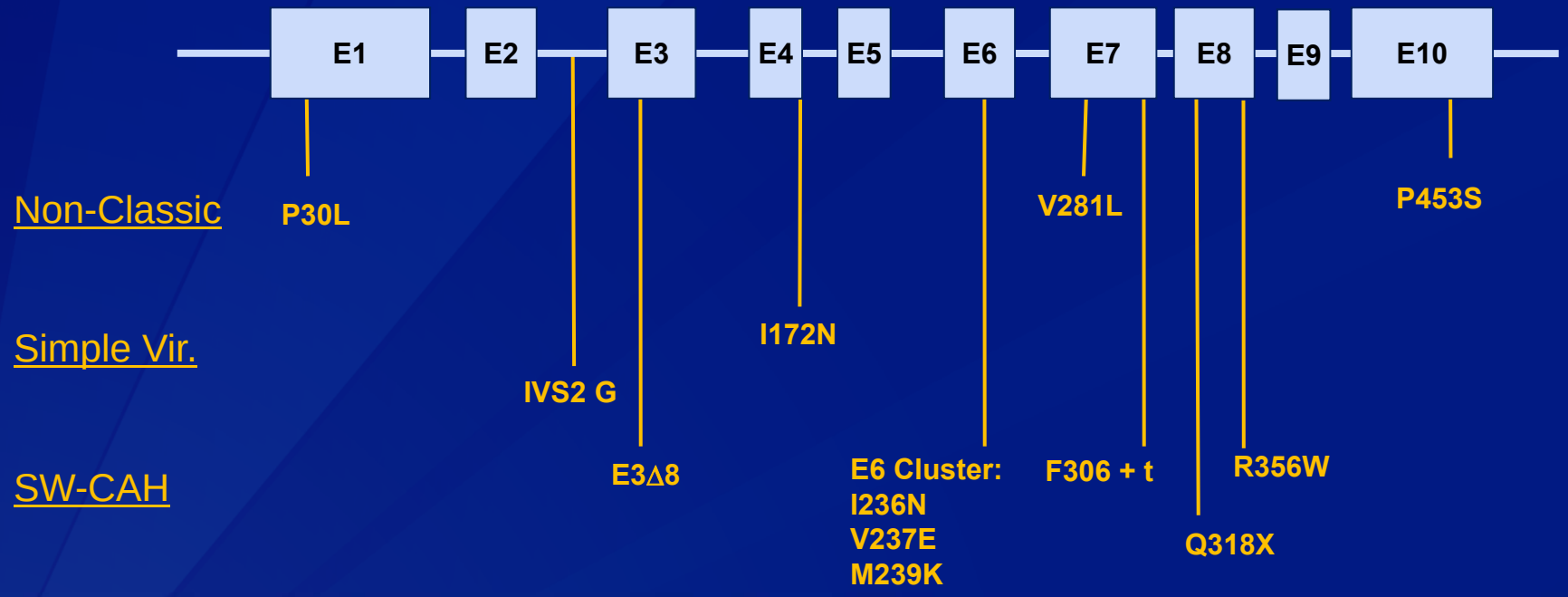
# Collaboration with California NBS

- **California has been screening for CAH since 2005**
  - Primary 17OHP screen with FIA - four birth weight cutoffs
  - 2nd 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

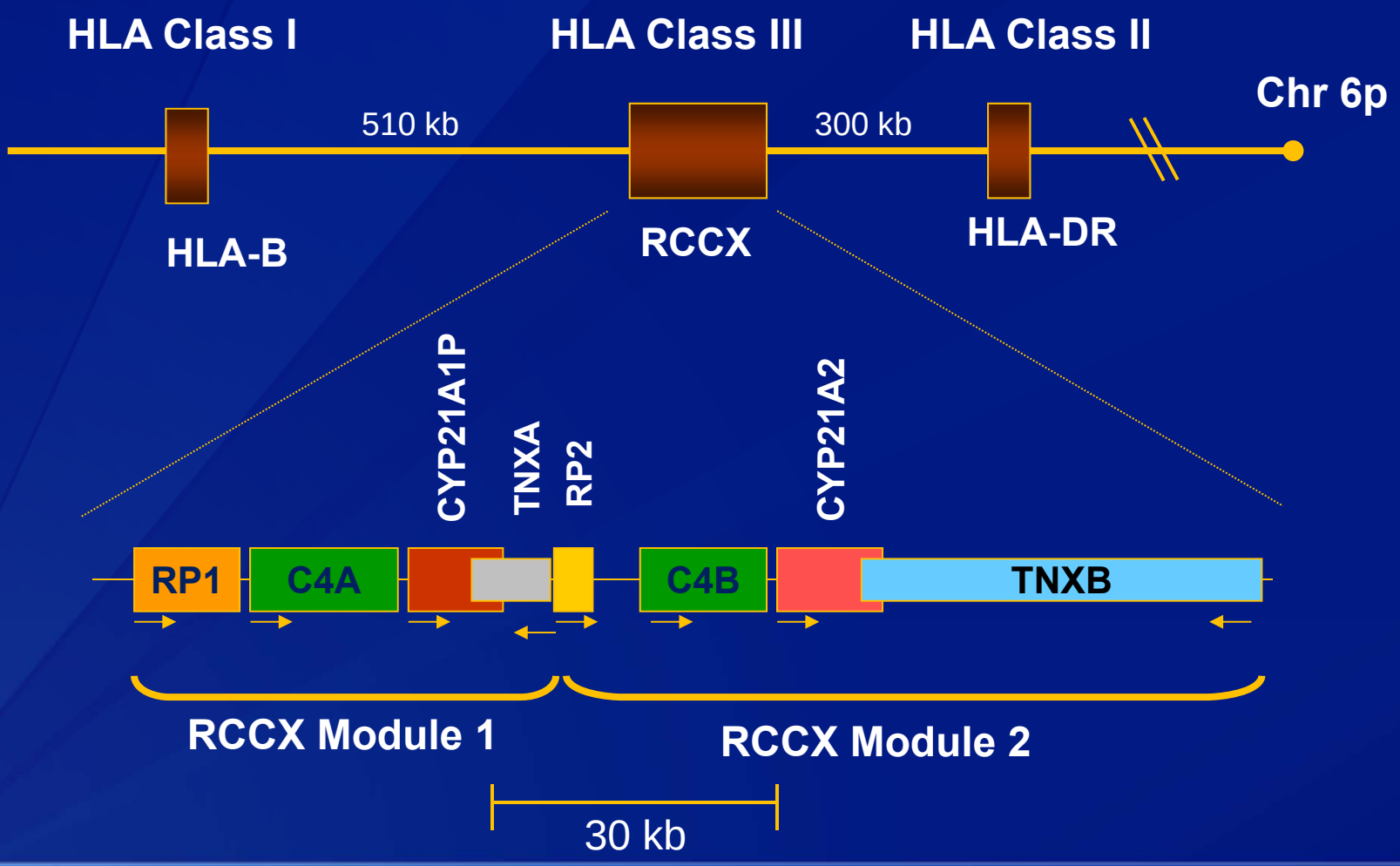
- **CAH is a multi-gene disorder**
  - 90-95% due to 21OH deficiency – CYP21A2
  - 5% due to 11β-hydroxylase – CYP11B1
  - 17α-hydroxylase, 3β-hydroxysteroid dehydrogenase, lipoid CAH
- **Chromosomal region is complex**
  - RCCX gene module repeats
  - CYP21A1P pseudogene sequence 98% identical to CYP21A2
- **Not known if common mutation panel adequately covers the California population**

# Common CYP21A2 Mutation Panel



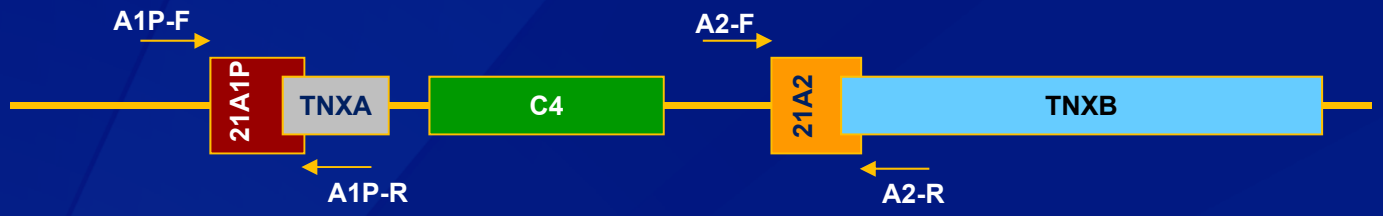

**Gene deletions ( 30kb Δ and intragenic Δ ) plus gene conversions account for ~30% of CAH-causing mutations**

# CYP21A2 Genomic Region



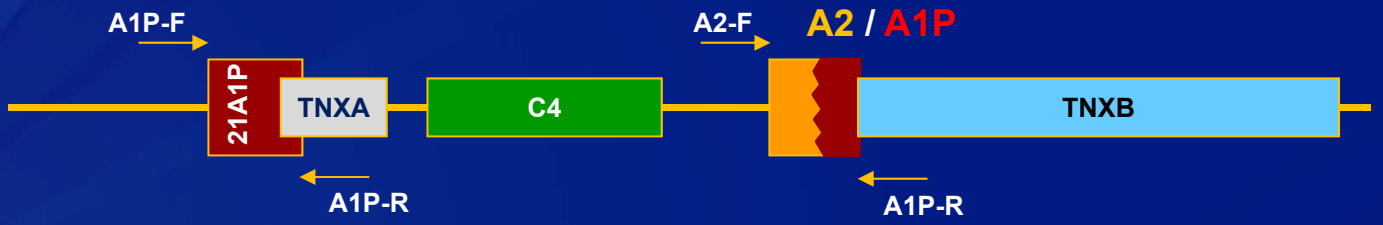
# PCR-Based Detection of Chromosome Deletion and Gene Conversion Alleles

Most-common chromosome arrangement

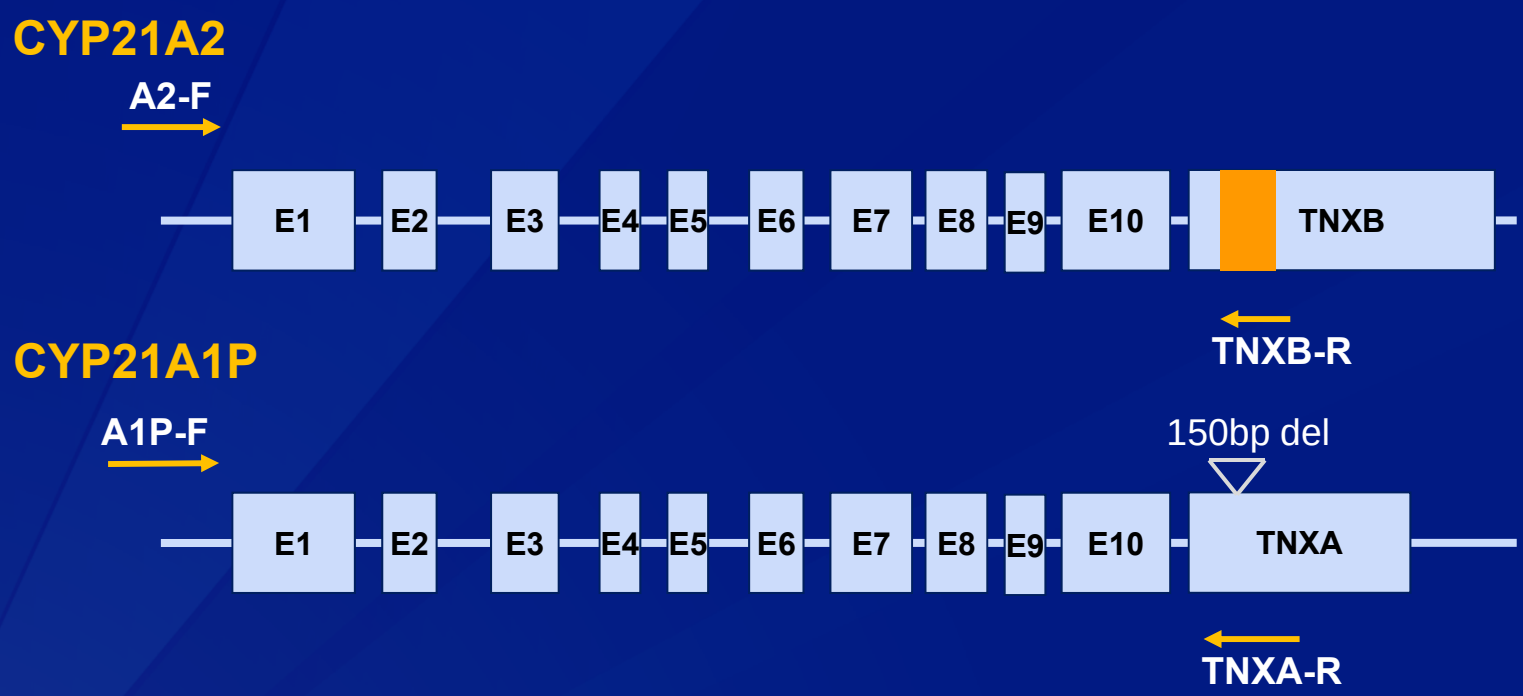



30Kb Deletion


Gene Conversion



# CYP21A2 and CYP21A1P PCR




| | | |
|---|---|---|
| CYP21A2 | A2-F + 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

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

| CYP21A2 Gene Recombinants | Phenotype | Count | % | US Frequency (%)* |
|---|---|---|---|---|
| 30 KB Deletion | Salt Wasting | 47 | 20.4 | 30.5 - Combined |
| A2 Deletion - non 30 KB del PCR | Salt Wasting | 12 | 5.2 | |
| Large Scale Gene Conversion | Salt Wasting | 4 | 1.7 | |

*Finkielstain et al. (2011). Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrin Metab 96, E161–172

# CYP21A2 Mutations not on Panel

| Additional Mutations | Phenotype | Count | % | US Frequency (%)* |
|---|---|---|---|---|
| c.-4C>T, c.738+74T | Undetermined | 1 | 0.43 | |
| T201A | Predicted Benign | 1 | 0.43 | |
| I291N | Predicted Damaging | 1 | 0.43 | |
| R316X | Salt Wasting | 1 | 0.43 | |
| H366Y | Salt Wasting | 3 | 1.30 | 0.8 |
| H366Y, c.*13A>G | Salt Wasting | 1 | 0.43 | |
| R427C | Salt Wasting/S. Virilizing | 1 | 0.43 | 0.3 |
| R483Δ1nt | Salt Wasting | 5 | 2.17 | |
| R483W, c.*13A>G | Salt Wasting | 1 | 0.43 | |
| c.*13A>G | Non-Classical | 1 | 0.43 | |

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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