Universal Newborn Screening for Severe Combined Immunodeficiency

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Mei Baker, M.D., FACMG
Associate Professor, Department of Pediatrics
Co-Director, NBS Laboratory at WSLH
University of Wisconsin School of Medicine and Public Health
Severe Combined Immunodeficiency (SCID)

Then…

Now…
Learning Objectives

• Understand SCID from a clinical perspective

• Understand the rationale for screening SCID in newborns

• Understand the screening process and confirmatory test for SCID
Severe Combined Immunodeficiency (SCID)

- **Infections in first year of life**
  - recurrent, etiology bacterial, viral and fungal
  - persistent despite routine treatment
  - severe--including sepsis, meningitis
  - opportunistic pathogens, such as PCP (pneumonia)
- **Failure to thrive, chronic diarrhea**
- **T cells decreased or absent**
  - poor proliferation *in vitro* to mitogens
- **B cells absent or non-functional**
  - low Ig’s after maternal IgG wanes; no specific antibody responses
- **Fatal without immune reconstitution**
SCID Genetic Analysis

- X-linked SCID is most common form (males)
- Specific gene defect can be found in 80% of cases (15-20 genes known)
- Clinical applications:
  - Carrier and prenatal dx
  - Predict response to BMT
  - Gene therapy

Buckley Ann. Rev Imm 2004
Available Curative Treatment Modalities for SCID

- Bone Marrow Transplantation

- Gene Therapy (X-linked and ADA SCID)
Does SCID fulfill NBS criteria?

- Prevalence of the disease (1:100,000 or greater)
  - SCID: 1:66,000 (conservative estimate)

- Can the disorder be detected by routine physical exam?
  - SCID: No, SCID baby appears normal at birth.

- Does the disorder have a short asymptomatic period after birth?
  - SCID: Yes, SCID baby can be protected by passive maternal immunity.

- Does the disease cause serious medical complications?
  - SCID: Yes, universally fatal within the first year of life

- Is there potential for successful treatment?
  - SCID: Yes, hematopoietic stem cell transplantation

- Is there a confirmatory test?
  - SCID: Yes, lymphocyte subpopulation analysis (flow cytometry)

- Does early intervention leads better outcome?
  - SCID: Yes!

- Is there a screening test?
  - SCID: Yes, measurement of TRECs using real-time qPCR
SCID: Benefits of Early Diagnosis

113 SCID infants with HSCT at greater than 3.5 months of age or less
46 SCID infants with HSCT at than 3.5 months of age or less

66%

96%

Screening for SCID in Newborns
Considerations

• Many genes

• Many mutations in each known gene

• Some genotypes still not known
TRECs are reduced in nearly ALL forms of SCID

T Cell Receptor Recombination During Development in the Thymus

Generation of T cell receptor excision circles (TRECs) occur in >70% of all new (naïve) T cells and can be detected by PCR.

T-cell Generation in Newborns

• Two mechanisms:
  – Thymic output
  – Postthymic T-cell proliferation

• Consequences:
  – Majority of T-cells are naïve T cells in newborns.
  – TREC s are diluted out, and 10% T cells contain TREC s in newborns.

Gent et al, Clinical Immunology. 2009; 133: 95-107
TagMan Probe Real-time qPCR

1. Polymerisation

2. Strand displacement

3. Cleavage

4. Polymerisation completed
Figure 2. Phases of the PCR amplification curve. The PCR amplification curve charts the accumulation of fluorescent emission at each reaction cycle. The curve can be broken into four different phases: the linear ground, early exponential, log-linear, and plateau phases. Data gathered from these phases are important for calculating background signal, cycle threshold (Ct), and amplification efficiency. Rn is the intensity of fluorescent emission of the reporter dye divided by the intensity of fluorescent emission of the passive dye (a reference dye incorporated into the PCR master mix to control for differences in master mix volume). ΔRn is calculated as the difference in Rn values of a sample and either no template control or background, and thus represents the magnitude of signal generated during PCR. This graph was generated with ABI Prism SDS version 1.9 software (Applied Biosystems).
Overall Analysis Scheme

NBS Card (NSC) a.k.a. Guthrie Card
Dried blood spots (DBS)

3 mm punch

96 well plate

Extract DNA → Amplify TREC by real-time QPCR → Analyze

TREC plasmid calibrators

ΔRn (amplification)

ABI 7900HT Fast Real-Time PCR System
Real-time PCR to Measure TRECs

TREC plasmid calibrators

\[ \Delta R_n \] (amplification)
Real-time PCR to Measure TRECs

**Calibrator Curve/Samples**

- **Slope:** -3.55
- **$R^2$:** 0.999

**Calibrators:** □

**Unknowns:** ✗

**Cycle Number**

**Quantity of TRECs**
Multiplexing _384-well Plate

Michael Cogley
**TREC Copy Numbers**

- **Number of Infants Screened**
- **True Negatives**
- **True Positives**
- **Normal Population**
- **Affected**
- **Unaffected**
- **False positives**
- **False negatives**

**Test Positive**
- **Affected**: TP
- **Unaffected**: FP

**Test Negative**
- **Affected**: FN
- **Unaffected**: TN

**Sensitivity** = \( \frac{TP}{TP + FN} \)

**Positive Predictive Value** = \( \frac{TP}{TP + FP} \)

**Specificity** = \( \frac{TN}{TN + FP} \)
**SCID Testing Algorithm**

- **Full term <40**
  - **Preterm <25**
    - TREC & Actin Assay on 2 additional punches
      - **Full term:** TREC < 25, Actin > 10,000
        - Screening Abnormal
      - **Full term:** TREC 25-40, Actin > 10,000
        - Screening Abnormal
      - **Full term:** TREC < 40, Actin < 10,000
        - Screening inconclusive
      - **Preterm:** TREC < 25, Actin < 10,000
        - Screening Abnormal
      - **Preterm:** TREC < 25, Actin > 10,000
        - Screening inconclusive

- **Full term ≥ 40**
  - **Preterm ≥ 25**
    - Normal
SCID Reporting Algorithm

**Full term screening Abnormal**
- Call out results to clinical consultants
  - Confirmatory Dx
  - Work up
- Call out results to PCP

**Full term screening possible Abn. and inconclusive**
- Written report and request a repeating NBS specimen

**Preterm screening inconclusive**
- Written Report and recommending repeat NBS following NICU procedure

**Preterm screening Abn.**
Confirmatory testing

- Flow cytometry
  lymphocyte subset enumeration for T, B and NK cell quantitation

- Lymphocyte (T and/or B) proliferation tests

- Quantitative immunoglobulin assessment (IgG, IgA, IgM and IgE)

- HIV testing (to rule out secondary causes of T-cell lymphopenia)

- Genetics testing

- Others: enzymes, Fluorescence in situ hybridization (FISH)
Special Considerations

• TREC copy numbers
  – Measurement units
  – DNA extraction
  – Calibrators

• TREC assay platform
  – Multiplexing vs. single target
  – 384-well vs. 96-well

• Automation

• QA/QC issues

• Premature Newborns
Wisconsin’s Laboratory Experience

TREC Assay Performance in Full Term Babies

- Sensitivity: 100% (No known false negatives reported)
- Positive Predictive Value for T cell lymphopenia: 40-60% (based on Flow results)
- Specificity: > 99%
Conclusions

• The NBS TREC assay allows for high-throughput, population based screening for SCID on a State level.

• The NBS TREC assay is relatively inexpensive and highly reproducible.

• The NBS TREC assay has a low screening positive rate (<0.03%).

• The TREC assay successfully identifies infants with SCID and other T cell related primary immunodeficiency.