NEWBORN SCREENING
SEVERE COMBINED IMMUNODEFICIENCY
T CELL LYMPHOPENIA

APHL Webinar
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Disclosures

- Baxter: PI in clinical trials sponsored by Baxter
What is Severe Combined Immune Deficiency: SCID?

- A group of disorders caused by single gene defects resulting in a combined immune deficiency
- *Estimated Prevalence: 1:50,00 to 1:100,000 (U.S)*
  - Ranges from 0.89 to 2.43 cases/100,000 live births
- Over 13 different genetic forms
- All have profound defects in T lymphocyte differentiation and function
- Some (not all) have defects in B cell and/or NK cell differentiation as well
- Affected individuals have severe defects in humoral and cellular immunity
SCID: Clinical presentation

- Present in first 6 months
- Opportunistic infections
  - PJP, CMV, RSV, adenovirus, Candida
- Chronic diarrhea - rotavirus, giardia
- Dermatitis – severe erythroderma
- Persistent thrush – candidal esophagitis
- Failure to thrive
- Absence of lymphoid tissue
- Sepsis - gram negatives
- Family history
Why do newborn screening for SCID?

- Universally fatal without treatment
- Asymptomatic at birth
- Curative therapy is readily available for most
- Early treatment (HSCT before 3 months of age) improves outcomes, decrease morbidity, mortality (Probable savings in healthcare costs)
- Ideal screening test - very high sensitivity, high specificity
- Screening test can be done on newborn dried blood spots
- Test must be inexpensive
- Confirmatory tests are widely available
Public Health Intervention

Outcomes: Better health, reduced morbidity, mortality

Newborn screening
Symptomatic screening

Analytic Validity, Clinical Validity, Clinical Utility, Cost Effectiveness, Value Added, Risk:Benefit

NATURAL HISTORY OF DISEASE

Early sequelae: morbidity
Late sequelae
Disability
Death
Asymptomatic
Early clinical disease
SCID classification

- **X-linked SCID:** Mutation in the gamma chain common to IL-2, IL-4, IL-7 and IL-9 receptors -

- **Autosomal Recessive SCID:**
  - Adenosine Deaminase deficiency
  - Jak3 tyrosine kinase deficiency
  - RAG 1 or 2 defect
  - IL-7R deficiency (α chain)
  - Purine Nucleoside Phosphorylase deficiency
  - MHC II deficiency
  - Artemis (T-B+)
  - ZAP-70 deficiency-
  - CD3γ and CD3ε mutations
  - CD45 deficiency
Genetic Defects

- gc, 45%
- JAK3, 11%
- RAG 1/2, 10%
- ADA, 11%
- IL7a, 9%
- T-B-, 10%
- RD, 1.50%
- T-B+, 2%
- CD3, 0.50%
- T-B+, 2%
- CD3, 0.50%
- T-B-, 10%
- IL7a, 9%
- ADA, 11%
- RAG 1/2, 10%
- JAK3, 11%
**Lymphocyte Stem Cell**

- **Bone Marrow**
  - pre-B-cell
  - NK precursor
  - CD4- CD8-
  - CD4+ CD8+
  - TCR \( \alpha \beta \)
  - IL2RG
  - JAK3
  - ADA
  - RAG1
  - RAG2
  - ARTEMIS
  - CD45

- **Thymus**
  - CD4- CD8-
  - IL-7R
  - CD4- CD8-
  - CD4+ CD8+ TCR\( \beta \)
  - CD4+ CD8+ TCR\( \alpha \beta \)
  - CD45

- **Blood**
  - Naïve B-cell
  - NK-cell
  - CD4+ TCR\( \alpha \beta \+)
  - T helper
  - Naïve T-cells
  - CD4+ TCR\( \alpha \beta \+)
  - CD8+ TCR\( \alpha \beta \+)
  - CD8+ TCR\( \alpha \beta \+)

- * = T-cell receptor excision circle (TREC)
### SCID phenotypes

<table>
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<tr>
<th>TYPE</th>
<th>GENE</th>
<th>CD4</th>
<th>CD8</th>
<th>B</th>
<th>NK</th>
<th>Igs</th>
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<td>↓</td>
<td>NL/↑</td>
<td>↓</td>
<td>Low</td>
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<td>JAK3</td>
<td>19p13.1</td>
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<td>Low</td>
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<tr>
<td>ADA</td>
<td>20q13.2</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>+/-  -</td>
<td>Low</td>
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<tr>
<td>RAG 1/2</td>
<td>11p13</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NL</td>
<td>Low</td>
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<tr>
<td>IL7R alpha</td>
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<tr>
<td>CD3ε</td>
<td>11q23</td>
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<td>↓</td>
<td>NL</td>
<td>NL</td>
<td>Low/ nl</td>
</tr>
<tr>
<td>CD3delta</td>
<td>11q23</td>
<td>↓</td>
<td>↓</td>
<td>NL/↑</td>
<td>NL/↑</td>
<td>Low/ nl</td>
</tr>
<tr>
<td>Artemis</td>
<td>10p</td>
<td>↓</td>
<td>↓</td>
<td>NL</td>
<td>NL</td>
<td>Low</td>
</tr>
<tr>
<td>AR - others</td>
<td></td>
<td>↓*</td>
<td>↓†</td>
<td>NL</td>
<td>NL</td>
<td>Low*</td>
</tr>
</tbody>
</table>

*Except ZAP70 def – 2q12
†except MHC II def- 16p13, 1q21
SCID: Laboratory findings

- Nearly all forms have severe lymphopenia
- All forms have low CD4 cells
- *in vitro* tests of T cell function abnormal
- IgA and M very low - IgG normal (maternal levels)
- no thymic shadow on CXR
- no specific antibodies/isoheamagglutinins
- Very low/absent TREC}s
Newborn Screening

- Early diagnosis is possible by identifying infants with no T cells

- Very sensitive
- Very specific

SCID
DGS
HIV

Lindegren. MMWR: 53:2004
Optimal Screening Test

- Must detect low/absent T cells
- Use existing NBS screening cards
  - Doesn’t require whole blood sample
- Inexpensive, sensitive and specific
  - No missed cases
  - Low rate of false positive tests
  - Little need for retesting
- Real Time quantitative PCR (qPCR): enumeration of T cell receptor excision circles (TREC) using DNA extracted from newborn blood spots = thymic output of naïve T cells
Screening algorithm

Initial screen abnormal (low TREC/Ns)

- Repeat screen abnormal
  - Low TREC/N Control
    - Premature infants: repeat testing until 36 wk [GA]
      - Notify PCP/family secondary testing
        - Whole blood sample
        - CBC diff
        - Flow cytometry
    - Repeat screen normal
      - No further testing*

* May choose to collect another sample
Confirmatory (Secondary) testing

- **Whole blood sample** (heparinized) sent to Clinical Immunology Laboratory + CBC with differential
  - Absolute lymphocyte count
  - Markers/Subsets evaluated by flow cytometry
    - cell % and Absolute counts: CD3CD4, CD3CD8, CD56(NK), CD19 (B)
- **Abnormal flow cytometry:**
  - immediate evaluation by PI specialist for diagnostic testing
  - Avoid live vaccines (rotavirus)
Screening for SCID/T cell lymphopenia

- Screening underway since 2008
- >800,000 infants screened to date in 5 states + PR
- Diagnoses
  - SCID: X-linked, JAK3, IL-7R, RAG1/2, ADA, PNP, CD45, Artemis, CD3, Reticular dysgenesis, DNA ligaseIV, Cernunnos, CHH, Other
  - SCID variants: Omenn, ILRa, ADA, other
  - Non-SCID*: Rac2 deficiency, Complete DiGeorge, Idiopathic T cell lymphopenia, Congenital chylothorax, Gastroschisis, Jacobsen syndrome, Trisomy 21, Ataxia telangiectasia, CD3CD25 defect, CHARGE syndrome

*Non-SCID all have clinically significant T cell lymphopenia
**TREC assay**

- **Sensitivity:**
  - *All known SCID babies identified in WI had abnormal (low-absent) TREC*
  - “Leaky” SCID could have sufficient TREC to be missed (Omenn, ZAP-70)
  - Maternal engraftment of T cells should still result in low TREC#

- **Lymphopenia not SCID** (primary and secondary forms)
  - **Primary causes:** DiGeorge Syndrome, rac2 defect, Trisomy 21, Jacobsen syndrome, idiopathic T cell lymphopenia, CD3CD25 defects, CHARGE
  - Premature infants (AGA <37 weeks): **transient**
  - **Secondary causes:** Cardiac bypass (dilution), 3rd spacing of lymphocytes (chylothorax, gastroschisis, anasarca)
  - **T cell destruction:** intrauterine infection with HIV

- **Screen failures:** no amplification of control
  - DNA template integrity
  - PCR inhibitors
  - Technical errors
SCID variants

- SCID phenotype: T cell lymphopenia (incomplete), immune deficient (severe infections), fatal without treatment
- Caused by hypomorphomic mutations in genes known to cause “classic” SCID (e.g. RAG, ADA, IL7Ra)
- Omenn: severe dermatitis, lymphadenopathy, failure to thrive, infections, Low (not absent) T cells, poor T cell function, low B cells
Non-SCID T cell lymphopenia

- **DiGeorge syndrome**: conotruncal heart defect, hypoparathyroidism, absence of thymus
- **CHARGE**: coloboma, heart defect, coanal atresia, retardation, genital, ear abnormalities
- Other syndromes: Trisomy 21, Jacobsen, Ataxia-telangiectasia
- Loss of T cells
  - Through lymphatics: chylothorax, lymphangiectasia
  - From gut: gastroschisis
Diagnostic testing for SCID

- CBC with differential showing lymphopenia
- Flow cytometry showing T cell lymphopenia, +/- B, +/- NK cell lymphopenia
- Additional testing may be done by the clinicians:
  - Lymphocyte proliferation after mitogen stimulation
  - Flow cytometry for CD45, CD45Ra/Ro
  - DNA analysis: sequencing of candidate gene
SCID: Therapy

- Bone Marrow Transplantation:
  - use of T cell depleted marrow
  - success for HLA-identical sibling donor > 90%

- Enzyme Therapy
  - PEG-ADA replacement therapy

- Gene Therapy
  - modifying T cells with normal gene for ADA, IL-2RG, Jak3 and reinfusing into patients

**Early diagnosis is key**
Once diagnosis of SCID is established:

- Referral to clinical center
- Initiation of protective isolation – no day care
- **Avoid live vaccines** (rotavirus, MMR, varicella)
- *Pneumocystis jirovecii* prophylaxis
- Gammaglobulin replacement (IVIG or SCIG)
- Identification of match for Hematopoietic Stem Cell Transplant
  - Transplants can be done as early as 3 weeks of age
    - In utero transplants have been done
  - National registries source of unrelated potential donors
Challenges

- Abnormal TREC screens in premature neonates
- Increasing awareness/education of healthcare providers
- Identification of clinicians/centers for diagnosis and treatment
  - Development of ACT sheet for ACMG
  - Collaboration with American Academy Pediatrics, American College Medical Genetics, Regional Screening groups, state newborn screening labs