Comprehensive Newborn Screening for Severe Combined Immunodeficiency in Manitoba, Canada

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Our Unique Populations

- Aboriginal
- First Nations
- Inuit
- Métis
- Mennonite
- Hutterite
- Amish
Severe Combined Immunodeficiency

- Is the most profound form of the Primary Immunodeficiency Diseases (PID) (~ 200 congenital disorders)
- Characterized by profound impairment in T-cell development and function and lack of an adaptive immune system
- Fatal within the 1st year without BMT
- Early treatment is associated with better outcome
- “Classic SCID”
  - Clinical features – chronic diarrhea, pneumonia, failure to thrive, persistent thrush
  - And one of:
    - Absolute lymphocyte count < 3 x 10^9/L
    - Family history
    - Exclusion – HIV infection or cystic fibrosis

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SCID Immunophenotype TBNK Classification

- **T- B+ NK+**
  - Common gamma chain; JAK3 defect
- **T- B- NK+**
  - RAG1, RAG2 – Omenn’s syndrome
- **T- B+ NK-**
  - CD3δ defect
  - Di George Syndrome (del22q11.2)
- **T- B- NK-**
  - ADA
- **T+ B+ NK+**
  - Mennonites
  - First Nations
## Comparative Summary

<table>
<thead>
<tr>
<th></th>
<th>Classic</th>
<th>Mennonite</th>
<th>Northern Cree</th>
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</thead>
<tbody>
<tr>
<td><strong>Age at presentation</strong></td>
<td>&lt; 1 year</td>
<td>6-18 months</td>
<td>2-6 months</td>
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<tr>
<td><strong>Clinical picture</strong></td>
<td>Infection</td>
<td>Respiratory</td>
<td>Overwhelming sepsis</td>
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<tr>
<td><strong>Lymphocyte count</strong></td>
<td>&lt; 2</td>
<td>5-14</td>
<td>4-9</td>
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<tr>
<td><strong>Immunoglobulins</strong></td>
<td>Decreased or absent</td>
<td>Normal or increased</td>
<td>Absent</td>
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<tr>
<td><strong>T+ B+ NK+</strong></td>
<td>Absent T cells</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td><strong>CD8+ cells</strong></td>
<td>Absent</td>
<td>Decreased</td>
<td>Normal</td>
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<tr>
<td><strong>Mitogen response</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Low to normal</td>
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<tr>
<td><strong>Mutation</strong></td>
<td>Multiple</td>
<td>ZAP70</td>
<td>IKBKB</td>
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**TRECS**

- **Extra-chromosomal T-cell receptor excision circles**
  - TRECs are formed during maturation of T lymphocytes in the thymus (TCR gene rearrangement; normal cutting and splicing events with end-joining of genomic DNA segments)

- Newborn babies normally have high numbers of naïve T-cells and TRECs

- Typically, SCID babies have few or no naïve T-cells and TRECs

- Detects not only SCID, but other forms of T cell lymphopenia
  - Absent in X-SCID
  - Decreased / variable in other SCID variants

- Decreased in premature infants

- Accepted test for newborn screening
Retrospective Pilot Study

- Overall incidence of SCID/PID in Manitoba is ~1:15,000, 3X more frequent than the national average and is overrepresented in 2 groups: Mennonites (ZAP70) and First Nations (IKBKB) babies that have T cells.

- Challenge: TREC method would likely not flag these babies unless their TRECs were significantly decreased.

- Retrospective pilot study* to establish normative TREC data and determine whether archived newborn specimens from 18 SCID/PID babies born between 1992 and 2010 could be identified from 982 normal, age matched control specimens by the TREC assay.

*Jilkina et. al. Molecular Genetics and Metabolism Reports 1 (2014) 324-333
Retrospective Pilot Study

- 18 SCID/PID babies all requiring BMT

- (5) T-cell NEG. SCID  - 3 ADA, 1 CD3, 1 Clinical SCID
- (8) T-cell POS. SCID  - 5 ZAP70, 3 IKBKB
- (1) T-cell NEG. PID   - CHH
- (4) T-cell POS. PID   - 2 CVID, 1 WAS, 1 XLP

Screen Positive (9)
- TREC/ul (0)        - 3 ADA, 1 CHH, 1 CD3
- TREC/ul (<252)     - 1 Clinical SCID (no molecular diagnosis)

- 2 ZAP70 zeta chain-associated protein kinase
- 1 CVID

False Positive
- 5 preterm babies, 1 twin, 4 unknown

Screen Negative
- TREC/ul (>252)     - 3 ZAP70, 1 WAS, 3 IKBKB, 1 CVID, 1 XLP
**IKBKB Sequencing**

**Exon 13**

**Control**

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**F1P1**

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**c.1292_1293insG**

**p.Gln432ProfsX62**
ZAP 70 MUTATION

Normal 3’ intronic sequence

Mennonite mutation

The newly created splice acceptor sequence results in the insertion of 9 nts to the mRNA and three additional amino acids to the protein product (inactivating the kinase).
High Resolution Melt Analysis

- Designed primers to produce short amplicons containing the respective mutation sites utilizing the same extract of DNA prepared for the TREC assay
- Amplify a 60bp fragment flanking the mutated IKBKB sequence
- Amplify 96bp fragment flanking the mutated Zap70 sequence
- **Goal:** use melt curves to exploit the difference in melt temperature (Tm) of the amplicons created by the presence of the mutation in order to genotype patient samples
- Amplified in the presence of SYBR green dye, which binds double stranded DNA
Raw Melt Curve

- Software produces a raw melt curve adjusted by the negative first derivative to visualize the dissociation temperatures (50% dissociation) of the amplicons.
Precision analysis

- HRM software manipulates raw fluorescent data by normalization to correct for background signal
- Areas of stable pre- and post-melt fluorescence intensity are set to relative values of 1.0 and 0
- Produces Normalized Melt Curve for clear differentiation of both sets of amplicons
Difference Curve

- Software generates an easy to read difference curve to visually magnify the melt profile differences between different clusters of the same genotype
- Includes percent confidence of cluster assignment
Zap 70
IKBKB Product Sequencing
IKBKB Heterozygote
Conclusions

- Universal application of the multiplex high resolution DNA melt analysis for mutation genotyping in combination with TREC quantification on all newborns is planned for implementation and should provide comprehensive detection of SCID in the newborn population of Manitoba.

- Screen positive criteria, follow up investigation and downstream protocols are under development.