Molecular Diagnosis of Severe Congenital Immunodeficiencies

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Severe Combined Immunodeficiency (SCID)

- severe defects in cellular and humoral immunity
- different immunological phenotypes
- common clinical presentation
- genetic heterogeneity
- common treatment options

‘Bubble babies’
### Causes of pneumonitis in SCID

- **Pneumocystis carinii (PCP)**
- **Cytomegalovirus (CMV)**
- **RSV**
- **Adenovirus**
- **Parainfluenza virus**

### Severe combined immunodeficiency (SCID)

- **Virus**
- **Bacteria**
- **Fungi**

- Pneumonia, diarrhoea, repeated infections

### Molecular defects in SCID

**SCID/CID**

- γc, IL7Rα, JAK3, ZAP-70
- RAG1/2, Artemis, DNA ligase IV, Cernunnos
- ADA, PNP
- MHC I/II, CD3δεζ, CD45, ORAI1, Coronin 1A

### Immunophenotypes in SCID

- **T-**
- **B-**
- **NK-**
- γc-JAK3 SCID
- IL-7Rα deficiency, Coronin-1A, ZAP-70, CD3δεζ
- ADA SCID, Reticular Dysgenesis
- RAG1/2 deficiency, Artemis, Cernunnos, DNA ligase IV, DNApolcs

- **T+**
- **B+**
- **NK+**

### Molecular defects in SCID

- Cytokine signaling defects – γc, JAK3, IL-7Rα
- TCR defects – CD3δεζ, ZAP-70
- VDJ recombination defects – RAG1/2, Artemis, DNA ligase IV, Cernunnos, DNApolcs
- Defects of metabolism – ADA, PNP, AK2 (reticular dysgenesis)
- Other – MHC class II def, Coronin 1A, Ora 1
Primary disease in SCID patients (n=477)

- ADA def: 11%
- B(-): 29%
- B(+): 48%
- Other SCID: 9%
- Reticular dysgenesis: 3%

X-linked SCID

- Most common form of SCID ~ 40-50%
- Classical T-B-NK phenotype
- Carriers show non-random X-inactivation in T cell lineage
- Defect in common γ chain (Noguchi et al. 1993)
- Common component of IL-2, IL-4, IL-7, IL-9, IL-15 cytokine receptors

Flowcytometric analysis of γc expression in XSCID patients

- White Cell Count: 9.74 x10^9/L
- Lymphocyte Count: 1.30 x10^9/L
- CD3: 0.0% 0.00 x10^9/L
- CD19: 96.0% 1.25 x10^9/L
- CD16+CD56+: 1.0% 0.01 x10^9/L
- CD3+CD4+: 0.0% 0.00 x10^9/L
- CD3+CD8+: 0.0% 0.00 x10^9/L
- IgG: 2.4 G/L (3 – 9)
- IgA: <0.06 G/L (0.15 - 0.7)
- IgM: 0.19 G/L (0.4 - 1.6)
Diagnosis of T-B+NK- SCID

γc expression

Phospho STAT5

Mutation (γc) R298X
Diagnosis: X-SCID

Defective IL7R expression in T-B+NK+ severe combined immunodeficiency

Diagnosis of T-B+NK- SCID

IL-7

αα αα αα

γγ γγ γγ

defects in IL-7R

Classical presentation at 2-4mths of age

Treated by HSCT

T-B+NK+ SCID 3

Control

T-B+NK+ SCID 3

• Normal γc expression
• Normal STAT5 ptyr to IL-2 and IL-15
• Abnormal STAT5 ptyr to IL-7
• Sequences IL-7Ra:
  – c.83-2A>G/heterozygote
  – p.Lys128X/heterozygote

Change in percent STAT5 tyrosine phosphorylated cells post IL-2 stimulation shown by diagnosis/therapy

Details of pre-therapy α and α-JAK3 are in the Table.

Some used CD8(+) T-cell: immunodeficient/normal counts reconstituted: normal counts excludes α and α-JAK3 defects, but progesterone (αc)-2αc, γc αc/o, αc post p2αK Dc post p5α therapy. Post IL-2 run 1 TCD-cell post bone cell transplant.

Effect of CD34 Deficiency on Mutation of α and β T-Cell Lineages in SCID Combined Immunodeficiency

Inherited and Somatic CD4 Mutations in a Patient with T-Cell Deficiency

T-B+NK+ SCID

TCR defects

Detective T Cell Receptor Signaling and CD8 Thymic Selection in Humans Lacking Zap-70 Kinase

Defective T-B+NK+ SCID CD4 absent/low
Low poorly functional CD4+
A Human Severe Combined Immunodeficiency (SCID) Condition with Increased Sensitivity to Ionizing Radiations and Impaired V(D)J Rearrangements Defines a New DNA Recombination/Repair Deficiency

By Nathalie Nicolas,* Denis Mottier,⁎ Marcella Cerutti-Caillé,* Dena Papathanopoulou,* Marie de Clercq,* Blaise L. Illei,* Alain Fache,* and Jean-Pierre de Villers⁹

Radiosensitive forms of SCID

<table>
<thead>
<tr>
<th>Defective gene</th>
<th>Mutation</th>
<th>Radiosensitivity</th>
<th>Immure defect</th>
<th>Growth delay</th>
<th>Microcephaly</th>
<th>Cancer predisposition</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Artemis</td>
<td>Null</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>DNA-PKcs</td>
<td>Homozygous</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
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</table>

SDC: sometimes immuno-deficiency.
DNA-PKcs recently described

novel defect in T-B- SCID

Radiosensitivity in comparison to RAG

Fibroblast radiation sensitivity in GOSH patients
Patient with florid rash, lymphadenopathy, FTT and pneumonitis

**LYMPHOCYTE PANEL**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>HEM RESULTS: WBC</td>
<td>8.3 ABS LY 6.64</td>
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<tr>
<td>CD3 44 %</td>
<td></td>
</tr>
<tr>
<td>CD19 45 %</td>
<td></td>
</tr>
<tr>
<td>CD16+CD56+ 0 %</td>
<td></td>
</tr>
<tr>
<td>CD4 4 %</td>
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<td>CD8 42 %</td>
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**PHA STIMULATION**

<table>
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<tr>
<th>PHA ug/ml</th>
<th>MEAN DPM</th>
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<tr>
<td>0.0</td>
<td>295</td>
</tr>
<tr>
<td>1.0</td>
<td>312</td>
</tr>
<tr>
<td>2.0</td>
<td>336</td>
</tr>
<tr>
<td>4.0</td>
<td>403</td>
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<td>8.0</td>
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Stimulation Index 1.49

**Immunoglobulins**

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<tr>
<td>IgG</td>
<td>4.1 G/L</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;0.06 G/L</td>
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<td>IgM</td>
<td>0.09 G/L</td>
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<tr>
<td>Total IgE</td>
<td>826 IU/ML</td>
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**Omenn’s Syndrome**

- Clinical presentation of SCID
- Erythroderma, eosinophilia, hepatosplenomegaly, lymphadenopathy
- Increased IgE, activated T cells, low/absent B cells
- Restricted TCR Vβ usage
- Mutations found in RAG1 and RAG2
- Mainly missense mutations with stable protein expression
- Partial V(D)J activity retained
- OS mutants affect the formation of RSS/RAG1/RAG2 complex

**Restricted TCRVβ clonality in Omenn’s syndrome**

Peggs and Mackinnon, BJH 2004

**TRECs**

Results

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<tr>
<th>Measure</th>
<th>Progression</th>
<th>Results per million cells</th>
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<tbody>
<tr>
<td>ND</td>
<td>T cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NK cell</td>
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</tr>
<tr>
<td></td>
<td>B cell</td>
<td></td>
</tr>
<tr>
<td>56981</td>
<td>T cell</td>
<td>3.11 x 10^7</td>
</tr>
<tr>
<td>54</td>
<td>NK cell</td>
<td>6.23 x 10^6</td>
</tr>
<tr>
<td>56981</td>
<td>B cell</td>
<td>2.83 x 10^6</td>
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</tr>
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SCID is diagnosed in all SCID DBS TREC samples. Includes patients with maternal engraftment and atypical feature CID/evans.

Omenn’s Syndrome

- Clinical and Immunological syndrome
- Heterogeneous molecular basis
- Defects in RAG1/2, Artemis, gamma c, ADA, CHH, IL-7Rα, etc
- Restricted TCR Vβ usage
- Low TREC levels

2 month old child with severe FTT, diarrhoea and recurrent cough

Spinal Type: SARAH
PHENOMEDICAL BILATERAL

- T 7.79 GL 0.2 - 10
- a2M GL 6.0 0.0 - 0.5
- IgG 6.65 GL 0.0 - 2.0
- CD16+CD56+ 2.0 % 0.00 x 10^9/L
- CD19 9.3 % 0.91 x 10^9/L
- CD14+CD16+ 0.01 % 0.00 x 10^9/L
- CD16 CD56 0.0 % 0.00 x 10^9/L
- CD16 CD56 0.0 % 0.00 x 10^9/L
- CD14 0.0 % 0.00 x 10^9/L

Spinal Type: Blood (peripheral)
PHA STIMULATION

- CD15 CD3 0.0 %
- T cell 0.00 x 10^9/L
- CD19 9.3 %
- b cell 0.91 x 10^9/L
- CD14+CD16+ 0.01 %
- d cell 0.00 x 10^9/L
- CD16 CD56 0.0 %
- d-adenosine 0.00 x 10^9/L
- CD16 CD56 0.0 %
- d-adenosine 0.00 x 10^9/L
- CD14 0.0 %
- d-adenosine 0.00 x 10^9/L

ADA deficiency

- Enzyme expressed in all body cells - purine salvage pathway
- Deficiency results in abnormalities of lymphocyte function and proliferation
- 10-15% of all cases of SCID
- Variability in clinical presentation

Biochemical defect in ADA deficiency

Immunodeficiency in ADA deficiency

- Cell mediated and humoral abnormality
- IL-2, IL-12, NK
- 85-90% present in the first year of life
- Pneumonitis, diarrhoea, skin infection, FTT
- 15-30% delayed onset partial ADA deficiency
- Present 0-3 years of life
- less severe infective episodes
- less severe immunophenotype and metabolic abnormalities
Defects in MHC class II expression

Absence of DR expression on immunophenotype

The actin regulator coronin-1A is mutated in a thymic region
deficient mouse strain and in a T/B/MK-SCID patient

Immunophenotypes in SCID

Cytotoxic T cell

Figure 1.

A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function

Immunological synapse

Figure 2.

Perforin

SAP

Figure 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>A91V/R410W</th>
<th>Deletion</th>
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<tbody>
<tr>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>SAP</td>
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<td>Reduced</td>
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<tr>
<td>Perforin</td>
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</table>
Figure 3a: Control (17)  Munc13-4 deficient (1)  STX11 deficient (3)

Figure 3b: Munc 13-4 mutation in patient: P72fsX75