Severe Combined Immunodeficiency (SCID)
including Omenn Syndrome and Zap-70 Deficiency
Severe Combined Immunodeficiency (SCID) including Omenn Syndrome (SCID/OS) and Zap-70 deficiency

Frequently used abbreviations: **ADA** - adenosine deaminase; **Ado** - adenosine; **D** - diversity; **dAdo** - 2′-deoxyadenosine; **dATP** - deoxyadenosine triphosphate; **GVHD** - graft-versus-host disease; **HSCT** - hematopoietic stem cell transplantation; **IL** - interleukin; **IL-7R** - interleukin-7 receptor; **J** - joining; **Jak** - Janus kinase; **NHEJ** - non-homologous end-joining; **NK** - natural killer cell; **OS** - Omenn Syndrome; **RAG1/2** - recombination-activating proteins 1/2; **RSS** - recombination signal sequences; **RS-SCID** - radiation-sensitive SCID; **SCID** - Severe Combined Immunodeficiency; **STAT** - signal transducer and activator of transcription proteins; **TCR** - T-cell receptor; **V** - variable; **XSCID** - X-linked SCID; **Zap-70** - ζ-chain associated protein of 70 kDa

Introduction

Severe Combined Immunodeficiency (SCID) is characterized by severe lymphopenia and lack of adaptive immunity and, if untreated, leads to death through infection. SCID occurs with an estimated incidence of 1 in 75,000 births (1) and is considered a pediatric emergency because of the potentially lethal outcome of recurrent or persistent infections suffered by SCID patients. Several monogenic causes with different modes of inheritance have been identified for SCID (reviewed in 2).

Genetic testing for SCID can allow distinction between the various forms of this syndrome. Knowledge of the defective gene may have implications for treatment and prognosis. This knowledge may also enable more effective genetic counseling, in addition to facilitating identification of asymptomatic carriers and timely initiation of treatment in affected descendants of carriers.

Types and Causes of SCID

Depending on the underlying genetic defect, four different primary phenotypes associated with SCID have been characterized (for comprehensive reviews, refer to references 1-3). Categorization is based on the classes of lymphocytes that are absent or severely reduced. T-cell lymphopenia is generally common to all forms of SCID, but levels of B and natural killer (NK) cells vary depending on the genetic defect. For certain rare subtypes of SCID, T cells may be present, but their function is impaired.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Product</th>
<th>Lymphocyte Phenotype</th>
<th>Associated Mode of Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL2RG</strong></td>
<td>Common γ chain of IL receptors (γc)</td>
<td>T− B+ NK−</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td><strong>JAK3</strong></td>
<td>Janus kinase 3 (Jak3)</td>
<td>T− B− NK+</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>ADA</strong></td>
<td>Adenosine deaminase (ADA)</td>
<td>T− B− NK−</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>RAG1</strong></td>
<td>Recombination-activating protein 1 (RAG1)</td>
<td>T− B+ NK−</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>RAG2</strong></td>
<td>Recombination-activating protein 2 (RAG2)</td>
<td>T− B− NK+</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>DCLRE1C</strong></td>
<td>DNA-cross-link repair protein 1C (Artemis)</td>
<td>T− B+ NK+</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>IL7R</strong></td>
<td>IL-7 receptor α chain (IL-R α, CD127)</td>
<td>T− B+ NK+</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>CD3D</strong></td>
<td>CD3 δ chain</td>
<td>T− B− NK−</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td><strong>CD3E</strong></td>
<td>CD3 ε chain</td>
<td>T*(CD4*CD8−)B+ NK+</td>
<td>autosomal recessive</td>
</tr>
</tbody>
</table>
While B and T cells, NK-cell development is normal (reviewed in 1). Notably, mutations in DCLRE1C, commonly known as Omenn Syndrome (OS) (16, 17) (see below). DCLRE1C, also known as DNA double-strand break repair protein 1C (ARTEMIS), is recruited to the DNA double-strand break (DSB) repair pathway, forming a stable complex with RAG1 and RAG2. This complex then recruits Artemis, a critical component of the non-homologous end joining (NHEJ) DNA repair machinery. Upon joining the NHEJ complex, Artemis cleaves the DNA hairpin structures (22), allowing other NHEJ components to repair the damaged DNA, thus completing the recombination process. Defects in either RAG1, RAG2, or Artemis prevent productive recombination of both the B- and T-cell receptors, which is a prerequisite for B and T cell maturation, thereby blocking development of B and T cells at very early stages (23, 24). The resulting T- and B-cell lymphopenia leads to increased susceptibility to a wide range of infections, including opportunistic pathogens. NK-cell levels are normal or elevated. Defects in Artemis also cause cellular sensitivity to ionizing radiation, likely due to a role for Artemis in the general DNA double-strand break repair pathway (25). DCLRE1C-related SCID is therefore also known as radiation-sensitive SCID (RS-SCID).
**T B'NK' SCID**

T B'NK' SCID is often associated with autosomal recessive loss-of-function mutations in *IL7R*, which encodes the α subunit of the IL-7 receptor (reviewed in 6). Additional causes of this type of SCID include autosomal recessive loss-of-function mutations in *CD3D* and *CD3E*, which code for proteins necessary for signaling through the pre-T-cell receptor (pre-TCR) or the TCR (reviewed in 1). Autosomal recessive loss-of-function mutations in *ZAP70*, which encodes a signaling protein that associates with the CD3 complex, give rise to a rare subtype of SCID characterized by the selective absence of CD8⁺ T cells (reviewed in 26, 27).

IL-7R α, the *IL7R* gene product, is a component of the interleukin-7 receptor (IL-7R) (reviewed in 28, 29). Interaction of IL-7 with IL-7R leads to recruitment of the intracellular signaling molecules Jak1 and Jak3. Phosphorylation of IL-7R α by Jak proteins activates multiple downstream signaling pathways which are important for transcriptional activation of genes involved in T-cell differentiation (30), T-cell survival and maturation (31), and TCR rearrangement (32). Disruption of IL-7 signaling arrests T-cell development at the double negative (CD4⁻CD8⁻) stage, preventing productive TCR rearrangement (33) and leading to T-cell lymphopenia.

The CD3D and CD3E gene products, CD3 δ and CD3 ε, respectively, are components of the invariant CD3 protein complex that pairs with the variable antigen-recognition subunits to form both the pre-TCR and the TCR. The CD3 complex is comprised of the CD3 γ, δ, ε, and ζ transmembrane protein subunits; each subunit contains at least one immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic tail (reviewed in 34). Upon ligand binding to the pre-TCR or the TCR, these ITAMs are phosphorylated, initiating a signaling cascade that results in transcriptional activation of genes involved in T-cell differentiation and proliferation. CD3 association with the pre-TCR is required for the transition from double negative to double positive T cells during thymocyte maturation and for rearrangement of the TCRα chain (35). Surface expression of and signaling by the αβTCR/CD3 complex is necessary for thymocyte maturation from double positive to single positive CD4 or CD8 T cells (reviewed in 36). Defects in any of the CD3 subunits can result in impaired TCR surface expression and/or signaling, thereby hindering thymocyte maturation. Defects in CD3 δ or CD3 ε lead to an arrest of thymocyte development at the double negative (CD4⁻CD8⁻) stage, causing a severe deficiency of both αβ and γδ T cells (36, 37).

Loss-of-function mutations in *IL7R*, *CD3D*, or *CD3E* result in severe T-cell lymphopenia. NK and B cells are present at normal levels, but B-cell activation and class-switch recombination are impaired, probably due to the failure of T-cell help. The T-cell lymphopenia associated with *IL7R*-, *CD3D*-, and *CD3E*-related SCID leads to recurrent opportunistic infections, while the defect in B-cell function further contributes to susceptibility to severe or recurrent infections.

Zap-70 is a Syk family protein tyrosine kinase expressed exclusively in T cells and NK cells. Zap-70 is recruited to the ζ chain of the invariant CD3 protein complex of the TCR in response to TCR engagement (38, 39). Once associated with the TCR/CD3 complex, Zap-70 is phosphorylated and activated. Activation of Zap-70 leads to recruitment of additional downstream signaling molecules, inducing a signaling cascade that results in transcriptional activation of genes required for T-cell activation, differentiation, proliferation, and function (reviewed in 40, 41). Loss-of-function mutations in *ZAP-70* disrupt T-cell development at the transition from double positive (CD4⁺CD8⁺) to single positive T cells, leading to a selective absence of peripheral CD8⁺ T cells, likely due to a defect in the positive selection of these cells (42). CD4⁺ T cells are present at normal or elevated levels, but do not function properly, indicating that Zap-70 is not essential for CD4⁺ T-cell development (43), but required for their activation and proliferation. As in T B'NK' SCID, NK and B cells are present at normal levels, but in most cases, B-cell function is impaired (26).

**Omenn Syndrome**

Mutations in *RAG1* or *RAG2* that allow residual enzyme activity (so called hypomorphic mutations) result in impaired V(D)J recombination and limited TCR diversity and can give rise to a distinct SCID phenotype commonly known as Omenn Syndrome (OS), which is distinguished from classic SCID by the presence of oligoclonal T cells, elevated levels of serum IgE, and eosinophilia (reviewed in 17).

Although hypomorphic mutations in *RAG1* or *RAG2* are the best characterized cause of OS, they do not account for all cases of OS (44). Hypomorphic mutations in *IL7R* (45) and *DCLRE1C* (ARTEMIS) (16) have also been implicated in OS, and the identification of additional causes is anticipated.

**Clinical Presentation of SCID**

Symptoms of SCID usually appear within the first few months after birth, following a brief asymptomatic period due to the presence of maternal Igs. Infants with SCID typically suffer from severe and persistent infections, intractable diarrhea, and failure to thrive (2). Infections are characteristically caused by opportunistic organisms such as...
Pseudomonas, Salmonella, Candida albicans, or Pneumocystis carinii or viruses such as respiratory syncytial virus, adenovirus, or cytomegalovirus. Lymph nodes and tonsils are absent. X-ray imaging will usually reveal a small thymus lacking thymocytes; however, in rare cases, a nearly normal-sized thymus may also be observed (36). SCID-associated deficiency in endogenous T cells can be initially masked by the temporary presence of maternal lymphocytes that have crossed the placenta during gestation (46). Engraftment of such maternal T cells or T cells received through postnatal blood transfusions can lead to graft-versus-host disease (GVHD) (47). The defect in B-cell function results in hypogammaglobulinemia and failure to mount a specific antibody response to immunization (48).

**ADA-related SCID** (T⁻B⁺NK⁻ SCID) is further characterized by the presence of skeletal and neurological abnormalities and/or excretion of large amounts of dAdo in the urine (12). The level of clinical severity and immune dysfunction associated with mutations in ADA varies substantially, with mutations that allow some ADA activity giving rise to milder disease or later onset of symptoms (12).

**DCLRE1C-related SCID** (T⁻B⁻NK⁺ SCID or RS-SCID) is also associated with increased sensitivity of both bone marrow and skin fibroblasts to ionizing radiation (49, 50), B-cell lymphomas (51), and oral and genital ulcers (52).

**Zap-70 deficiency** is clinically similar to classic SCID, but is distinguished by the selective absence of CD8⁺ T cells in the presence of normal or elevated CD4⁺ T cells levels. Although CD4⁺ T cells are present, proliferative responses to antigens are severely reduced. In most cases of Zap-70 deficiency, lymph nodes and tonsils are palpable and X-ray imaging will show a normal-sized thymus (26). Thymic biopsy will reveal the presence of double positive (CD4⁺CD8⁺) thymocytes in the cortex, while CD4⁺, but not CD8⁺ single positive cells are present in the medulla (42).

**Omenn Syndrome** can be distinguished from SCID by the presence normal or elevated T-cell levels with a restricted, oligoclonal T-cell repertoire, elevated levels of serum IgE, and eosinophilia. In rare cases, B cells are also present; however both B cells and T cells are oligoclonal and non-functional (53). Lymph nodes, liver, and spleen are enlarged (17). Patients with OS also suffer from exudative erythematous chronic diarrhea due to infiltration of the skin and intestines by activated T cells, resulting in symptoms that resemble graft-versus-host disease (GVHD) (25).

### Diagnosis of SCID

Patients with SCID and related SCID subtypes are usually diagnosed during the first few months after birth. SCID is suspected in infants presenting with recurrent, severe, or unusual infections and lymphopenia. Diagnosis currently relies on detection of a reduction in total lymphocyte counts, detection of diminished T-cell levels by flow cytometry, and lymphocyte functional tests, and is supported by a family history of death in infancy due to infection or a family history of SCID. Biochemical testing may be considered to identify ADA deficiency (12).

Diagnosis of SCID and its subtypes can also be achieved through genetic testing. Importantly, genetic testing allows detection of carriers, allowing more effective genetic counseling. Genetic testing of newborns known to have a genetic predisposition for SCID can allow diagnosis and treatment of SCID before any life-threatening infections develop. Such early treatment has been correlated to increased survival (47).

### Treatment of SCID

Hematopoietic stem cell transfer (HSCT) has proven successful in controlling the defect in T-cell mediated immunity (2), although continued intravenous immunoglobulin therapy is often necessary if B cells do not engraft. HSCT is also the recommended treatment for OS (17) and Zap-70 deficiency (26). When performed before serious infection develops, HSCT can result in a survival rate as high as 97% (3), making early diagnosis critical.

ADA-related SCID patients who are unable to receive HSCT may benefit from enzyme replacement with polyethylene glycol-conjugated ADA (PEG-ADA), which has been shown to increase T-lymphocyte levels and improve cellular immune function.

Recent clinical trials suggest that gene therapy will be another promising treatment option for both ADA-related SCID and IL2RG-related XSCID; however, clinical trials are currently on hold due to development of a form of leukemia in two IL2RG gene-therapy recipients (2, 47). Research into the development of gene therapy-based replacement of both Jak3 (5) and Zap-70 (54, 55) is also ongoing, but has not yet yielded viable treatment options.
Genetics of SCID

IL2RG-related XSCID is an X-linked recessive disorder, exclusively affecting males. Of note, mutations in IL2RG resulting in a milder form of XSCID, distinguished by the presence of circulating T cells and delayed onset of infections, have also been characterized (56-59).

Loss-of-function mutations in CD3E that allow residual expression of CD3ε are associated with milder immunodeficiency characterized by recurrent bacterial and viral infections (36, 60).

Except for IL2RG-related XSCID, all other known types of SCID are inherited in an autosomal recessive manner, affecting both males and females.

Although very rare in the general population, DCLRE1C-related SCID occurs with an incidence of 1 in 2000 live births within the Athabascan-speaking Navajo and Apache Native American population (often referred to as Athabascan SCID or SCIDA) due to a founder mutation in DCLRE1C (52).

Table 2

<table>
<thead>
<tr>
<th>Lymphocyte Phenotype</th>
<th>Affected Gene</th>
<th>Relative Frequency (3)</th>
<th>Affects</th>
</tr>
</thead>
<tbody>
<tr>
<td>T− B+ NK−</td>
<td>IL2RG</td>
<td>46%</td>
<td>males only</td>
</tr>
<tr>
<td></td>
<td>JAK3</td>
<td>6.9%</td>
<td>males and females</td>
</tr>
<tr>
<td>T− B− NK−</td>
<td>ADA</td>
<td>16.1%</td>
<td>males and females</td>
</tr>
<tr>
<td>T− B− NK+</td>
<td>RAG1</td>
<td>3.4%</td>
<td>males and females</td>
</tr>
<tr>
<td></td>
<td>RAG2</td>
<td>3.4%</td>
<td>males and females</td>
</tr>
<tr>
<td></td>
<td>DCLRE1C</td>
<td>1.1%</td>
<td>males and females</td>
</tr>
<tr>
<td>T− B+ NK+</td>
<td>IL7R</td>
<td>10.3%</td>
<td>males and females</td>
</tr>
<tr>
<td></td>
<td>CD3D</td>
<td>0.6%</td>
<td>males and females</td>
</tr>
<tr>
<td></td>
<td>CD3E</td>
<td>0.6%</td>
<td>males and females</td>
</tr>
<tr>
<td>T+ (CD4+CD8−) B+ NK+</td>
<td>ZAP70</td>
<td>n/a</td>
<td>males and females</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>~15%</td>
<td></td>
</tr>
</tbody>
</table>

Omenn Syndrome

Loss-of-function mutations in RAG1, RAG2, DCLRE1C, or IL7R can result in either SCID or OS (16, 17, 45). Both SCID and OS are inherited in an autosomal recessive manner, that is, both copies of the affected gene must harbor a loss-of-function mutation for a disease phenotype to be expressed. If both gene copies contain a mutation that leads to complete loss of enzyme function, the resulting phenotype is SCID. If at least one copy of the gene contains a mutation that allows residual enzyme activity, the phenotype will be OS. Thus, a patient affected by OS could also be a carrier of a SCID-associated mutation. With only one exception (a frameshift mutation in RAG1), all known OS-associated mutations in RAG1 and RAG2 are missense mutations (reviewed in 17, 53).

Of note, hypomorphic mutations in both copies of DCLRE1C can give rise to milder disease characterized by residual V(D)J recombination activity in vitro and partial T- and B- lymphocyte deficiency. These patients also exhibit increased frequency of B-cell lymphomas (51).

OS and SCID have been reported within the same family (49), implying that the identical genetic defect can cause both phenotypes. However, GVHD in SCID patients may also lead to OS-like symptoms (17).
Testing for SCID

Genetic testing can confirm a diagnosis of SCID or establish a diagnosis before infections develop. Such early diagnosis is important, since timely treatment has been shown to increase survival. Genetic testing can also distinguish between the different molecular causes of SCID, with important implications for genetic counseling. Importantly, genetic testing allows detection of asymptomatic carriers of SCID-associated mutations. This is critical in cases of X-linked diseases such as XSCID, since female carriers are asymptomatic, while their sons are at a 50% risk of being affected.

How is Genetic Testing for SCID Performed?
DNA for sequencing is obtained from leukocytes present in a small blood sample. The coding sequences of the genes in question are amplified in a highly specific manner through a polymerase chain reaction (PCR), and all PCR products are fully sequenced. Sequencing results are interpreted, and a detailed result report is sent to the patient's physician.

References