Mutations in genes required for T-cell development: *IL7R*, *CD45*, *IL2RG*, *JAK3*, *RAG1*, *RAG2*, *ARTEMIS*, and *ADA* and severe combined immunodeficiency: HuGE review

Lisa Kalman, PhD¹, Mary Lou Lindegren, MD², Lisa Kobrynski, MD³, Robert Vogt, PhD¹, Harry Hannon, PhD¹, Joelyn Tonkin Howard, MSPH⁴, and Rebecca Buckley, MD⁵

Severe combined immunodeficiency (SCID) is an inherited immune disorder characterized by T-cell lymphopenia (TCLP), a profound lack of cellular (T-cell) and humoral (B-cell) immunity and, in some cases, decreased NK-cell number and function. Affected children develop severe bacterial and viral infections within the first 6 months of life and die before 1 year of age without treatment. Mutations in any of eight known genes: *IL2RG*, *ARTEMIS*, *RAG1*, *RAG2*, *ADA*, *CD45*, *JAK3*, and *IL7R* cause SCID. Mutations in unidentified genes may also cause SCID. Population-based genotype and allelic frequencies of these gene defects have not been measured. Some minimal estimates of SCID prevalence are presented. Currently, hematopoietic stem cell transplants are the standard treatment. In clinical trials, gene therapy has been used to reconstitute immune function in patients with *IL2RG* and *ADA* defects. The availability of effective therapies, plus the short asymptomatic period after birth, (when stem-cell transplantation is most effective), make SCID a potentially good candidate for newborn screening. Dried blood spots are currently collected from all infants at birth for newborn metabolic screening. Tests for TCLP on dried blood spots could be developed as a screen for SCID. Because SCID may be unrecognized, with infant deaths from infection attributed to other causes, newborn screening is the only way to ascertain true birth prevalence. Validated tests and pilot population studies are necessary to determine newborn screening's potential for identifying infants with SCID. **Genet Med 2004;6(1):16–26.**

Key Words: severe combined immunodeficiency disorder, *IL2RG*, *RAG1*, *ADA*, *JAK3*

GENES

For this review, we chose to examine SCID disorders that are characterized by severe T-cell lymphopenia (TCLP) caused by defects in a known gene. The genes reviewed in this article are *IL7R*, *CD45*, *IL2RG*, *JAK3*, *RAG1*, *RAG2*, *ARTEMIS*, and *ADA*. Their mode of inheritance, chromosomal location, and function are listed in Table 1. Figure 1 shows the stage of T-cell and NK-cell development that is blocked by gene mutations that cause SCID. The eight genes examined in this review have multiple mutations that cause SCID. Many of these mutations are unique because they are present in only one case. Population-

based genotype and allele frequencies of these genes have not been measured.

We used several methods to identify articles relevant to SCID. We searched PubMed for all articles published from 1997 to 2001 that included the following key words: “severe combined immune deficiency,” “adenosine deaminase deficiency,” and “combined B- and T-cell immunodeficiencies.” We obtained additional articles by personally locating information in libraries. These papers were reviewed and presented at a CDC-sponsored conference, “Applying Genetic and Public Health Strategies to Primary Immunodeficiency Diseases,” which took place November 8–9, 2001 (see <http://www.cdc.gov/genomics/info/conference/PI/PIMeeting.htm>). We subsequently did a comprehensive PubMed search (all years covered in the database) for articles about each of the eight genes. We also used several search algorithms to locate genetic and epidemiological data from 1990 to 2003. These algorithms included all genes and gene-related phrases as well as epidemiologic terms. A title search verified that the information in these articles was relevant. We then read the articles' abstracts to determine which articles were relevant for this particular review. We conducted an additional search to identify publications describing registries that collected data about patients

From the ¹Centers for Disease Control and Prevention, Newborn Screening Quality Assurance Program, ²Office of Genomics and Disease Prevention, Atlanta, Georgia; ³Emory University, Atlanta, Georgia; ⁴University of Texas Southwestern Medical Center, Dallas, Texas; ⁵Duke University, Durham, North Carolina.

Lisa Kalman, PhD, Newborn Screening Quality Assurance Program, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mailstop F-43, Atlanta, GA 30341-3724.

Received: August 4, 2003.

Accepted: October 6, 2003.

DOI: 10.1097/01.GIM.0000105752.80592.A3

Table 1
Characteristics of SCID^{36,40,101}

Gene	Locus	Gene product/function	Presence of			Mode of inheritance ^a	No. unique mutations identified	OMIM No.	References
			T-cell	B-cell	NK-cell				
<i>IL7R</i>	5p13	IL7 receptor. Needed for T-cell development. Activates JAK3 kinase	—	+	+	AR	5	146661	2,3
<i>CD45</i>	1q31-q32	Protein tyrosine phosphatase. Regulates Src kinases required for T-cell and B-cell antigen receptor signal transduction	—	+	+	AR	3	151460	5–8
<i>IL2RG</i>	Xq13.1	gamma-c chain of IL2, 4, 7, 9, 15 cytokine receptors. Needed to activate JAK3 for intracellular signaling	—	+	—	XLR	169	308380	9, 96
<i>JAK3</i>	19p13.1	Tyrosine Kinase. Needed for differentiation of hematopoietic cells	—	+	—	AR	27	600173	14,15,97
<i>RAG1</i>	11p13	DNA recombinase. RAG1/RAG2 mediate DNA recombination during B-cell and T-cell development	—	—	+	AR	44	179615	16,98
<i>RAG2</i>	11p13	DNA recombinase. Rag1/Rag2 mediate DNA recombination during B-cell and T-cell development	—	—	+	AR	18	179616	17,99
<i>ARTEMIS</i>	10p	Involved in DNA repair during V(D)J recombination	—	—	+	AR	9	605988	18,19,100
<i>ADA</i>	20q13.11	Part of the purine salvage and methylation pathways. Needed for removal of toxic metabolites (e.g. ATP, S-adenosyl homocysteine) that inhibit lymphoid cells.	—	—	—	AR	54	102700	23–25

^aMode of inheritance: AR, autosomal recessive; XLR, X-linked recessive.

from populations in a defined geographic area because these articles did not appear in a population-based epidemiology search.

Gene variants

IL7R

The *IL7R* gene comprises eight exons spanning 20,738 base pairs.^{1,2} Defects in *IL7R* have been recently implicated in SCID. Few patients with *IL7R* mutations have been studied. A number of mutation sites have been identified, including three nucleotide substitutions that resulted in missense mutations, one nonsense mutation, and one substitution that subsequently resulted in a splice-site mutation.^{3,4} The majority of these mutations are located in exon 4.^{2,3}

CD45

The *CD45* gene spans over 120 kb. Mutations in *CD45* were identified in three patients with SCID.^{5–8} One patient was homozygous for a six base-pair deletion that removed a tyrosine residue that is essential to protein function. Another patient had one allele with a large deletion in *CD45* and a point mutation that disrupted a splice site in the other allele.

IL2RG

The *IL2RG* gene comprises eight exons spanning 5447 base pairs.¹ There are 264 mutations in *IL2RG* that are known to cause SCID. Of these mutations, 169 have been documented in

only one patient.⁹ Types of (unique) mutations in *IL2RG* vary and include 55 missense mutations, 33 nonsense mutations, 10 insertions, 32 deletions, and 33 splice mutations; types also include complex, large deletions, and mutations that affect RNA processing and translation. Mutations are not evenly distributed throughout the gene. Hotspots for mutation occur at CG dinucleotides at cDNA 690–691 and at cDNA 879.¹⁰ Another two mutations are associated with a milder form of SCID.^{11–13}

JAK3

The *JAK3* gene is comprises 25 exons spanning 24,029 base pairs.¹ Twenty-seven unique mutations have been identified in *JAK3*, occurring in all seven structural domains.¹⁴ No hotspots have been reported and multiple types of mutations have been identified: 13 missense mutations, 7 nonsense mutations, 3 splice site mutations, 2 deletions, and 1 insertion.^{14,15}

RAG1

The *RAG1* gene comprises 2 exons spanning 12,544 base pairs.¹ Fifty-nine independent mutations in *RAG1* are known to cause SCID, 44 of which are unique molecular events.¹⁶ Most of the mutations were missense (36 independent mutations), 12 were frameshift deletions, 10 were nonsense mutations, and one was a frameshift insertion.

Fig. 1 Defects in human T-cell development resulting in SCID phenotype. A simplified depiction of lymphocyte differentiation is shown. B cells and NK cells mature in the bone marrow, whereas T cells mature in the thymus. Normally, only the mature forms of these cells are released into the peripheral blood. Various stages in NK and T-cell development that are blocked by mutations in the genes known to cause SCID (*IL2RG*, *JAK3*, *ADA*, *IL7R*, *RAG1*, *RAG2*, *ARTEMIS*, and *CD45*) are indicated by X and dashed lines. Presence or absence of T-cell-specific antigenic markers (CD4, CD8, TCRβ, and TCRαβ) is also indicated. Effects of these mutations on B-cell development is not shown.

RAG2

The *RAG2* gene comprises 2 exons and spans 7092 base pairs.¹ Of the 21 independent mutations identified in *RAG2* that were shown to cause SCID, 18 represent unique molecular events. Fifteen of these mutations were missense, two were nonsense, and one of each of the following: frameshift deletion, inframe deletion, gross deletion, and a frameshift insertion.¹⁷

ARTEMIS

The *ARTEMIS* gene comprises 18 exons and spans 56,665 base pairs.¹ Mutations in the *ARTEMIS* gene cause SCID. In Athabaskan-speaking Native Americans, a single nonsense mutation in exon 8 of this gene has been identified in 20 of 21 patients with SCID (called SCIDA in this population). One of these patients was heterozygous at this locus; the paternal allele contained the nonsense mutation, and the maternal allele was not expressed.¹⁸ Haplotype analysis suggests that the high incidence of SCIDA in this population is due to a founder mutation.¹⁸ In a study of Europeans with SCID caused by mutations in the *ARTEMIS* gene (RS-SCID), Moshous et al.¹⁹ have found eight separate mutations in patients from 11 families.

Three of the mutations identified were genomic deletions of two or more exons resulting in a frameshift deletion. One was a one-base deletion that resulted in a frameshift deletion, three were splice-site mutations, and one was a nonsense mutation. No missense mutations were identified.

An epidemiologic study was conducted by Jones et al.²⁰ concerning SCID among the Navajo population on the Navajo Reservation, which includes areas of Arizona, New Mexico, and Utah. Death certificate data for 1969 to 1982 indicated that infectious disease-related deaths increased among Navajo children and decreased among non-Navajo children living in the same area. Cases of SCID or probable SCID were identified by chart review. On the basis of the number of deaths attributed to SCID on the Navajo Reservation, the authors estimate that the incidence of SCID in this population was 52 per 100,000 live births. Segregation analysis of 24 families was done to obtain a disease-gene frequency of 2.1%. The authors considered this percentage to be a conservative estimate.²⁰ Another study examined SCIDA alleles in unrelated Navajo adults who were not related to SCIDA-affected families. Three of the 30 individuals tested were carriers for the SCIDA mutation. This finding suggests that the gene frequency may be higher than 2.1%.²¹

ADA

The *ADA* gene comprises 10 exons spanning 33,003 base pairs.¹ Sixty *ADA* mutations in 59 unrelated families have been identified in SCID-affected individuals. Fifty-four of the mutations are unique.²⁴ Of these 54 mutations, 37 are missense mutations, 9 are splice-site mutations, 4 are frameshift deletions, 3 are nonsense mutations, and 1 is an inframe and gross deletion.^{22,24} There is evidence of a genotype-phenotype correlation for severe and mild forms of *ADA* related SCID. Milder forms of SCID are thought to be caused by mutations that result in decreased, but not absent, *ADA* enzyme function.^{22–25}

Buckley⁵ reported on the genetic distribution in 160 SCID infants treated consecutively over a period of three decades at Duke University Medical Center in Durham, NC, a major referral center in the southeastern United States. In this population, 44% of the cases were attributed to mutations in *IL2RG*, 10% to mutations in *IL7R*, 16% to mutations in *ADA*, 6% to mutations in *JAK3*, and 3% to mutations in *RAG*. The remainder of the cases were attributed to unknown genes.⁵ A retrospective single-center study of 117 SCID patients in Europe reported that patients with X-linked SCID (presumably *IL2RG* mutations) represented 28.2% of the cases, and *ADA* deficiency represented 13.7%. Autosomal-recessive non-*ADA* SCID, in which the patients lacked both T cells and B cells (T^-B^-) accounted for at least 30.8% of the cases, and Omenn syndrome (specific *RAG1* or 2 defects) represented 11.1%.²⁶

Although comparison of these two series is difficult, it appears that autosomal recessive, non-*ADA*, T^-B^- forms of SCID (*RAG1/2*, *ARTEMIS*) have a different genetic distribution in Europe than in the United States.

Prevalence of SCID

Although no population-based prevalence data exist on gene variants or on clinical SCID, several countries have developed registries to collect information about cases of primary immunodeficiency diseases, including SCID. The minimum prevalence of SCID (clinical cases) was estimated using data collected from these immunodeficiency registries. We reviewed data from registries (the only available epidemiologic data existing for SCID) that met certain criteria. These registries included a description of methods describing an attempt to collect facility-based data on clinically recognized SCID cases in a defined geographic area to estimate the minimum prevalence of SCID. The authors stated that they used a standard case definition, such as the one specified by the World Health Organization (WHO)²⁷ for the case definition of SCID. These studies are identified in Table 2. Generally, data for these registries were collected by surveying hospital personnel and immunologists based in these countries. The cases in each registry were categorized by disorder, as defined by WHO. Minimum prevalence estimates were based on the frequency of reported cases of SCID per estimated population at risk. The

Table 2
Registries

Study site (Ref)	Population sampled	Years included	Response	SCID estimated incidence and/or prevalence	No. of cases SCID/PID	Limitations
Australia (28)	Longitudinal cross-sectional survey of immunologists (ASCIa members) and tertiary referral from all clinical immunology departments in pediatrics and general hospitals. Patients currently receiving care or recently deceased.	Jan 1990–March 1994	First 500 cases registered 23/27 hospitals and 6 clinicians	Prevalence of PID = 2.82/100,000 Prevalence of SCID = 0.15/100,000 ^a	26/500 5.2%	Ascertainment bias Under-reporting Facility-based, not population-based
Norway (30)	Solicited PID cases from survey of 60 hospitals (all nonpsychiatric hospitals in Norway).	1961–1998	35/60 responded. Most of the other 25 were contacted; none had any cases.	Birth prevalence SCID = 8.9 (1.5–9.3) per 1,000,000 live births Prevalence SCID = 0.045/100,000 ^b	7/372 1.9%	Ascertainment bias Under-reporting ICD9 code limitations Poor response rate
Sweden (31)	Survey of all 41 children's hospitals in Sweden	1974–1979 (children ≤ 16 yrs)	All responded	Birth prevalence SCID = 1.4/100,000 live births	8/174 4.6%	Ascertainment bias Under-reporting
Switzerland (29)	Survey of 12 medical centers or departments	January 1975–Jan 1985	Unknown	Birth prevalence SCID = 2.43/100,000 live births Prevalence SCID = 0.47/100,000 ^c	28/313 8.9%	Ascertainment bias Under-reporting

^aAustralia: Primary immunodeficiency (PID) prevalence = 2.82/100,000. SCID represented 5.2% of PID; calculated prevalence of SCID = (0.052)(2.82/100,000) = 0.15/100,000.

^bNorway: Prevalence of combined immunodeficiency disorders (CID) = 0.45/100,000, SCID represented 10% of CID; calculated prevalence of SCID = (0.1)(0.45/100,000) = 0.045/100,000.

^cSwitzerland: Three living SCID patients/6,423,100 population = 0.47/100,000.

prevalence of SCID was estimated to be 0.11/100,000, 0.47/100,000, or 0.045/100,000 in Australia, Switzerland, and Norway, respectively.^{28–30} Switzerland, Norway, and Sweden estimated the birth prevalence of SCID to be 2.43/100,000, 0.89/100,000, or 1.4/100,000 live births, respectively^{29–31} (Table 2).

Prevalence is a measure of the proportion of affected patients who are alive at a particular point in time compared with the overall population. The prevalence of a disorder is affected by how long an individual with the disease survives after birth. The survival of SCID patients after birth is dependent on such factors as timely diagnosis and the availability of appropriate medical care and treatment. Therefore, population-based data on birth prevalence are needed. These data could be obtained through population-based surveillance or through assessment of population-based blood specimens to evaluate TCLP or clinical phenotype.

Unfortunately, these registry data underestimate the true prevalence of SCID. This underestimate can be attributed to lack of clinical recognition, lack of reporting to the registries, overrepresentation of certain referral centers, lack of a standardized case definition, and death of the infants before diagnosis. In addition, none of these studies are truly population based. The effect of these factors may vary between studies and between different countries and is correlated with the accessibility to or availability of medical care to the population. Finally, these studies define SCID phenotypically and not genotypically and may therefore include or exclude different disorders in their SCID category, making comparisons difficult.

Clinical features of SCID

SCID is a primary immunodeficiency disease caused by mutations in a number of genes involved in various aspects of B-cell and T-cell development. SCID is fatal without treatment and is characterized by the lack of a functional cellular and humoral immune system. Almost all patients have absent or very low T-cell number and function (TCLP). Total lymphocyte counts in these patients are usually $< 1,500$ to $2,000$ cells/ mm^3 (normal $4,000$ – $13,500$ cells/ mm^3); CD3+ T-cell counts are usually < 500 cells/ mm^3 (normal range $3,000$ – $6,500$ cells/ mm^3).^{32–34} The number of B and NK cells varies according to the underlying genetic defect.^{33–35} (Table 1). In a few rare cases, maternal engraftment of T cells may cause near-normal T-cell counts.

Symptoms at presentation

Patients with SCID usually present in the first few months after birth with a typical constellation of symptoms and signs, including failure to thrive as well as severe and persistent or recurrent infections. Other suggestive symptoms are persistent diarrhea, rashes, recurrent sinopulmonary infections, recurrent skin infections, abscesses, and poor wound healing.^{36,37} Pathogens include microbial organisms, such as *Pseudomonas* and *Salmonella* species; protozoa; and fungi, such as *Pneumocystis carinii* and *Candida albicans*; as well as viruses, such as

respiratory syncytial virus, parainfluenza, cytomegalovirus, and adenovirus. SCID patients may develop graft-versus-host disease caused by maternal T-cell engraftment or postnatal transfusions.³⁸ Physical examination reveals that these children do not have lymph nodes or tonsils.³⁹ X-ray imaging and autopsies have shown that these patients have a small thymus lacking thymocytes.

SCID caused by certain genotypes can also result in unique symptoms. For example, mutations in the *IL2RG* gene is X-linked and occurs in males. Approximately 85% to 90% of patients with mutations in *ADA* present as infants with severe immunodeficiency. The remaining cases are milder, with a progressive onset after 2 years of age and even as late as adulthood.^{23,40} About 50% of children with infantile-onset *ADA* deficiency have skeletal deformities.^{23,40–42} These children also may have neurological symptoms, behavior problems, and a decreased IQ.^{43,44} Children affected with SCIDA often have oral or genital ulcers not seen in other forms.⁴⁵ Further population-based data are needed on genotype-phenotype relations.

The pattern of lymphopenia characteristic of SCID varies with the type of SCID (Table 1). Patients with SCID caused by *ADA* deficiency have very few T, B, and NK cells or none at all (T-B-NK-SCID). SCID caused by mutations in genes involved in DNA recombination (*RAG1*, *RAG2*, and *ARTEMIS*) is T^-B^- but NK^+ . Mutations in genes needed for multiple cytokine receptor signaling (*IL2RG* and *JAK3*) cause $T^-B^+NK^-$ SCID, whereas mutations in *IL7R* and *CD45* cause $T^-B^+NK^+$ SCID (Fig. 1). Lymphocyte profiles are determined by subset analysis using flow cytometry.⁴⁶ Patients with B^+ forms of SCID often have normal or elevated levels of nonfunctional B cells. These lymphocyte profiles may be altered by the presence of maternal lymphocytes that cross the placenta during gestation.³³

Treatment

Successful treatment of SCID involves transplantation of hematopoietic stem cells from HLA-identical or haploidentical T-cell-depleted bone marrow. Myers et al.⁴⁷ and Buckley et al.⁴⁸ analyzed the survival of infants who received transplants within the first 3 months of life. The survival rate for infants transplanted at this center, before about 3 months of age, was 95%, compared with a survival rate of approximately 75% for infants receiving treatment at a later age. These children, who are more than 8 months to 19 years posttransplant, developed normal levels of T cells and low-to-normal levels of NK cells. Unlike T cells, B-cell function was not normal after transplantation in many cases; thus, posttransplant recipients may require ongoing immunoglobulin replacement (IVIG) and antibiotics. Treatment earlier in infancy may be more successful, because it is administered before the onset of opportunistic infections that may cause death or morbidity before immune reconstitution can be achieved.

A recent report analyzed long-term survival of 475 SCID patients from 37 centers in 18 European countries who were treated between 1968 and 1999.⁴⁹ This group includes 51

(11%) with *ADA*, 137 (29%) who were phenotypically $T^{-}B^{-}$, 217 (46%) who were phenotypically $T^{-}B^{+}$, 58 (12%) identified as "other," and 12 (3%) with reticular dysgenesis. Three-year survival and engraftment was 77% and 54% for HLA-identical or HLA-haploidentical transplantation, respectively. Variables that affected survival rate for HLA-identical transplants included age at transplantation and the use of chemoprophylaxis. The 3-year survival rate for HLA-identical transplant recipient was 85% for patients younger than 6 months of age at the time of the transplant, 73% for those who were 6 to 11 months, and 53% for those who were 12 months or older. For non-HLA-identical transplants, patients with a B^{-} phenotype had lower survival rate than SCID patients with a B^{+} phenotype. Patients in this group also had a higher survival rate if they received care in a protected environment and did not have a pulmonary infection prior to transplant. Survival rates for all types of SCID patients have increased over time.

There are many differences in treatment protocols among different doctors, centers, and countries, making comparisons of outcomes challenging. These differences include the protocols for T-cell depletion of donor bone marrow as well as pre-transplant and posttransplant chemotherapy.^{50,51} Currently, multicenter trials have started to evaluate different protocols for hematopoietic stem cell transplant for SCID (L. Kobrynski, Emory University, personal communication, 2003).

Gene therapy has been used in clinical trials to treat 11 patients with SCID who had mutations in *IL2RG* (γc).^{52,53} Autologous $CD34^{+}$ bone marrow cells were transduced ex vivo with a replication incompetent retroviral vector containing a normal gene encoding the γc -chain. Initial results (2.5 years after treatment) in four patients indicate that they had nearly normal T-cell numbers and phenotypes. These T cells also had nearly normal repertoires of T-cell receptors and in vitro proliferative responses to antigens. Although the frequency of transduced B cells was low, these children did not require immunoglobulin replacement.⁵³ One patient underwent stem-cell transplantation 8 months after gene therapy because T-cell reconstitution failed.⁵³ Two of the patients developed a form of leukemia because the retroviral gene construct of the *IL2RG* gene was inserted near the *LMO2* gene,^{54–56} which resulted in insertional mutagenesis.

Gene therapy is being developed for other forms of SCID,⁵⁷ including SCID caused by mutations in the *ADA* gene.^{58–61} In one study, two patients with *ADA*-SCID received gene therapy using a retroviral vector containing the normal *ADA* gene. After treatment, both children had increased lymphocyte counts, improved immune functions, and lower levels of *ADA* and purine metabolites. Both patients were clinically well and developing normally. Neither patient required PEG-*ADA* therapy.⁶⁰

Enzyme replacement is an option for patients who have SCID that is caused by mutations in the *ADA* gene and who are unable to receive hematopoietic stem cell transplants. Twice-weekly injections of PEG-*ADA*, although very costly, have been shown to improve cellular immune function and increase the number of T lymphocytes.^{62–64}

INTERACTIONS

Infectious disease

Because of the lack of a functional immune system, SCID patients are unusually susceptible to bacterial sepsis and to viral and fungal infections. Patients present in the first few months of life with frequent, severe, and persistent infections that do not respond to usual therapy. Without hematopoietic stem cell transplants, enzyme replacement, or gene therapy to correct their T-cell defects, patients with SCID will most likely die from a severe infection within the first year of life.

Immunizations

Vaccination with live attenuated vaccines such as oral poliovirus vaccine, smallpox, tuberculosis, measles and mumps and rubella, varicella, typhoid, and yellow fever can cause severe or fatal infections in SCID patients and should be avoided.^{65–69} Killed or inactivated vaccines such as inactivated poliovirus vaccine, diphtheria and tetanus toxoids and pertussis, and *Haemophilus influenzae* type b may be given to SCID patients, although these children are unlikely to produce specific antibodies because of their immune defect. Passive immunoprophylaxis can be achieved by administering specific immune globulins after exposure to diseases such as measles, chicken pox, hepatitis B, tetanus, smallpox, and rabies.⁶⁶ Passive antibody protection can be provided through monthly infusions of IVIG.

Age of diagnosis

Most children are diagnosed after developing severe or persistent infection, usually before 6 months of age (symptomatic screening). Children with affected siblings or other family members may be tested prenatally or at birth (asymptomatic screening).

Family history

Family history is an important diagnostic tool for identifying of patients affected with rare disorders. A patient who has previously diagnosed siblings or siblings who died of infections in infancy is suggestive of either X-linked or autosomal recessive SCID. A family history of unexplained deaths of male infants along maternal bloodlines may suggest an X-linked recessive disorder such as *IL2RG* SCID. Autosomal recessive forms of SCID may occur in children of either sex. Family history may be ambiguous, however, because of undiagnosed cases in the past or because of de novo mutations.^{13,37} A study by Puck et al.¹³ examined the frequency and variety of *IL2RG* mutations in 103 unrelated SCID-affected males and their relatives. In 62% of the *IL2RG* cases, there was no family history of X-linked SCID. In 13% of all *IL2RG* cases studied (and 23% of sporadic *IL2RG* cases), the mother of the SCID patient did not show skewed X chromosome inactivation, which is indicative of an *IL2RG* carrier. These new *IL2RG* mutations occurred as a result of maternal mosaicism or within the oocyte.¹³ There are no population-based data for the allele frequencies of any of the mutations that cause SCID.

ASSOCIATIONS

Associations between SCID and the genes in this review have been determined by molecular and DNA-based methods. Currently, no literature on epidemiologic-based associations exists. The relative, absolute, and attributable risks for these genes related to SCID are unknown. All mutations reported in the literature cause SCID. The penetrance of mutations in these genes is unknown. Current literature deals with case-only series.

Because of the lack of information on the distribution of genotypes in the population, data are scarce regarding genotype-phenotype relations. Different mutations in the same gene can cause a spectrum of disease phenotypes; therefore, we are probably only aware of the more severe cases.⁷⁰ Mutations that cause a less severe phenotype have been identified in *RAG1*, *RAG2*, *ADA*, *JAK3*, and *IL2RG*. More severe mutations, such as deletions or frameshifts of the *RAG1* or *RAG2* genes, result in the SCID phenotype.⁷¹ Some mutations (mostly missense) in *RAG1* and *RAG2* impair, but do not abolish, the function of these enzymes. These mutations cause Omenn syndrome, a primary immune deficiency affecting the maturation of T and B lymphocytes that is clinically distinct from SCID.^{40,71–73} Patients with Omenn syndrome have a variable number of nonfunctional T cells and undetectable levels of B cells. These patients also have a number of other clinical features, including erythroderma, lymphadenopathy, and hepatosplenomegaly. Corneo et al.⁷⁴ have reported on several patients with the same *RAG1* or *RAG2* mutations. Some of these patients had SCID, and others had Omenn syndrome. Other factors, such as timing of treatment, stochastic events, environmental factors, or other genetic factors may also affect the phenotype of *RAG1* and *RAG2* mutations.^{71,74}

Mutations in *ADA* that decrease but do not abolish enzyme activity can cause immune deficiencies with later onset and more mild phenotypes.^{22,75–77} Siblings and unrelated individuals who carry the same *ADA* mutations can have different phenotypes. These differences may be due to differences in environmental factors or to other genetic factors.⁷⁸ Somatic mosaicism has also been observed in two cases of *ADA* SCID. These patients showed clinical improvement without therapy. In one case, not all of the cells carried the *ADA* mutation; in the other case, the point mutation reverted in somatic cells.^{40,79} Other patients, who had no *ADA* enzyme activity in erythrocytes, were immunologically normal as a result of activity in other cell types.^{77,80,81}

Frucht et al.⁸² have described two and possibly three siblings with identical mutations in *JAK3* who had different clinical phenotypes. Each sibling had an A96G initiation start-site mutation that encoded a dysfunctional product and another allele with a splice donor-site mutation that is predicted to cause premature termination of *JAK3*. One sibling had extremely low *JAK3* levels and the other had no detectable *JAK3*, consistent with his more severe phenotype. The authors speculate that other genetic factors may have affected the phenotypes in these siblings.⁸² Other groups have also reported milder forms

of *JAK3* SCID.¹⁴ Mutations in *IL2RG* that confer a mild SCID phenotype have also been identified.⁷⁰ Standardized clinical case definitions for SCID are needed. A less severe form of SCID may also need to be defined to more broadly assess genotype-phenotype relations.

Laboratory tests

Most SCID patients have lymphopenia with total lymphocyte counts of $< 2,000$ lymphocytes/mm³ (normal newborn range is 2,000–5,500 lymphocytes/mm³; normal range at 6 months is 4,000–13,500 lymphocytes/mm³). All patients have few T cells or none at all and, depending on the form of SCID, some may have few B and NK cells or none at all. Lymphopenia can be detected prenatally or after birth by obtaining a white-blood-cell count and differential. Patients with some forms of SCID can have abnormally high levels of B cells, which can mask TCLP and may cause the total lymphocyte count to appear normal. Subset analysis by flow cytometry is therefore necessary to enumerate T, B, and NK cells.⁴⁶ Diagnostic tests analyze T-cell function by using in vitro responses of peripheral blood lymphocytes to phytohemagglutinin and common antigens. B-cell function is measured by looking at serum immunoglobulins and specific antibodies. Both T-cell and B-cell function are abnormal in SCID; however, patients may appear to have some function as a result of transplacental passage of maternal cells and immunoglobulins. *ADA* enzyme activity in leukocytes can be measured to confirm diagnosis of SCID caused by defects in the *ADA* gene. This test has a very high sensitivity and specificity for SCID caused by *ADA* mutations.

Diagnosis can be confirmed by DNA-sequence analysis of the gene(s) suggested by the lymphocyte profile. Mutations in unidentified gene(s) cause approximately 18% of SCID.⁵ Sequence analysis of the known genes will not confirm a diagnosis in these cases. Many mutations known to cause SCID are due to genetic alterations affecting the sequence of the protein, which results in the production of a full-length but nonfunctional protein. Thus, other potential tests, such as protein detection may not be diagnostic.

In families that have previously affected members, SCID can be diagnosed prenatally if the mutated gene is known. DNA sequence analysis can be performed on fetal cells collected by chorionic villus biopsy or amniocentesis. Umbilical-cord blood can be analyzed for *ADA* enzyme levels and TCLP.^{36,77,83} DNA-based tests have been developed to diagnose SCID caused by mutations in *ADA*, *IL2RG*, *SCIDA*, *RAG*, and *JAK3* prenatally.^{21,77,84–86}

Carriers in high-risk families can be identified by DNA-sequence analysis if the affected gene is known. Female carriers of *IL2RG* mutations can be identified by nonrandom X-chromosome inactivation in lymphoid cells⁸⁷ and a variety of other molecular techniques.⁸⁶

Population testing

Many characteristics of SCID make this disorder a good candidate for newborn screening, as defined by Wilson and Jungner⁸⁸: (1) SCID is an important health problem, and it is fatal in

infancy without immune reconstitution; (2) a short asymptomatic period exists after birth; (3) effective treatments are available; (4) early intervention improves outcome; and (5) profound deficiencies of cellular and humoral immunity may be detectable with screening tests. No population testing currently exists for SCID, although New York had a program to detect children with ADA deficiencies in the 1970s using a colorimetric test based on ADA enzyme activity.^{89,90} Of the 2.56 million newborns screened over a 12-year period, no cases of ADA SCID were detected. Two cases that were not detected by screening were reported during this time period. Twelve cases of partial ADA deficiency were detected by the program. However, all of these patients who lacked ADA in erythrocytes, but who had substantial ADA activity in other cell types, had normal immune function.^{77,91} These results lead to the discontinuation of ADA screening in New York.

Nearly every newborn in the United States is screened for a minimum of four and a maximum of about 30 metabolic, hematologic, or endocrinological disorders depending on the state in which they are born. Several drops of blood collected from each baby are applied to filter paper and dried. This simple, easily transported, and inexpensive specimen-collection method allows for the assay of over 30 disorders from a single drop of blood. Detecting TCLP from dried blood spots (DBS) would be the most efficient way to integrate SCID screening into the existing newborn screening system. This practice is possible, in principle, because DBS contain mummified T cells (and other leukocytes); however, a high-throughput test based on direct visualization from the DBS is not feasible.

Work by Dr. R. Buckley at Duke University compared lymphocyte levels between normal and SCID babies at birth has shown that most SCID patients had low absolute lymphocyte counts at birth (range of absolute lymphocyte counts was 114–2210 lymphocytes/mm³ for 25 SCID newborns and 1670–8910 lymphocytes/mm³ for 14 normal infants at birth) (Fig. 2). All of the patients had very low T-cell counts (range of T-cell counts for SCID infants was 0–84 lymphocytes/mm³ and for normal infants was 903–7226 lymphocytes/mm³ at

birth). These data suggest that T-cell counts could be a sensitive indicator of SCID and that total lymphocyte counts may not identify the 10% to 20% of SCID patients whose high B-cell counts gave them a misleadingly normal lymphocyte count.

Several types of soluble T-cell-specific biomarkers, which can theoretically be recovered from DBS, may serve as potential surrogates for a T-cell count. The first type of biomarker is the family of cell membrane proteins unique to T cells, most notably CD3. Measurements of CD3 eluted from cultured T cells have shown strong correlation with T-cell count,⁹² but this method has not yet been tested in whole blood or blood spots. Measurements of eluted CD4 also correlate strongly with T-cell counts, and this method has been applied successfully to whole blood and to DBS.⁹³ Because the CD4 protein is also expressed (at lower levels) on monocytes, this marker is not as T-cell specific as CD3, but it offers another viable option.

The second type of T-cell biomarker is the circular DNA that is removed when T-cell receptor variable genes rearrange, often called T-cell antigen receptor excision circles (TRECs).^{47,94} DNA is well preserved on DBS, and detection of TRECs should be possible,⁹⁵ but accurate quantification may be difficult after polymerase chain reaction amplification, especially for high throughput applications, such as newborn screening.

Although DNA is well preserved on DBS, the wide variety of genetic mutations that can cause SCID and the lack of knowledge about the effects and severity of particular mutations rule out screening tests based on genomic DNA analysis. Total lymphocyte counts, when obtained in a complete blood count, would probably not be an adequate screen for SCID, because affected newborns can have increased B-cell counts, producing a 10% to 20% overlap with the normal lymphocyte distributions.⁴⁷ Subset analysis by flow cytometry would be necessary to enumerate T, B, and NK cells.⁴⁶ This test could not be done on DBS.

To be clinically useful, each of these tests must be developed and must undergo population-based evaluation to determine its specificity and validity. Potential confounding variables include the possible detection of lymphopenia due to genetic

Fig. 2 Absolute lymphocyte and T-cell counts in SCID and control infants at birth. Absolute lymphocyte count and the T-cell count for 25 SCID and 14 normal newborns are shown.

causes, such as DiGeorge syndrome or cartilage hair hypoplasia, or by HIV infection. However, although these children do not have SCID, they nevertheless are affected by a serious immunologic deficiency that requires evaluation. Screening tests not only would identify children with a fatal but treatable disease, but also could provide valuable information regarding the incidence of these disorders in the population, information about their penetrance, and the frequency of mutations among affected individuals. Population-based data on the frequency of mutations in each of the genes that cause SCID could also be obtained.

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Without prompt diagnosis and treatment, children with SCID die in their first year of life. Data suggest that children born with this condition would benefit from presymptomatic detection and treatment. Much work needs to be done to assess the epidemiology of the genetic conditions that underlie SCID. An active case ascertainment that uses a standardized case definition, which includes genetic tests in a defined geographical area over a defined time period, would provide a more complete accounting of cases and an accurate estimate of the incidence and prevalence of SCID. An ascertainment of the prevalence of SCID-associated mutations and disease in different populations is also needed. Tests need to be developed that use DBS to detect TCLP. Pilot studies regarding newborn screening could provide data on the incidence of SCID in the population as well as information on the phenotype of various mutations.

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