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NEWBORN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCY (SCID): A REVIEW

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1. ABSTRACT

Because prompt intervention may prevent complications, early diagnosis is important in many inherited metabolic diseases. Early diagnosis of Severe Combined Immunodeficiency (SCID) is critical - because chances for successful treatment are highest for infants who have not yet experienced severe opportunistic infections. SCID is a rare disease that can be detected in newborn infants (i.e., those < or = 1 month of age) by automated blood count and manual differential. Early diagnosis of SCID is rare since, because estimates of the incidence rate range from one in 50,000 to 100,000 births, most pediatricians do not routinely count white blood cells in newborns. Tests for T-cell lymphopenia (TCLP) using dried blood spots (DBS) could be used to identify children with SCID - as well as for other immunodeficiencies that would not be apparent until after the child developed an infection. Screening newborns for SCID would allow early diagnosis and treatment -- as well as genetic counseling for the family.

2. INTRODUCTION

SCID is a group of disorders caused by gene mutations (1-9). It is characterized by profound deficiencies in T, B, and, in some cases, NK cell function (10-12). SCID infants are lymphopenic; their small thymus lacks thymocytes; their spleen is deficient in T cell areas; and tonsils and lymph nodes are not formed (10). Such infants rarely survive beyond 1 year of age without therapeutic intervention such as bone marrow transplantation (BMT) (3) or gene therapy (11, 13-14).

SCID was identified in 1950 (15). Immune function was first successfully restored by BMT in 1968 (16). Since 1981, development of BMT techniques - especially T cell depletion (TCD) (17) - allowed successful use of haplo-identical (parental) and unrelated mismatched donors (18). BMT, intravenous immunoglobins, medications, and gene therapy offer hope for a partial or complete cure if the child lives long enough to benefit. Newborn screening for SCID is technically possible, and in

the near future, accurate and inexpensive screening technologies may become available.

3. THE EARLY DAYS OF NEWBORN SCREENING

Newborn screening in the US became a reality in the 1960s when Dr. Robert Guthrie developed a screening test for phenylketonuria (PKU). This test uses a few drops of dried blood on filter paper. PKU is an inherited disorder in which children are unable to metabolize the amino acid phenylalanine. If untreated, affected children will become severely mentally retarded and will experience neurological symptoms. Dietary therapy, when started soon after birth, reduces symptoms and allows affected children to develop normally. The incidence of PKU is 1/16,000 births (19).

The history of PKU screening and the creation of the current system of screening policy and practice were documented by the Newborn Screening Task Force of the American Academy of Pediatrics (20). PKU screening illustrates the complexities of using research and technological innovations to influence policy and change institutional practice. Despite processing features that would seem to make PKU screening an easy decision, it took more than 10 years for most states to mandate screening. Two barriers to implementing PKU screening were: 1.) the scientific community had not developed standards and procedures for evaluating the accuracy of screening tests or the efficacy of treatments. Advocacy outpaced science in that research validating the PKU screening test and evaluating the safety and efficacy of dietary treatment were not accomplished until after state laws were passed for mandatory screening (20); and 2.) the United States had no public health infrastructure at either the state or federal level by which newborn screening could be implemented. The roles of state and federal governments had not been articulated and there was no precedent for who would conduct screening, pay for it, or provide treatment (21). Advocacy efforts at the state and national levels led to mandated newborn screening for PKU in most states by the mid-1970s. Guthrie's research and advocacy efforts ushered in a new era of public health medicine focusing on preventing debilitating effects of a disorder through early identification and treatment.

Another break-through in newborn screening occurred in the early 1970s when Dussault *et al* (22) developed a radioimmunoassay for thyroxine (T4) using DBS to screen for congenital hypothyroidism. McCabe *et al* (23) also took an important step by using polymerase chain reaction and DNA extracted from DBS to screen for mutations in hemoglobin genes. With the decoding of the human genome, DBS may also be used to screen for other genetic disorders (20, 24).

4. NEWBORN SCREENING TODAY

Efforts to develop a tandem mass spectrometry (MS/MS) newborn-screening assay using DBS began in the 1990s (25). MS/MS is used in many screening programs to analyze amino acids and acyl carnitines in blood to detect disorders of amino acids, organic acids, and fatty-acid metabolism. The selection of analyses determines which disorders can be screened. This simple - and inexpensive - DBS specimen-collection method allows for an assay of more than 30 disorders (26). Improvements in ionization, automation, and data processing have enabled some laboratories to screen for newborn metabolic diseases in as many as a thousand patients per day (25).

In many countries it is routine to screen infants for congenital hypothyroidism, PKU, and other disorders. The aim is early detection and treatment to minimize morbidity and mortality (27). For example, multidistrict studies in the UK suggested coverage of 99.9% of infants (28), where MS/MS is now used to screen for PKU, replacing older methods. In addition to PKU, it can detect other compounds, including acyl carnitines, which may identify inborn metabolism errors, such as organic acidaemias and disorders of fatty acid oxidation (of which medium chain acyl CoA dehydrogenase deficiency [MCADD] is important).

To appraise MCADD screening, the Child Health Support Group (CHSG), a subgroup of the United Kingdom's National Health Service (NHS), drew on British research (29-31), reviews commissioned by the NHS Research and Development programs (32, 33), and reports from established or pilot programs in North America, Australia, and Europe (34-37). Despite international screening of over a million infants, questions and uncertainties remain about the performance and outcome of newborn screening for MCADD. There has been no long term follow up of infants detected by screening. The National Screening Committee (NSC) recommended that research be carried out in the UK to determine the program's performance (38).

In Australia, screening of newborns by MS/MS was introduced in New South Wales and the Australian Capital Territory in early 1998, South Australia in 1999, and in Victoria in 2002. Wilcken (27) examined newborn screening by MS/MS for diagnosing 31 disorders in Australia. The technology is being introduced in the US and some European countries (27, 37, 39-44).

In the US, newborn screening programs exist in 50 states and the District of Columbia. Each identifies a contact person knowledgeable about the program and follow-up issues and a second individual whose responsibility is laboratory protocol. DBS are collected from over 95% of all U.S. newborns. The National Newborn Screening and Genetics Resource Center maintains a website listing the status of newborn screening by state and disorder (see http://genes-r-us.uthscsa.edu/resources/newborn/screenstatus.html). Each year four million infants are born in the United States. Nearly every newborn is screened for as many as 30 metabolic, hematologic, or endocrinological disorders (26).

The American Association of Pediatrics has called for a national agenda on newborn screening programs at the state level. All states screen for PKU, hypothyroidism, galactosemia, sickle cell anemia,

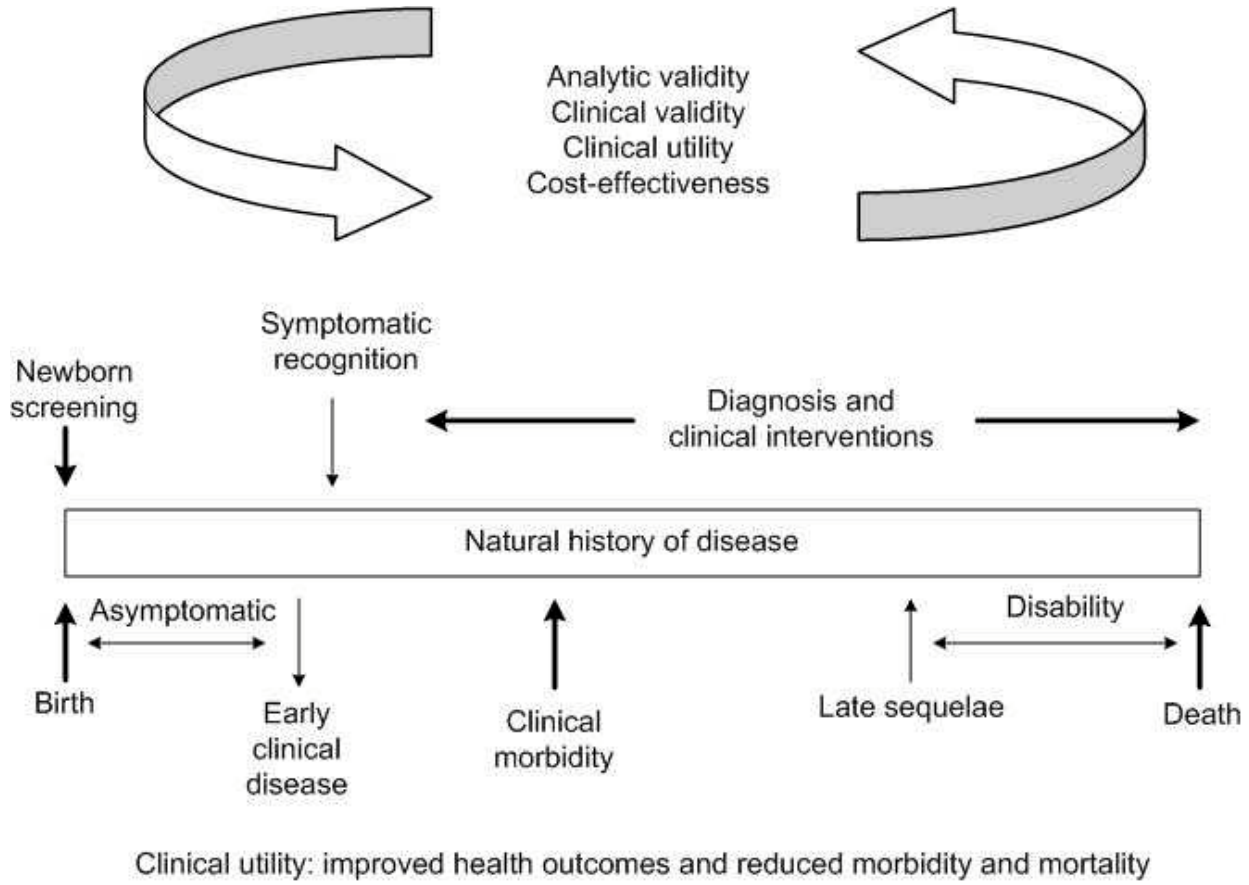

Figure 1. Potential public health interventions regarding genetic diseases (Source: 45).

congenital adrenal hyperplasia, biotinidase deficiency, maple syrup urine disease, homocystinuria, MCADD, and hearing loss. To standardize screening, the American College of Medical Genetics formed an expert group to establish guidelines and recommend a group of disorders for screening in all states. Because of changes in technology, the discovery of new genetic causes of disease and disability, the tendency of states to make their own decisions, and the variable role of advocacy groups within each state, variability in screening will continue for the foreseeable future (21).

5. FEASIBILITY OF CONDUCTING A NEWBORN SCREENING FOR SCID

Some physicians believe that screening for SCID should be a standard neonatal test. Advances in technology and genetics have produced significant changes in newborn screening. It is now possible to use one test for detecting multiple metabolic or genetic conditions. For example, tests for TCLP using dried blood spots (DBS) can be used to screen for SCID, a syndrome of diverse genetic origin (1-9). This process could identify SCID children - as well as those with other immune deficiencies (e.g., purine nucleoside phosphorylase deficiency, DiGeorge's syndrome and perhaps congenital HIV infections) - that are not apparent until after the child develops an infection.

Although because of the low incidence of SCID, most pediatricians do not routinely order lymphocyte counts. SCID and other genetic diseases (e.g., single-gene disorders with high penetrance) can be detected (both prenatally and at birth) using methods such as: 1) screening tests to evaluate newborns for conditions requiring early intervention and 2) clinical algorithms for early recognition of symptomatic persons - before the onset of clinical morbidity and with confirmatory laboratory diagnosis (including genetic testing) (Figure 1). Treatment regimens can then be initiated early to reduce morbidity, disability, and mortality (45).

5.1. Clinical presentation, laboratory tests and prevalence of SCID

The classic signs of SCID include an increased susceptibility of the baby to infection and failure to thrive (failure to grow and gain weight as expected). Infants with SCID may have recurrent infections, such as ear infections (acute otitis media), sinus infections (sinusitis), bronchitis, oral thrush (a type of yeast that multiplies rapidly, creating white, sore areas in the mouth), and pneumonia. They may also have chronic diarrhea. Children with signs and symptoms suggestive of SCID are usually seen first by their pediatrician or family practitioner; therefore, it is essential that primary care physicians be able to recognize signs and symptoms of SCID and be knowledgeable about

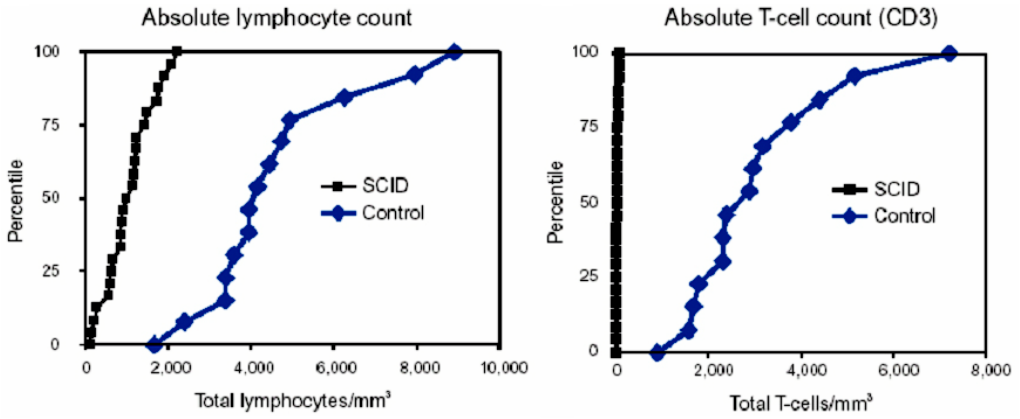

Figure 2. Absolute lymphocyte count distributions in severe combined immunodeficiency (SCID) – 25 newborns with SCID and 14 healthy newborns at birth evaluated at Duke University (Source: 78).

Table 1. Number of lymphocytes at birth and different ages

Age	Number	Reference
Range of absolute lymphocyte counts (cells/mm3) at birth		**26**
• 25 SCID newborns (age 0–16 days)	114–2,210	
• 14 normal infants (age 0–8 days)	1,670–8,910	
• Range of T-cell counts (cells/mm3) at birth for*		
• 25 SCID infants (age 0–16 days)	0–84	
• 14 normal infants (age 0–8 days)	903–7,226	
Normal number of lymphocytes (percentage of total leukocytes) at different ages		**47**
• Birth	5,500 (2,000–11,000) cells/mm3 (31%)	
• 6 months	7,300 (4,000–13,500) cells/mm3 (61%)	
• 21 years	2,500 (1,000–4,800) cells/mm3 (34%)	
Distribution of total lymphocytes and T-cell subsets in normal healthy children at ages 0–3 months		**51**
• Median total lymphocyte counts (N = 800)	5,400 cells/μL (10th–90th percentile 3,400–7,600 cells/μL)	
• Median CD3 T-cell counts (N = 699)	3,680 cells/μL (10th–90th percentile 2,500–5,500 cells/μL)	
• Median CD4 T-cells counts (N = 699)	2,610 cells/μL (10th–90th percentile 1,600–4,000 cells/μL)	

screening procedures for their diagnosis. This process is complicated by the diverse presentations of immunodeficiency and the lack of specificity and by the relative inaccessibility of screening (46).

The phenotypic hallmark of SCID is profound T-cell lymphopenia, with counts below the first percentile of normal (although transplacental maternal T-cell engraftment might cause this number to be higher in some cases). Compared with healthy infants whose total lymphocyte counts at birth are 2,000 - 11,000 cells/μL (47), counts in SCID babies are usually <1,500 cells/μL (Figure 2, Table 1). $CD3^+$ T-cell counts in SCID are typically <500 cells/μL (normal: 3,000--6,500 cells/μL) (26, 48-50). In an urban, primarily minority, cohort of 800 healthy children, median total lymphocyte counts at ages 0 - 3 months were 5,400 cells/μL (10^{th}-90^{th} percentile, 3,400-7,600 cells/μL); median $CD3^+$ T-cell counts were 3,680 cells/μL (10^{th}-90^{th} percentile, 2,500-5,500 cells/μL); and $CD4^+$ T-cells were 2,610 cells/μL (10^{th}-90^{th} percentile, 1,600--4,000 cells/μL) (51). In a study by Myers *et al* (52), two neonates had an absolute lymphocyte count (ALC) just over 2000/mm^3, and the cells were predominantly B lymphocytes. For those with a family history of SCID, lymphocyte phenotyping and T-cell function studies should be performed (either prenatally or at birth). Regardless of genotype, nearly all SCID neonates were lymphopenic, and lymphocyte phenotypes at birth were typical for the defect (52). In all cases, a lymphocyte count may be useful in diagnosing SCID. Very low counts (less than 2.7 x 10^9/1) may be seen in T(-) B(-) SCID. In T (-) B (+) SCID, the lymphocyte count is usually below the lower end of the age-related range (53).

SCID is a pediatric emergency. Thirty years ago, all SCID babies died. A pathogen with little effect on a healthy baby can kill one with SCID. The key to improving the chances of a baby with SCID is early detection, since early BMT can provide a cure and avoid severe morbidity or mortality. Nearly all cases could be diagnosed at birth if routine blood counts and manual differentials were done and if flow cytometry and T-cell functional studies were performed if lymphocyte counts are below the newborn normal range (2,000-11,000/mm^3) (10, 47). Prenatal diagnosis can be made if there is a family history of SCID. Treatment can begin shortly after birth (52).

5.2. Population testing and screening tools

No population testing exists for SCID, although in the 1970s New York had a program to detect children with ADA deficiencies using a colorimetric test based on ADA enzyme activity (54, 55). Of 2.56 million newborns screened over 12-years, no cases of *ADA* SCID were detected. Two cases - not detected by screening - were

reported, and twelve cases of partial ADA deficiency were detected. However, all patients lacking ADA in erythrocytes, but who had ADA activity in other cell types, had normal immune function (56, 57). These results caused ADA screening in New York to be discontinued (26).

Other studies identified a high incidence of SCID in the Navajo Native American population. To determine incidence, Jones *et al* (58) reviewed the death certificates of children who died between 1969 and 1982, identified cases that met the study criteria, and interviewed families of the children. Segregation parameter estimates of 0.27-0.38 were obtained from data for 24 families, suggesting a gene frequency of 2.1% (arguing against multifactorial inheritance). SCID cases that were referred to specialty centers lacked T and B cells in blood, and serum immunoglobulins ranged from absent to near normal.

Although several countries have registries to collect information on primary immunodeficiency diseases, including SCID, no population-based prevalence data exist on gene variants or on clinical SCID. Newborn studies in Australia, Switzerland, and Norway showed SCID prevalence to be 0.11, 0.47, or 0.045 per 100,000 live births, respectively (59-61). Switzerland, Norway, and Sweden estimated SCID prevalence to be 2.43, 0.89, or 1.4 per 100,000 live births, respectively (26, 60-62).

Diagnosing SCID at birth requires high-throughput screening tests. Data indicate that a T-cell count might be an effective screening tool. Development of a DBS-based, high-throughput test for T-cell lymphopenia would make it possible to integrate screening for SCID into the existing DBS system. Screening tests might detect markers on mummified T-cells (and other leukocytes) that are present on DBS.

Multiple types of soluble T-cell-specific biomarkers recoverable from DBS are potential surrogates for T-cell counts. One of these is the family of cell-membrane antigens unique to T-cells (notably CD3, CD4, and CD8). Measurements of markers from DBS might be possible using antibody-based detection assays (63). Another biomarker is the circular DNA that is removed when T-cell-receptor variable genes are rearranged during development. These are called T-cell antigen receptor excision circles (TRECs) (52, 64). TRECs are used to measure recent thymic emigrants (RTEs) levels to assess thymic output. $CD4^+$ and $CD8^+$ T cells progress through several stages in their lifespan. Mature $CD4^+$ and $CD8^+$ thymocytes emigrate from the thymus to the periphery as RTEs (65). After RTEs mature, they are classified as naive T cells, which circulate through blood and lymphoid tissues. The rate of RTE production by the thymus contributes to the peripheral T cell pool. It is important to monitor thymic production under conditions influencing T cell depletion and reconstitution (66). Using PCR amplification, it is possible to detect and quantitate TRECs from DBS (67). TRECs, located in new T-cells, are abundant in normal newborns but absent in newborns with SCID. Quantitation of TRECs in a newborn screening with high-throughput has not yet been developed (45).

Total lymphocyte counts have been proposed as a screen for lymphopenia. However, affected newborns often have increased B-cell counts, producing a 20% overlap with normal lymphocyte distributions. This situation can cause cases of SCID to be overlooked, requiring additional testing for some normal newborns (52). Detection of all cases requires enumeration of lymphocyte counts with a manual differential and subsequent subset analysis using flow cytometry - neither of which can be performed on DBS. Detection of DNA sequences from DBS is possible. However, although DNA-based tests for detecting disease-causing alleles can detect one mutation or a limited number of them, the number and wide spectrum of molecular defects and the lack of data regarding genotype-phenotype relations in SCID currently precludes development of a DNA test (45).

5.3. Disease: genes and the environment

Genetic variations - even so-called single–gene disorders - develop from the interaction of genetic and environmental factors. These interactions modulate an individual's susceptibility to certain diseases/disorders. For example, PKU results from a genetic variant that leads to deficient metabolism of the amino acid phenylalanine In the presence of normal protein intake, phenylalanine accumulates and is neurotoxic. PKU occurs only when both the genetic variant (phenylananine hydoxylase deficiency) and the environmental exposure (dietary phenylalanine) are present (68). Genetic variations do not cause disease, but they do influence a person's susceptibility to environmental factors. All human diseases result from the interaction of genetic susceptibility and (sometimes modifiable) environmental factors, such as infectious, chemical, physical, nutritional, and behavioral exposure. This is the most important fact in understanding the role of genetics and environment in disease development (69).

For example, male children who have a polymorphism in the monoamine oxidase, a gene conferring low enzyme activity, show non-aggressive behavior when raised in a non-abusive environment. When raised in an abusive environment male children with this polymorphism show aggressive and antisocial behavior. However, males with normal enzyme activity do not become violent offenders when raised in the same abusive/maltreated environment (70). Similar results have been found in animals. Mice lacking a functional corticotroping-releasing hormone 1 receptor do not differ from wild-type mice in alcohol intake under stress-free conditions; however, under stress, the knockout mice increase alcohol consumption (71). Monkeys with a polymorphism in the regulatory region of the serotonin transporter gene show no differences from wild-type monkeys when reared with their mothers. On the other hand, monkeys with the polymorphism that were nursery-raised have attention and orientation deficits (72).

A 1998 study revealed defects in the predominant pathway for a double-strand break repair called nonhomologous DNA end joining (NHEJ). V(D)J recombination is a double-strand DNA breakage and rejoining process that relies on NHEJ (73). Progress in the

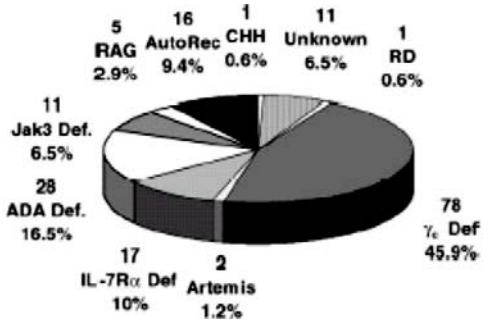

Figure 3. Relative frequencies of genetic types of SCID in 170 patients (Source: 78) .

biochemistry and genetics of NHEJ and of human SCID has proven to be synergistic and covers the range from biochemical etiology to possible gene therapy for B(-) SCID patients (e.g., RAG1-or RAG2-deficient SCID patients frequently fail to develop B cells after BMT) (74).

RAG1, RAG2, and Artemis (a novel V(D)J recombination/DNA repair factor that belongs to the metallo-β lactamase superfamily) are essential for antigen receptor gene rearrangement. RAG1 or RAG2 mutations result in the inability to form antigen receptors through genetic recombination. Some mutations in RAG1 or RAG2 genes lead to partially impaired V(D)J recombinational activity (resulting in Omenn's syndrome [11, 49]). An Artemis deficiency results in an inability to repair DNA after double stranded cuts have been made by RAG1 or RAG2 gene products in rearranging antigen receptor genes from their germline configuration.

In mammalian cells, double strand breaks in DNA (due to ionizing radiation, radiomimetic drugs, or occurring during gene rearrangements) are repaired by NHEJ or illegitimate recombination. In yeast, homologous recombination is the predominant mechanism for repairing double strand DNA breaks, but NHEJ is detectable when homologous recombination is inactivated (e.g., by a mutation in Rad52 [75, 76]). During NHEJ, two broken DNA ends are directly joined with no overlap (end-to-end) or with minimal overlap and the use of short fortuitous homologies near the two ends. Thus, the term "nonhomologous" refers to the absence of extended segments of homologies between the two recombined DNA molecules. "Simple ligation" (the joining of two ends with cohesive protruding single strands [PSSs]) or two blunt ends - a process that conceivably can be achieved by a DNA ligase alone) is a form of NHEJ (77).

The SCID molecular basis has been identified (see Table 2), but the mechanisms by which environmental influences might translate into SCID are unclear. Population-based data on the frequency of mutations in each of the genes that cause SCID and the incidence of these disorders have never been studied. Population-based data on the frequency of mutations in each gene causing SCID and the incidence of these disorders in different populations – especially in populations that are exposed to damaged environments (for example, radiation exposure [e.g., Chernobyl] and chemical pollution [e.g., Bhopal]) have also never been studied. Even though RAG1 or RAG2 mutations type of SCID are more common in Europe than in the United States, only 5 such patients have been found among 170 SCID patients evaluated by Buckley (78) (Figure 3). Genetic and environmental interactions modulate an individual's susceptibility to certain diseases/disorders. Newborn screening for SCID will provide opportunities to explore the contributions of gene–gene and gene-environment interactions in SCID and to create models for guiding future programs in medicine and genetic epidemiology.

5. 4. Economic, scientific and social significance of newborn screening for SCID

Newborn screening requires a public health infrastructure integrated with the health care delivery system. Follow-up is an integral part of any comprehensive screening program (24) to answer the questions: a) does the disease occur frequently in the population? and b) does the disease result in significant health consequences for affected individuals?

PKU was ideal for screening for three reasons: First, untreated, PKU has a devastating effect on development, usually resulting in severe mental retardation. Second, Guthrie created an accurate and inexpensive screening method (19). Finally, there was a simple treatment (dietary change) that, if implemented, could prevent the effects of PKU. The treatment improved quality of life and saved millions of dollars that families and society would otherwise have been forced to invest in the care of individuals with PKU-based mental retardation.

The incremental cost of MS/MS for laboratories where screening and genetic testing were conducted was $0.70 per newborn, including the cost of reagents, microtiter plates and other consumables, maintenance and depreciation of instruments, staffing, and confirmatory tests. The mean cost of confirmatory testing for infants who required it was $217. The cost per disorder detected (excluding PKU) was $3,939. Including PKU, the cost was $2,519. Data on costs incurred outside of newborn-screening and laboratories are not available (27). In 2000, fees charged for newborn screening in the US are presented in Table 3 (79).

In considering adding SCID to newborn screening, evidence-based criteria should be used. Whether a condition is a key public health problem is often decided on the basis of prevalence. Cost concerns (i.e., cost-effectiveness or cost-benefit of screening tests) are important. Low-prevalence disorders like SCID (1/100,000) might not be considered a critical public health concern; however (depending on the severity of the outcome, effectiveness of interventions, and cost of screening and treatment), detection of a disorder with a low prevalence might, in reality, be more cost-effective than detection of a more common disorder (80).

Identifying children with SCID at birth provides time to institute therapies for immune reconstitution before the onset of opportunistic and other infections. SCID is a

Table 1. Molecular defects in Human SCID and Characteristic lymphocyte phenotypes

Chromosome	Molecular defects	Lymphocyte phenotype	Inheritance	Reference	PMID
1q31–32	CD45 deficiency	T(-), B(+), NK(+)	AR	8	10700239
5p13	IL-7 receptor alpha chain deficiency	T(-), B(+), NK(+)	AR	7	9843216
10p13	Mutations in the Artemis gene (Radiation sensitive; Athabascan)	T(-), B(-), NK(+)	AR	9	11336668
11p13	RAG1 deficiencies	T(-), B(-), NK(+)	AR	6	8810255
11p13	RAG2 deficiencies	T(-), B(-), NK(+)	AR	6	8810255
11q23	CD3 delta chain deficiency	T(-), B(+), NK(+)	AR	112	14602880
19p13.1	Jak3 deficiency	T(-), B(+), NK(-)	AR	5	7481768
20q13.11	Adenosine deaminase (ADA) deficiency	T(-), B(-), NK(-)	AR	1	4117384
Xq13.1	Common gamma-chain (γc) deficiency	T(-), B(+), NK(-)	X-linked	3	8401490

AR: Autosomal Recessive Inheritance

Table 2. Summation of Fees Charged in 2000 for Newborn Screening

	State/Territory	Amount of Fee	Program Components covered by fee
1	Alabama	$24.00	Laboratory
2	Alaska	$24.00	Laboratory, Program Administration/Follow-up
3	Arizona	$20 / $15	Laboratory, Program Administration/Follow-up, Treatment, specialist consultation, nurses
4	Arkansas	$14.83	Laboratory
5	California	$42.00	Laboratory, Program Administration/Follow-up
6	Colorado	$33.50	Laboratory, Program Administration/Follow-up, Treatment and Genetic Counseling
7	Connecticut a	$18.00	Laboratory
8	Delaware	$40.69	Laboratory, Program Administration/Follow-up, Medical Consultant.
9	District of Columbia	No charge	
10	Florida d	$20.00	Laboratory, Program Administration/Follow-up
11		Georgia	No Charge
12	Hawaii	$27.00	Laboratory, Program Administration/Follow-up, Treatment, fed ex, education, consultants/genetics
13	Idaho	No Charge	
14	Illinois	$32.00	Laboratory, Program Administration/Follow-up, Treatment
15	Indiana	$28.50	Laboratory, Program Administration/Follow-up and Treatment
16	Iowa	$33.00	Laboratory, Program Administration/Follow-up
17	Kansas	No Charge	
18	Kentucky	$14.50	Laboratory
19	Louisiana	$18.00	Laboratory, Program Administration/Follow-up and Treatment, Surveillance, Education.
20	Maine	$26.75	Laboratory, Program administration/Follow-up and education.
21	Maryland	$15.75	Laboratory (reagents only).
22	Massachusetts	$49.55	Laboratory, Program Administration/Follow-up, in home trait counseling
23	Michigan	$39.00	Laboratory, Program Administration/Follow-up and some treatment.
24	Minnesota	$21.00	Laboratory, Program Administration/Follow-up
25	Mississippi	$35.00	Laboratory, Program Administration/Follow-up and Treatment
26	Missouri	$13.00	Laboratory
27	Montana	$36.92 e	Laboratory
28	Nebraska	$53.00-$54.60	Laboratory, Treatment
29	Nevada	$30.00	Laboratory, Program administration/Follow-up
30	New Hampshire	$18.00	Laboratory
31	New Jersey	$34.00	Laboratory, Program Administration/Follow-up and Treatment
32	New Mexico	$20.00	Laboratory, Program Administration/Follow-up and Treatment, Education & Genetic Serv
33	New York	No Charge	
34	North Carolina	No Charge	
35	North Dakota	$17.00 c	Laboratory
36	Ohio	$27.00	Laboratory, Program administration/Follow-up, Treatment
37	Oklahoma	$10.50	Laboratory
38	Oregon	$32.00	Laboratory, Program Administration/Follow-up, Treatment
39	Pennsylvania		
40	Rhode Island	$59.00	Laboratory, Program Administration/Follow-up, Specialty formulas.
41	South Carolina	$21.00	Laboratory and Treatment
42	South Dakota	No Charge	
43	Tennessee	$17.50 b	Laboratory
44	Texas	$13.75	Laboratory
45	Utah	$27.00	Laboratory, Program Administration/Follow-up
46	Vermont	$27.00	Laboratory, Program Administration/Follow-up
47	Virginia	$16.00	Laboratory, Program Administration/Follow-up and Treatment, metabolic formula.
48	Washington	$39.25	Laboratory, Program Administration/follow-up, Treatment, Program evaluation and educ.
49	West Virginia	$20.46	Laboratory
50	Wisconsin	$55.50	Laboratory, Program Administration/Follow-up and Treatment.
51	Wyoming	No charge	
52	Puerto Rico	$18.00	Laboratory, Program Administration/Follow-up, Treatment.
53	Virgin Islands	No charge	
54	NeoGen Screening	$19.75	Laboratory, Program Administration/Follow-up

Source: 79. URL: http://genes-r-us.uthscsa.edu/resources/newborn/00/2000report.pdf a = Hosp. bill each infant tested, pass charge to insurance co. as part of mty fee; fees cover testing through state lab; b = increased to $17.50 10/00; c = 1/2000 to 6/2000 fee $16.00 - 7/2000 to 12/2000 fee $17.00; d = charge based on the number of live births occurring during the previous calendar year; e = Jan-June, 2000 fee $35.50, July-Dec, 2000 fee $36.92.

good candidate for development of a newborn screening protocol (81) because:

- It is fatal without immune reconstitution
- A short asymptomatic period exists after birth
- Effective treatments are available
- Profound deficiencies of cellular and humoral immunity might be detected (46)
- Early diagnosis and treatment can improve long-term quality of life and prevent rapid deterioration of the immune system (82)

A white cell count and manual differential costs $40 to test for SCID in the U.S. This could allow us to treat - and often cure - SCID at a reasonable cost. While genetic in origin, most cases of SCID appear to arise without a family history. It affects babies of both sexes and of all races, creeds, colors, countries, and cultures (the most common version of this condition, X-linked SCID, is unique to boys). No one knows how many babies dying of pneumonia, infections, measles, and other causes are - in reality - succumbing to this "rare" condition. After demonstrating that newborn screening for T-cell lymphopenia can be performed with precision (and at an acceptable cost) and that follow-up services and treatment can be provided to affected children, a recent proposal for newborn screening included the suggestion that a national-level body should recommend that the states include this test in newborn screening. Economic analysis can then be used to integrate and evaluate the results, based on the multiple screening criteria (83).

Early treatment also reduces costs and improves outcome. "A transplant in the first three months of life can cost less than $50,000, but the cost of care skyrockets up to millions of dollars for seriously ill patients- not including the cost of treatment (for diarrhea, pneumonia, and infections) from birth to diagnosis -, with less guarantee of success" (84). For example the cost (over a period of 2 1/2 years) of trying to save a single SCID child who was diagnosed at the age of 5 months - and who died at the age of 2 years 8 months - was $1,345,648.50 (85). SCID patients who received stem cell transplants from related donors within the first 28 days of life (before the onset of a life-threatening infection) developed a more robust immune system, with higher levels of T cell reconstitution and output from the thymus gland. (T cells are the white blood cells that are essential for normal function of the immune system) (84).

In our efforts to determine the cost of testing for SCID, we contacted the Centers for Disease Control and Prevention (CDC). According to a staff member,

"There is currently no screening test for TCLP from dried blood spots. A test is being developed at NIH (Dr. Jennifer Puck's lab) to measure TRECs in dried blood spots, but it is still in the developmental stage, we have not yet gained enough experience to provide cost estimates of the screening test based on the TRECs assay, but we expect the lab test itself to cost less than five dollars per sample. There are other costs involved, most notably the need to follow-up positive results, and those will depend on the specificity of the test (that is, the proportion of 'false' positive screening results) (86)."

6. ADVANCES IN SCID RESEARCH

During the past 10 years there have been advances in understanding the molecular basis of different forms of SCID. These have led to improvements in diagnosis and management. First, unambiguous assignment of a molecular diagnosis is now often possible. This is important in children with evidence of combined (cellular and humoral) immunodeficiency, but with "milder" clinical phenotypes than those in infants with classical SCID. Some children have molecular defects that are identical to those causing SCID. In these cases the long term outlook is poor enough to justify early BMT. Second, accurate carrier detection and first trimester prenatal diagnosis are possible in any family where the mutation is defined. In some cases prenatal diagnosis of an affected fetus may not lead to termination of the pregnancy, but can allow preparation for BMT early in the neonatal period - or even *in utero* in selected cases (87). Third, knowledge of the genetic defect permits better understanding of the molecular pathogenesis of disease with the possibility of designing more rational therapies and somatic gene therapy (88, 89).

6.1. Advances in research on molecular defects

Nine genetic variants determine four phenotypes (90): T(-) B(-)NK(-), T(-)B(-)NK(+), T(-)B(+)NK(-) and T(-)B(+)NK(+) SCID (7). Mutations in the following nine genes are known to cause SCID (see table 2):

ADA deficiency (10 to 20%) results in toxic amounts of deoxyadenosine accumulating in progenitors of lymphocytes and kills precursors of T, B, and NK cells by causing apoptosis.

Products of genes IL-2RG, Jak3, and IL-7R alpha are components of cytokine receptors. Absence of T and NK cells is the consequence of mutations in either the gene encoding the (gamma)c subunit of receptors for growth-promoting cytokines (X-linked SCID, 50%) or of the gene for Janus kinase 3 (5 to 10%).

Products of RAG1, RAG2, and Artemis are essential for antigen receptor gene rearrangement. Mutations in this variant affect three gene-encoding proteins of the recombination machinery (i.e., activating gene 1 or 2 [10%] or Artemis [10%]).
Three defects cause an isolated deficiency of T-cells

- Lack of IL-7Ralpha (5 to 10%)
- Deficiency of CD45 (a glycoprotein involved in T-cell signaling)
- A deficiency of CD3delta, a component of the T-cell antigen receptor, results in the absence of circulating mature CD3+ T-cells and gamma/delta T-cells (less than 1%)

These genetic defects account for 90% of SCID cases (Figure 3). Reticular dysgenesis (mixed myeloid-

lymphoid defect) and selective T-cell deficiencies are not yet understood (90) .

6.1.1. ADA deficiency

The ADA gene has been mapped to chromosome 20q13.2-q13.11, cloned, and sequenced (91). The absence of ADA (1) accounts for 17% of SCID cases (Figure 3) (11, 57).

ADA deficiency results in accumulations of adenosine, 2'-deoxyadenosine and 2'-O-methyladenosine. The latter directly or indirectly leads to lymphocyte apoptosis. Distinguishing features of ADA deficiency include skeletal abnormalities of chondro-osseous dysplasia (including flaring of the costochondral junctions and bone-in-bone anomalies in vertebral bodies). ADA-deficient patients have more profound lymphopenia than do infants with other types of SCID, with mean ALC of less than 500/mm^3 and a deficiency of all three types of immune cells (T[-]B[-]NK[-]SCID) (10,11). Milder forms of ADA SCID can lead to delayed diagnosis of immunodeficiency – even to adulthood (92). SCID should be suspected in any patient with recurrent infections and with severe lymphopenia (78).

6.1.2. Common gamma-chain deficiency

X-linked SCID (XSCID) accounts for almost 50% of SCID cases (10, 11, 78, 93) (see Figure 3). In XSCID, affected males typically have few T or NK cells, but have normal or increased numbers of B cells (T[-]B[-]NK[-]SCID); the B cells, however, are nonfunctional and exhibit defective class switching, due (in part) to the absence of T-cell help (93). Earlier work localized the defective gene in XSCID (in the SCIDX1 locus) to the chromosomal region between Xq11 and Xq13 (94). The gene encoding the IL-2Rγ chain was cloned (95) and localized to Xq13 at the SCIDX1 locus. DNA sequencing established mutations in IL2RG causing XSCID (2, 96).

Because XSCID patients exhibit a more severe immunological phenotype than patients with IL-2 deficiency, in which T and NK cell development is normal, it was at first hypothesized that IL-2Rγ is a component of more than one cytokine receptor (2) - at least one of which was required for lymphoid development. Later, IL-2Rγ was shown to be a shared component of receptors for IL-2, IL-4, IL-7, IL-9 and IL-15, and was renamed the common cytokine receptor γchain, γ_c (93, 96-98).

6.1.3. Janus kinase 3 (JAK3)

JAK3, the only member of the Janus family of intracellular protein tyrosine kinases expressed in hematopoetic cells (99-101), associates with γc (102) and is required for signal transduction by γc-containing receptors (93). Mutations in human JAK3 may result in autosomal recessive SCID, with a phenotype that is nearly identical to SCID-XI (2, 3) and is characterized by the absence of T and NK cells and with normal numbers of poorly functioning B cells (T[-]B[-]NK[-])(4, 5, 103) .

6.1.4. IL-7 receptor α chain deficiency

Patients with neither the γ_c nor the Jak3 deficiency were diagnosed with T(-)B(+)NK(+) SCID. Because mice whose genes for either the α chain of the IL-7 receptor or of IL-7 have mutated, they have deficient T- and B-cell function, but normal NK cell function (105). Mutations in these genes were also sought in human patients with SCID, and mutations in the gene for IL-7Rα on chromosome 5p13 were found in 17 (10 %), of patients (78). This suggests that the T cell - but not NK cell - defect in SCID-X1- and Jak3-deficient SCID results from an inability to signal through the IL-7 receptor (96).

6.1.5. Recombinase activating gene (RAG) deficiencies (*RAG1*- or *RAG2*-deficient SCID)

Infants with SCID due to mutations in *RAG1* or *RAG2* fail to rearrange either T-cell or B-cell antigen receptors and have a distinctive lymphocyte phenotype lacking B and T lymphocytes, but with NK cells (T[-]B[-]NK[+]SCID) (6). Five patients with RAG mutations have been seen in 161 infants with SCID at Duke. All but one was in RAG2.

Some patients with Omenn's syndrome also have mutations in *RAG1* or *RAG2*, resulting in partial and impaired V (D) J recombination (105). This syndrome is characterized by generalized erythroderma and desquamation, diarrhea, hepatosplenomegaly, hypereosinophilia, and markedly elevated serum IgE levels (but low levels of other immunoglobulin isotypes). The lymphocyte count is elevated because circulating, activated, oligoclonal, and autoreactive T lymphocytes are not responding normally to mitogens or antigens *in vitro* (106, 107). Circulating B cells are not found, and the lymph node architecture is abnormal due to the lack of germinal centers (108). The condition is fatal unless corrected by BMT (109).

6.1.6. CD45 deficiency

Another mutation that causes SCID occurs in the gene encoding leukocyte surface protein CD45 (8, 110). This hematopoietic-cell-specific transmembrane protein tyrosine phosphatase regulates Src kinases required for T and B cell antigen receptor signal transduction. A 2-month-old male infant presenting clinically with SCID had few T-cells, but normal numbers of B cells. The T cells failed to respond to mitogens, and serum immunoglobulins diminished with time. There was a large deletion at one *CD45* allele and a point mutation, causing alteration of the intervening sequence 13 donor splice site, at the other (8). A second case of SCID due to CD45 deficiency has also been reported (110).

6.1.7. Artemis gene mutation

A novel V(D)J recombination/NHEJ factor, Artemis, has been identified. Mutations in the Artemis gene cause human SCID with increased radiosensitivity (RS-SCID), an autosomal recessive disease characterized by absence of T and B lymphocytes and a defect in V(D)J recombination. Phenotypes of RS-SCID patients, and links to mutations, are described. Biochemical and structural properties of Artemis proteins are integrated into processes of V(D)J recombination and NHEJ. A genomic caretaker function is assigned to Artemis (111).

6.1.8. CD3 (delta) deficiency

Dadi *et al* (112) describe CD3 (delta) deficiency SCID. In this variant, there is a selective block in the differentiation of lymphocytes: development of T-cells is arrested; however, differentiation of other lymphocytes – NK and B cells - and other hematopoietic lineages appear normal. That two of the three affected infants in the SCID described by Dadi *et al* died from viral infections before four months of age demonstrates the essential role of T-cells in defending against viruses - even weakly pathogenic adenoviruses and cytomegaloviruses.

6.2. Advances in treatment

Approximately one-fourth (16.5%) of SCID cases are associated with ADA deficiency (see Figure 3). Treatment consists of red blood cell transfusions, enzyme replacement, pharmaceuticals, BMT, and gene therapy. ADA replacement is used to treat SCID in those who are not candidates for - or who have failed - BMT. It is not a replacement for Human leukocyte antigen (HLA)- identical BMT therapy. It can be used in infants from birth - and in children of any age - at time of diagnosis. Pegademase bovine is used for enzyme replacement therapy, administered intramuscularly to children. Few adverse effects or drug interactions have been documented. Although expensive (approximately $200,000-400,000 annually), pegademase bovine is a standard therapy that has been FDA-approved since 1990 (113).

6.2.1. Immune reconstitution

Approaches to immune reconstitution include BMT and gene therapy. BMT – both HLA identical unfractionated and T-cell-depleted HLA haploidentical – is successful in immune reconstitution if done in the first 3.5 months of life - and without pretransplant chemotherapy (78) - because the recipient is virtually devoid of T-cells. This eliminates adverse effects, including: neutropenia, red cell and platelet transfusion-dependency, mucositis, veno-occlusive disease, busulfan lung disease, growth suppression, sterility, and a 15% risk of later malignancy (114). Although Graft Versus Host Disease (GVHD) prophylaxis was not used for placental blood transplants (except for 1 month of cyclosporine given to two infants with GVHD), clinically significant GVHD was seldom seen. Omission of GVHD prophylaxis with cyclosporine permitted infants to develop T-cell function without hindrance (78).

6.2.1.1. Bone marrow transplantation (BMT)

For decades, BMT was the only hope for long-term survival in SCID patients (10, 115). This is true for the majority of these disorders. Depending on age at transplantation, the type of SCID and the donor (identical, vs haploidentical, vs unrelated), success rates vary from 50% to 100% (116). There appears to be no advantage in performing transplants *in utero* (117, 87), as opposed to soon after birth (52). *In utero* transplants carry risks associated with injecting the fetus and the inability to detect GVHD during gestation.

6.2.1.1.1. BMT in the United States

Dr. Rebecca Buckley has performed transplants in 132 infants with SCID, of which 102 (77%) were still alive (as of December, 2003). None showed evidence of susceptibility to opportunistic infections and most were in good general health. Follow-up age ranged from 2 months to 21.3 years. No pre-transplantation conditioning was given except to 3 infants who also received cord blood transplants. Of these 102 patients, 96 survived 1 or more years after BMT, 68 were alive 5+ years, and 37 for 10+ years. Median follow-up of survival patients was 5.4 years. All 15 recipients of marrow from HLA-identical donors, 87 of 117 recipients given T-cell-depleted haploidentical bone marrow from a related donor, and 2 of 5 infants from the latter group, who were also given unrelated placental blood transplants, survived. Survival rates were similar for different genetic types of SCID, except that only 1 of 4 male infants with SCID of unknown type survived. Factors affecting survival included race (more Caucasians survive, $p < 0.001$), sex (all but three females survive, $p < 0.05$), and age at time of transplant. Of 36 infants transplanted during the first 3.5 months of life, 35 (97%) survived, compared to 67 of 96 patients (70%) transplanted after that age. No patients died from GVHD, despite 87 patients receiving haploidentical BMT (78).

Between 1984 and 1999, 16 infants with Athabascan SCID (SCIDA) (due to Artemis mutations) (among Athabascan-speaking Native Americans, including Navajo and Apache Indians from the southwestern US and Dene Indians from the Canadian Northwest Territories) were given BMT at the University of California in San Francisco. Seven received HLA-identical sibling marrow, and nine received T-cell-depleted parental marrow. All but two received pre-transplant chemotherapy. All seven who received HLA-identical marrow survived; five of nine who received parental marrow survived. Three of four children who died received radiation or busulfan, and two of eight long-term survivors who received cytototoxic chemotherapy failed to develop secondary teeth. Children with this radiation-sensitive form of SCID had a poor outcome if given pre-transplant chemotherapy (118).

Between 1984 and 1997, at Children's Hospital of Los Angeles, 48 SCID infants received BMT. Eleven received HLA-identical related BMT, and 37 got T-cell-depleted haploidentical parental marrow. All received pre-transplant conditioning except one, who received HLA-identical sibling marrow. The 11 who received HLA-identical marrow survived, but only 17 survived of the 37 (47%) who received T-cell-depleted parental marrow (119).

6.2.1.2.1. BMT in other places

Aside from the United States, most studies of BMT for SCID come from the European Group for Blood and Marrow Transplantation and the European Society for Immunodeficiency (115, 120, 121). Registry data for 475 SCID patients came from 37 centers in 18 countries. Between 1968 and December 1999, long-term survival among SCID patients receiving stem-cell transplants improved, probably because of prevention of complications. In SCID, 3-year survival with engraftment was significantly better after HLA-identical transplantation than after mismatched transplantation (77% *vs.* 54%; p=0·002). Due to the development of T-cell-depletion

techniques, better antibiotics, and earlier recognition of SCID, survival improved over time. In HLA-mismatched stem cell transplantation, children with B (–) SCID had a poorer prognosis than those with B (+) SCID. Improvement with time occurred in both phenotypes. Except in related HLA-identical transplantation, acute GVHD caused a poor prognosis whatever the donor origin (120).

In a European retrospective study of long term immune reconstitution from 18 centers, of 193 patients with SCID who received haploidentical T-cell-depleted BMT between December 1982 and December 31, 1993 (most of whom had received pretransplantation chemotherapy), only 92, or 48% were long-term survivors. Eighty-nine of 116 (77%) patients who survived for 6 or more months had pre-transplant chemotherapy. Seventy-seven deaths occurred within 6 months after transplantation, and 24 more deaths were reported in the next 6 months (121).

Although approaches used for BMT in SCID infants differ from center to center, much has been learned about factors influencing success or failure. Although long term B cell function is better after chemotherapy, the mortality rate is higher using pre-transplant chemotherapy.

6.2.2. Gene therapy

Immune reconstitution using gene therapy in clinical trials was performed on several SCID patients (13, 14, 88, 89, 122). In 1990, two girls with ADA deficiency were enrolled in a clinical trial of gene therapy. They received infusions of *in vitro*–transduced autologous T lymphocytes. T-cell counts were restored, along with cutaneous delayed type hypersensitivity to antigens. Patients had normalization of isohemoagglutin titers and antibody responses to vaccines (122).

The first success of human gene therapy involved the correction of several XSCID patients by *ex vivo* transduction and reinfusion of stem cells with a functional copy of the γc gene (14). It was believed to be a step forward, because efforts to achieve gene correction of ADA SCID failed in the1990s. The group at Hospital Necker in Paris treated 11 XSCID children with gene-corrected autologous bone marrow cells; all of them survive. Nine had normal T- and B-cell function; two did not. Nine acquired normal immune function and did not require intravenous immunoglobulin infusions or medication.

Unfortunately, a serious adverse event - the unexpected complication of T cell leukemia – occurred in 2 of 11 children receiving gene therapy for XSCID (109, 123, 124). Shortly after varicella developed, the first patient was discovered to have a high white blood cell count due to an expanded clonal population of circulating (gamma)(delta)-positive T-cells. The white blood cell count increased and became a leukemia-like process that was treated with chemotherapy. The clone was shown to carry the inserted gene (110). The position of insertion is in an intron in a gene on chromosome 11 called LMO2. The product of the LMO2 gene is crucial for normal hematopoiesis and serves a regulatory function (125). However, LMO2 can also be an oncogene expressed in acute lymphoblastic leukemia of childhood. The second patient developed an (alpha)/([beta) T-cell proliferation with the gene also inserted near to the LMO2 gene.

Insertional oncogenesis is a potential complication of retroviral vector gene transfer, because integration occurs at random. This was thought unlikely with such vectors because they cannot reproduce themselves and repeatedly insert into the cell's chromosomes to increase the likelihood of malignant change. Before this case, malignant changes had not been seen in any human beings given retroviral vectors for gene transfer.

Nevertheless, similar therapies are considered promising for other immunodeficiencies (11, 45). Whether leukemia in these children indicates that the risk of insertional mutagenesis is higher than estimated is unknown. It is necessary to weigh this risk against the risks and benefits of other methods of treatment and determine which is best for a particular SCID patient (123).

7. CONCLUSION

Accurate diagnosis, precision in prognostication, genetic counseling, and new treatments have improved and extended the lives of children with SCID. Nine molecular defects responsible for SCID have been identified. Although population-based genotype and allelic frequencies of these gene defects have not been measured, they should be. Newborn screening could provide data on the population incidence of SCID as well as on the phenotype of various mutations. Tests need to be developed that use DBS to detect TCLP.

Although low-prevalence disorders such as SCID might not be considered a critical public health concern; detection and early treatment of disorders with a low prevalence might, in reality, be just as – or even more - cost-effective than detecting more common disorders.

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