Review

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Advances in the Molecular Etiology of Severe Combined Immunodeficiency and Its Screening

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Abstract

Severe combined immunodeficiency (SCID) is the most severe heterogeneous group of inherited disorders characterized by profound abnormalities such as humoral and cell-mediated immunity defects and hindered natural killer cell development and function. The knowledge of the molecular basis of SCID is essential for precise diagnosis and early treatment. In recent years, new genetic defects that cause SCID have been discovered, and the molecular and immunological mechanisms of SCID have been better understood. SCID symptoms include candidiasis, chronic diarrhea, failure to grow, and oral thrush. Hematopoietic stem cell transplantation, enzyme replacement therapy, and gene therapy are used to treat SCID. The prevalence of SCID varies worldwide. More than 80% of SCID infants have no family history of the condition. However, the development of a newborn screening test has enabled SCID detection before symptoms appear, ensuring that affected infants receive life-saving treatments. Countries that organize newborn screening programs for SCID can detect patients in their early stages of life and treat them accordingly. This review will serve as a source of up-to-date information on the identification of various genetic disorders that cause SCID, as well as their clinical characteristics, treatments, and diagnosis options, potentially saving the lives of many infants before pathogenic infections occur.

Keywords: Severe combined immunodeficiency, diagnosis, therapeutics, immunodeficiency

Introduction

Severe combined immunodeficiency (SCID) is a life-threatening, most severe heterogeneous group of inherited disorders. It represents a large spectrum of genetically and immunologically distinct syndromes correlated with devastating defects in humoral and cell-mediated immunity (1). SCID often accounts for malfunction or remarkable decrease in the number of T and B lymphocytes. The incidence of SCID is around 1 in 40.000 to 75.000 newborns (2). Due to various molecular defects, infants with SCID are unable to produce antibodies for their protection and hence are highly vulnerable to viral, bacterial, fungal infections, which often causes serious or life-threatening complications. In the first few months of life, mother's immunoglobulins (maternal antibodies) defend infants having SCID, preventing symptoms from showing up. The first signs of SCID occur in the first three to six months, after the metabolism of the maternal antibodies (3). The white blood cells are the functional cells of the working immune system which protect us from foreign pathogens. B and T-lymphocytes are the two types of lymphocytes produced in the bone marrow and thymus, respectively (4). A severe defect in T-lymphocytes differentiation leads to the remarkable decrease in the number of B-lymphocytes, T-lymphocytes, and natural killer (NK) cells that cause abnormal or weak action of the immune system (5). Complete absence of T-lymphocytes is also noticed in some cases (6). While the symptoms of leaky SCID are similar to those of regular SCID, the T-cell counts are not as low as those of typical SCID (7). Omenn Syndrome can occur on its own or due to SCID (8). In this syndrome, the SCID is associated with low immunoglobulin (IgG), IgA, and IgM

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and the virtual absence of B-cells. Although the number of T-cells is elevated, functional impairment of T-cells is seen (6). Infants with Omenn syndrome suffer from a lack of an immune system as well as autoimmune activity (9).

According to the International Union of Immunological Societies' (IUIS) expert committee on Inborn Errors of Immunity, the spectrum of clinical findings, including typical SCID, Omenn syndrome, leaky SCID, CID, granulomas with T-lymphopenia, autoimmunity, and CD4 T-lymphopenia, can be found in an allelic series of RAG1/2 and other SCID-associated genes (10). An algorithm for the 2022 update of IUIS phenotypical classification has also been published recently (11).

Though a vaccine is designed to give long-term protection from a disease, the live vaccine is a possible threat for babies with SCID due to their suppressed immune system (12). The very early common symptoms of SCID include pneumonia, BCGitis, eczema, diaper dermatitis, and dysmorphic face (13). Further, ear infections (otitis media), bronchitis, sinusitis (sinus infection), oral thrush, diarrhea, failure to grow, and weight gain are additional potential symptoms (14). In a study conducted on newborns with SCID, screening of 11 states of the United States indicated that the incidence of SCID was 1 out of 58.000 newborns (15). After analyzing the blood profile of SCID patients, it was found that autosomal recessive trait linked to SCID was more prominent in the society where marriage between the same blood origin took place (16). David Vetter, one of the most popular SCID patients, was born in 1971, and lived most of his life in a sterilized pathogen-free bubble to avoid the risk of infection. The Bubble Boy's life allowed doctors and researchers to learn a lot about patients with suppressed immune systems through his case, which in turn contributed to saving many lives (17).

David Vetter received histocompatibility leukocyte antigen-mismatched bone marrow transplantation from his sister at the age of 12 years (18). However, unfortunately, he developed Burkitt's lymphoma due to the traces of Epstein-Barr virus present in his sister's bone marrow which ultimately led to his death (19). By studying and analyzing some new cases, it was found that hematopoietic stem cell transplantation therapy should be started before the beginning of serious problems or pathogenic infections as early treatment gave significantly better results (20). On 1st January 2008, a screening methodology named T-cell receptor (TCR) excision circles (TRECs) was started for the first time in Wisconsin, which was conducted in Guthrie cards by polymerase chain reaction (PCR) (21). T-lymphocytes, which are undergoing development, produced a critical by-product called TRECs. The Journey of SCID has been shown in Figure 1.

Patients are categorized into groups with typical SCID, atypical SCID, and no SCID, based on the amount of TREC (22). Nowadays, many screening assays are being done globally to identify patients with primary immunodeficiency (PID). In Israel, Taiwan, Qatar, several Canadian regions, and the wide part of America (Puerto Rico, Columbia, and Navajo region), PID screening programs based on the TREC method have been started (23). Although TREC screening can detect most infants with SCID, cases with defects at the molecular level during T-lymphocyte receptor rearrangement cannot be identified (24). Such unidentified types of SCID include major histocompatibility complex (MHC)-class II and Zap-70 deficiency (25). As a result of the development of appropriate screening assays, it is now possible to determine the true incidence of SCID.

New and Rare SCID Forms

CORO1A mutation: A rare T-B+ SCID with profoundly reduced T-cell counts, normal B-cell counts, and low levels of immunoglobulin. The thymus is present and patients typically develop the symptoms of recurrent infections in infancy or early childhood (26). Coronin family proteins are important actin cytoskeleton regulators, and mutations in CORO1A, which encodes Coronin-1A, which is the predominant coronin expressed in lymphocytes, cause varying degrees of T-cell lymphopenia, susceptibility to infection, and immune dysregulation in humans (27).

SLP76 mutation: SLP76 is a key protein involved in TCR signaling and in other hematopoietic pathways (28). The TCR signaling pathway is an ensemble of numerous proteins that are crucial for an adequate immune response. Any protein disruption in this pathway results in severe immunodeficiency and unfavorable clinical outcomes (29). An infant with severe immunodeficiency, who was discovered to have novel biallelic SLP76 mutations, was reported (30).

Radiosensitive SCIDs (Artemis deficiency): Mutations in *DCLRE1C*, the gene encoding Artemis, cause T-B-NK+ SCID (ART-SCID) (31). Among the genetic defects that cause T-B- SCID are biallelic mutations in DCLRE1C, initially identified in a subset of T-B- SCID patients with increased radiosensitivity (32).

Furthermore, newborn screening (NBS) had saved the lives of many children by identifying them between the safety margins or before complications began. In USA, where the annual number of births are around 4 million, about three million newborns were passed through the screening test, and it was found that 1 in 5.800 newborns had SCID, while 1 in 7.300 had significant T-cells lymphopenia, which is much higher than expected (33). This review article provides complete information about the cause of disease, its clinical features and treatments, identification

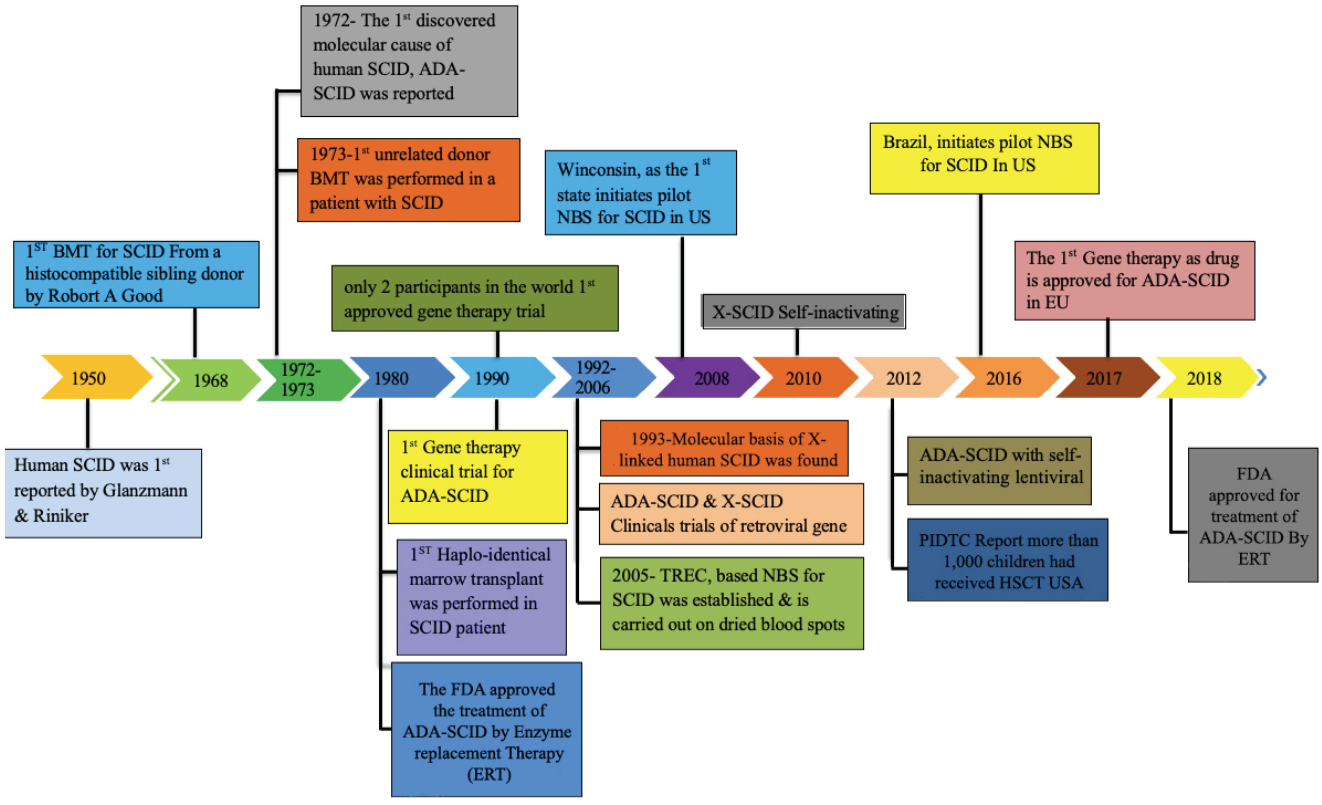


Figure 1. Chronological overview of the major events for screening and treatment of SCID.
Different SCID events began in 1950 when the first case of human SCID was reported. The first molecular cause of SCID was identified in 1972. The molecular basis of X-linked SC4ID was discovered in 1993. The first curative option for SCID, BMT, was discovered in 1968 from a histocompatible sibling donor. The first unrelated donor BMT was performed in a patient with SCID in 1973, and the first Gene Therapy clinical trial for ADA SCID was conducted in 1990, with Ashanti Desilva. She became one of only two participants in the world to receive the first approved gene therapy trial. The necessity of early diagnosis and treatment of SCID gave birth to the newborn screening program in 2005. TREC based Newborn Screening for SCID was established and is carried out on dried blood spots. In 2012, PIDTC reported that more than 1000 PID children had received HSCT in the USA. The first gene therapy as a drug is approved for ADA-SCID in the European Union (Strimvelis). By the end of 2018, all 50 states of the US started screening for SCID. One more event that occurred in 2018 is the FDA-approved treatment of ADA-SCID by ERT.
TREC: T-cell receptor excision circle, ERT: Enzyme replacement therapy, SCID: Severe combined immunodeficiency, BMT: Bone marrow transplant, PIDTC: Primary immune deficiency treatment consortium, PID: Primary immune deficiency, ADA: Adenosine deaminase deficiency

of various genetic disorders, and the diagnosis options available, which will aid in saving the lives of many infants before the pathogenic infections.

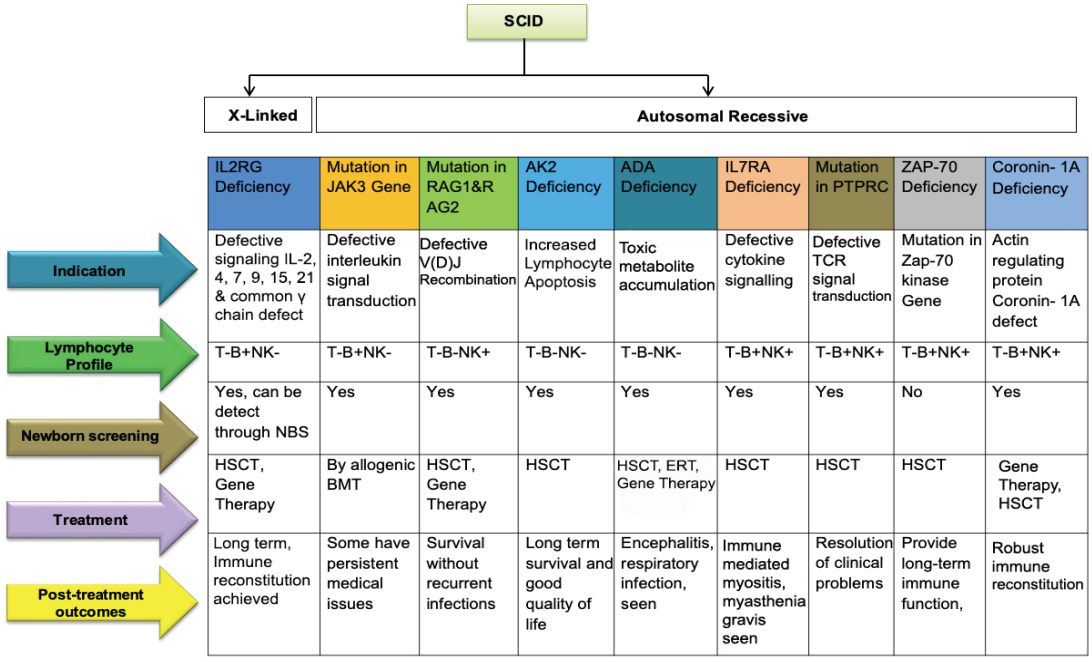
Inheritance Pattern

In SCID, due to the marked decrease in the number of lymphocytes, many pathogenic infections (bacteria, virus and fungus) which are otherwise treatable, cause lethal complications (34). SCID diminishes the affected individual's immune system, which further vigorously impacts T-lymphocytes, B-lymphocytes, and NK cells' development and maturation (35). It is now realized that about 20 different genetic mutations associated with immune dysfunction are responsible for SCID in newborns (36). SCID varies greatly based upon the congenital disabilities or the involvement of the gene. Detailed information on the inheritance of SCID, along with the screening, treatment and long-term outcomes has been depicted in Figure 2.

These SCID causing genetic defects can be inherited in two ways: An X-linked (sex-linked) recessive genetic trait or an autosomal recessive genetic trait (37,38). The most common inheritance pattern for SCID is X-linked recessive trait, with males being disproportionately affected in this type (39). Genetic counselling provides information on how genetic conditions might affect newborns, which is highly beneficial for families with SCID history. Counsellors collect the familial medical history, order genetic tests, conduct adequate study of the genetic conditions and evaluate the results, and provide information to parents about the inheritance pattern of SCID (40). Different types of genetic defects and their presumed pathogenesis were documented in Table 1.

X-Linked Recessive Genetic Trait

The most prevalent type of SCID is inherited through the X-linked Recessive pattern. The most common clinical



	IL2RG Deficiency	Mutation in JAK3 Gene	Mutation in RAG1&RAG2	AK2 Deficiency	ADA Deficiency	IL7RA Deficiency	Mutation in PTPRC	ZAP-70 Deficiency	Coronin- 1A Deficiency
Indication	Defective signaling IL-2, 4, 7, 9, 15, 21 & common γ chain defect	Defective interleukin signal transduction	Defective V(D)J Recombination	Increased Lymphocyte Apoptosis	Toxic metabolite accumulation	Defective cytokine signalling	Defective TCR signal transduction	Mutation in Zap-70 kinase Gene	Actin regulating protein Coronin- 1A defect
Lymphocyte Profile	T-B+NK-	T-B+NK-	T-B-NK+	T-B-NK-	T-B-NK-	T-B+NK+	T-B+NK+	T-B+NK+	T-B+NK+
Newborn screening	Yes, can be detect through NBS	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Treatment	HSCT, Gene Therapy	By allogenic BMT	HSCT, Gene Therapy	HSCT	HSCT, ERT, Gene Therapy	HSCT	HSCT	HSCT	Gene Therapy, HSCT
Post-treatment outcomes	Long term, Immune reconstitution achieved	Some have persistent medical issues	Survival without recurrent infections	Long term survival and good quality of life	Encephalitis, respiratory infection, seen	Immune mediated myositis, myasthenia gravis seen	Resolution of clinical problems	Provide long-term immune function,	Robust immune reconstitution

Figure 2. Numerous genetic mutations of severe combined immunodeficiency contributing to X-linked and autosomal recessive trait.
Several genetic mutations have a differential effect on T, B, and NK cells' population and function. The hallmark of Severe Combined Immunodeficiency is an absence of mature T-cells. They normally have four different types of lymphocyte profile, e.g., T-B+NK+ (T-cell absent, B-cell present, NK-cell present), T-B+NK- (T-cell absent, B-cell present, NK-cell absent), T-B-NK+ (T-cell absent, B-cell absent, NK-cell present), T-B-NK- (T-cell absent, B-cell absent, NK- cell absent). Early diagnosis is the best curative option. NBS is possible in all the genetic mutations except Zap-70 Deficiency. Treatment options available are HSCT, Gene therapy, ERT, and BMT. Post-treatment outcomes include that most of the affected newborns achieved a long-term immune reconstitution and a good quality of life.
IL-2RG: Interleukin-2 receptor gamma, JAK3: Janus Kinase 3, RAG1&RAG2: Recombinase activating gene 1&2, ADA: Adenosine deaminase deficiency, IL-7Ra: Interleukin-7 receptor alpha, Zap-70: Zeta chain associated protein kinase deficiency, IL: Interleukin, TCR: T-cell receptor, V(D)J: V(Variable) D(Diversity) J(Joining), NBS: Newborn screening, HSCT: Hematopoietic stem cell transplantation, ERT: Enzyme replacement therapy, BMT: Bone Marrow Transplant

finding of X-linked SCID is the defect in cell-mediated and humoral immunity. The pathogenesis of X-linked SCID is the deleterious mutations in IL-2 receptor subunit gamma (*IL2RG*), a gene that encodes common gamma chain (gc, CD132) of the cytokine receptors for interleukins IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These mutations result in a non-functional gamma chain which causes widespread defects in X-linked interleukin signaling and SCID (41,42). Furthermore, this mutation affects the development and maturation of B-lymphocytes and NK cells by reducing the number of T-lymphocytes (43). There are few treatment options for SCID, including hematopoietic stem cells transplantation (HSCT) and gene therapy, which restore the patient's immune system, provided given within two years of being diagnosed with SCID (44).

In gene therapy, the mutated gene is replaced by a healthy gene (gene of interest) to carry out a normal function in our body (45). In Western countries, approximately 25-40% of all SCID cases are X-linked (46,47). Two distinct cases of SCID are observed by analyzing the pedigree of SCID or how it is inherited from parents to offspring: (i) Case -1: If a carrier mother has one mutated X chromosome, her male child has a 50% chance of being affected and 50% chance of being normal, while her female child has a 50% chance of being a carrier and 50% chance of being normal if the father is normal. (ii) Case -2: from an affected father, the mutated gene is transferred to the female child only, not to the male child when the mother is normal. In families of SCID patients, prenatal diagnosis guidance is required to avoid pregnancies with such diseases (48). Besides this, prenatal testing gives sufficient time to prepare the matched donor for HSCT (46,49).

Mechanism of T-cell Activation Through IL-2 Receptors System

When an antigen enters into our immune system, IL-2 is secreted, and its receptors on the cell surface are also expressed (5). The association of these receptors and IL-2 controls the duration and magnitude of T-lymphocyte immune responses. The IL-2 receptor comprises three components that are readily found on Treg cells, namely IL-2Rα (CD25), IL-2Rβ (CD122), and γc (CD132) (50,51). Receptors with low affinity are made up of only the β chain, whereas intermediate affinity ($Kd=10^{-9}M$) receptors are made up of β and γ chains. Including

NK cells, some subsets of resting lymphocytes express intermediate affinity IL-2 receptors (52).

Out of low, intermediate and high-affinity receptors, IL-2 signals can be conducted by high & intermediate affinity receptors (53). A cascade reaction occurs due to the heterodimerization of domains and chains in the cytoplasm caused by the attachment of IL-2 to its receptor (54). The importance of the α chain is negligible for signal

Table 1. Different types of genetic defects and their presumed pathogenesis (41,186-189)

Designation	Mechanism	Presumed pathogenesis	Mutated gene	Type of mutation	Frequency of different genotypes In NBS	Effects
IL-2RG deficiency (γc deficiency)	Defective cytokine development and T-cells are unable to deliver the signal to B or NK cells, which prevent their maturation	Mutation in gamma (γ) chain of IL-2,4,7,9,15 Receptors	IL-2R gamma	Hypomorphic mutation in the *IL-2RG* gene, frameshift, missense	46%	Males only
JAK3 deficiency	Survival signaling	Mutation in Janus kinase3 and defective interleukin signal transduction	JAK3	Missense, non-sense	6.9%	Males and females
RAG1 and RAG2 deficiency	Defective V(D)J rearrangement	Missense mutations in *RAG1* and *RAG2* genes	RAG1 or RAG2	Missense, non-sense	3.4%	Males and females
Adenosine deaminase (ADA) deficiency	Premature cell death	T-cell and B-cell defects from toxic metabolites (dATP, S-adenosyl homocysteine) due to enzyme deficiency	ADA	Missense, frameshift	16.1%	Males and females
Reticular dysgenesis (AK2 deficiency)	Mutation in the AK2 protein alters the mitochondrial mechanism so that mitochondria cannot provide enough energy to the hematopoietic stem cells. As a result, HSC will not be able to differentiate	Defective maturation of T and B-cells and myeloid cells (stem cell defect) and increased lymphocyte apoptosis	AK2	Missense, deletion, non-sense, splice-site mutation	0.7%	Males and females
IL-7Rα Deficiency	Mutation in IL-7RA, prevent the development of T-cell and maturation of T-cell	IL-7 receptor alpha chain deficiency and defective cytokine signaling	IL-7R alpha	Frameshift	10.3%	Males and females
Zap-70 Deficiency	Abnormal T-cell receptor (TCR) signaling	Mutations in Zeta chain associated protein kinase (*Zap-70*) gene	Zap-70	Non-sense	1%	Males and females
DCLRE1C Deficiency (artemis deficiency)	Defective V(D)J Recombination during DNA repair process	Hypomorphic mutations in the non-homologous end-joining gene *DCLRE1C* (encoding Artemis)	DCLRE1C	Missense, non-sense, deletion	3.9%	Males and females
Coronin1A deficiency	The most potent coronin present on lymphocytes is coronin1A, a transcriptional product of the *CORO1A* gene. Any defect in this gene results in a decrease in the lymphocytes and immune system failure	Actin regulating protein coronin 1A deficiency	CORO1A	Null mutation in CORO1A	0.1%	Males and females
Mutation in the *PTPRC* gene	Defective CD45 deficiency	Defective TCR signal transduction	PTPRC	Deletion, point mutation	1%	Males and females

transduction. However, it increases the binding affinity towards the low level of IL-2 by 100 folds than that of cells having an intermediate relationship. γ and β chains come under a superfamily consisting of receptors for interleukin (55). Gamma chain (γc) family cytokines collectively regulate immune cells development, proliferation, survival, and differentiation (56).

Mutation of Gamma Chain in *IL-2RG* Gene

In 1993, it was found that the molecular defects caused by the interleukin-2 receptor gamma chain is associated with X-linked SCID (57). 4.2 kb *IL-2RG* genes contain 8 exons found in the Xq13.1 chromosome band. In the study of 8 exons of gene encoding IL-2R, occurrences of approximately 119 out of 200 mutations were found to be frameshift type mutations (58). The common gamma chain is crucial for all cytokines (IL-4, IL-9, IL-7, IL-21, IL-15) starting from IL-2 (59). IL-15 and IL-2 help in the development of NK and T-cells, respectively, for which the gamma portion of IL-2R is necessary. Molecular defects in the *IR2G* gene are responsible for the T-negative, B-positive, NK-cell negative (T-B+NK-) type of SCID (60). Transmembrane domain, the domain which binds to IL-6 receptor alpha, and domains that bind to fibrin nectin3 are present on IL-2RG (61). Mutations are commonly found in exon 5, exon 4, and exon 3. Approximately half of all mutations are non-sense and missense. Splice site, deletion, and insertion mutations are found to be less common (62). There is a failure to form protein due to a non-functional gamma chain, which results from alterations in the *IL-2RG* gene. Typical X- SCID does not have any functionally abnormal NK-cell, T-lymphocyte and B-lymphocytes due to mutations in IL-2RG, whereas atypical X-SCID with milder symptoms results from hypomorphic IL-2RG mutations (63). Clinical features include many pathogenic invasions, diarrhea, sepsis, pneumonia, and pyrexia. Patients with SCID eventually die if their immune system is not re-established by any possible treatments like gene therapy or hematopoietic stem cell transplant.

Gamma is Shared by Multiple Cytokine Receptors

T-cells are produced in bone marrow but they are mature in the thymus with the help of the gamma chain (64). The whole receptor of interleukin 2 constitutes of interleukin 2 receptor alpha beta and gamma chains, with IL-2RG encoding the gamma chain part (65). In the development of regulatory T-cells, the IL-2 attaches with the gamma chain part to perform the functions of increasing the cytolytic activity of NK cells and maintaining peripheral tolerance (66). Apart from IL-2 different cytokines like IL-21, IL-9, IL-15, IL-7, IL-4 also require the gamma chain part for their signaling (67). IL-7 is essential for B lymphocyte homeostasis and maturation. IL-15 is crucial for the development and maturation of NK-cells (68). Mice lacking IL-15 have been found to have a low number of $CD8^+$ T-cells and NK-cells (66). In antibody production and differentiation of helper T-cells, IL-21 and IL-4 play an essential role (69). Immune cells need a gamma chain for their growth, maturation, and other functions.

Autosomal Recessive Genetic Trait

There are many genes involved in this type of genetic defect (70). If the child gets both affected genes in a recessive inheritance pattern, then the child will be affected by the disease. However, when a child gets one healthy and one altered gene, the child is a carrier without any signs and symptoms (71). If both parents are the carrier, then they have a 25% chance to have a SCID-affected child (having 2 altered genes), a 50% chance to have a carrier child and a 25% chance to have a normal child. In autosomal recessive SCID, females and males both follow a similar pattern (72). Consanguineous marriage promotes an increase in the number of autosomal recessive SCID (73). Turkey has a high rate of consanguinity, which leads to more cases of AR type SCID (80%), compared to the USA (20%), which has a low rate of consanguinity (74,75). A recent study demonstrated that the high rate of consanguinity is directly linked with the higher incidence and prevalence of SCID (76).

Janus Kinase 3 (JAK3) Deficiency

JAK3 deficiency is another major cause of SCID (77). Although it shares similarity with X-linked SCID in terms of phenotype, it showcases an entirely different genotype (78). There is a complete absence of T-lymphocyte and NK-cells but an adequate number of B-lymphocytes (T-negative, B-positive, NK-negative) is present (60,79). The frequency of JAK3 deficient SCID is quite rare in America and much more in Europe (80). As it ranges from 1 per 1,00,000 to 1 per 1,000,000, it comes under the category of uncommon diseases (81). Classical clinical features of JAK3 deficient SCID include failure to thrive, bronchitis, and diarrhea for a more extended period (82). Other signs and symptoms include various pathogenic invasions and hypersensitivity reactions against mother's antibodies, causing rashes, liver damage, and a decrease in the number of all immune cells (83). In this disease, tyrosine kinase, JAK3, is defective in its structural and functional level because of its abnormalities in the genetic level (84). Almost all hematopoietic cells require the JAK3 enzyme to signal transduction pathways carried out by cytokines (85). JAK3 is an inactive enzyme. Its activation should attach to the gamma c portion of an interleukin receptor while a cytokine interacts with another portion of that receptor. γc is not only a part of the receptor for IL-2 but also an important part of receptors for IL-4, IL-7, IL-9, IL-15

(86). Cytokines induce the JAK3 phosphorylation, which is attached to the gamma chain of the receptor (87). This is followed by the phosphorylation of STAT (a transcriptional factor that transduces and activates transcription) by the phosphorus-containing JAK3. Furthermore, in order to induce the process of transcription, it dimerizes and enters into the nucleus (40). JAK3 deficiency SCID is also considered to be a major problem for pediatric patients. They tend to have extremely low chances of survival unless and until appropriate treatment, such as histocompatible HSCT, is performed (88).

Abnormalities in V(D)J Recombination

Mutation in the Recombinase Activating Gene (*RAG1* and *RAG2*)

The endonuclease protein, which is transcribed from the *recombinase activating genes 1* (*RAG1*) and *RAG2* genes, is essential for the formation of the receptors of T-lymphocytes (89). Any defect, like the non-sense type of mutation in these two genes, causes abnormal receptor function of lymphocytes, which ultimately causes SCID (90). The hallmark of this type of SCID is the lymphocyte profile: T-negative, B-negative, NK-cells positive which is inherited in an autosomal recessive manner (91). Different receptors of lymphocytes are formed by the association of V (variable), D (diversity), and J (joining). These receptors play a prominent role in the growth and maturation of immune cells (92). During this process, the combination of *VDJ* genes produces unique sequences that code for specific receptor chains. RAG1 and RAG2 unwind a double-stranded DNA and splice at particular sites by its endonuclease activities (93). DNA-PKcs (DNA dependent protein kinase), Ku80, DNA ligase IV, Ku70, XRCC4 molecules actively participating in DNA repairing are also required to help the endonuclease activities of the *RAG* genes (94). Programmed cell death of T-lymphocytes occurs due to defective VDJ recombination activities (95). The *RAG1* gene consists of a non-core and core region (89). Domains needed for the recombination of VDJ are located on the core part of RAG1 including C-terminus domain (CTD), the nonamer binding domain (NBD), the dimerization domain, DNA binding domain, and the zinc binding domain (ZBD) (96). Protein RAG2 comprises PHD (Plant Homeo Domain), situated in the non-core part (97). In the core part of RAG1 in ZBD, maximum mutations (missense) are concentrated, followed by NBD and CTD, and rest of the mutations predominantly affect the non-core part. Non-sense and frameshift mutations follow the missense mutation in the core domain of RAG2 (98). It is interesting to note that the RAG1 and RAG2 mutations are most common in countries with high rates of consanguinity (99). Hyper IgM syndrome, idiopathic CD4 lymphocytopenia, CVID and chronic bone deformities are the other clinical phenotypes associated with RAG defects (74).

Omenn Syndrome

Molecular defects of the RAG are responsible for causing Omenn syndrome, a form of SCID (100). Mainly represented clinical features with this defect include loss of hair, exfoliation of the skin, rashes, and scaling. Elevated IgE levels are the marker of Omenn syndrome (101,102). Frequent clinical findings include the occurrence of inguinal and axillary lymphadenopathy, and hepatosplenomegaly in SCID patients (103). The patients may develop hepatitis, inflammatory pneumonitis, or enteritis (104). Usually, coexisting infection with the opportunistic or conventional pathogen is demonstrated (105). In individuals having Omenn syndrome, there is a markedly low count of naive T-lymphocytes and an increased or normal count of $CD3^+$ T-lymphocytes (7). When 2 RAG alleles undergo the null mutation, it gives rise to T-negative B-negative type of SCID (106). When only one allele undergoes missense mutation on a RAG, it causes classical Omenn syndrome by allowing the partial recombination of VDJ (107).

Abnormalities in the Purine Metabolism

The multifunctional enzyme adenosine deaminase (ADA) affects the purine metabolism and affects the maturation of immune cells. Any defect in its amount and function due to *ADA* gene mutation causes the most frequent autosomal recessive form of SCID named ADA-SCID (T-negative, B-negative and NK-negative) (108). The metabolic enzyme ADA is found in almost every cell, but it performs a variety of functions. It has the highest function in the brain, lymphoid tissues like the thymus, and gastrointestinal tract (109). ADA is a monomeric soluble enzyme of 41KDa, consisting of 363 amino acids (110). ADA deaminates adenosine and 2' deoxyadenosine (produced during high cell turnover by DNA degradation) to inosine and deoxyinosine respectively (111). The cytoplasm of lymphocytes and erythrocytes have been found to be high in ADA (112). The 20q13.11 chromosome band contains *ADA* genes, which encode the ADA enzyme (113). A structure (>200 kDa) made up of monomers of ADA is present in mature T-lymphocytes, cells present in the medulla of the thymus, and epithelial cells. This complex binds to the CD26 receptors and has functions the same as ectopeptidase, hormone regulations and cytokines (114). Toxicity due to accumulation of 2'deoxyadenosine and adenosine in lymphoid tissue and bone marrow occurs because of absence in ADA function (115). Signs and symptoms of ADA-SCID include skeletal system abnormalities, neurodevelopmental abnormalities, and deafness due to sensorineural loss (116). The late

form of this disease is common and this entity can be presented with end-organ damage like bronchiectasis, delay in neurological development (117). The autosomal recessive form of SCID is around 11-20% of all forms of SCID (1 per 2,00,000) (118). The time of appearance of signs and symptoms of ADA-SCID may vary significantly. It is advised to appear for ADA function test to detect the presence of this disease, especially because it might go undetected in screening due to the presence of an adequate amount of lymphocytes (119). To evaluate ADA-SCID, the use of enzyme replacement therapy (ERT) is prevalent at the enzyme level, gene therapy at a genetic level, and HSCT at the cellular level (120).

IL-7 RA Deficiency

Another cause of autosomal recessive SCID is a deleterious mutation in the IL-7 receptor alpha chain. In this type of SCID, T-lymphocytes are absent, but B lymphocytes and NK-cells are present (T-negative, B-positive, NK-positive). It is seen in both males and females. The frequency of this type of SCID is approximately 10% of all forms of SCID (121). IL-7 receptor alpha gene is present in 5p13 chromosome (122). It is composed of 8 exons which constitute an 18 kb region of the genome. It encodes for a type-1 membrane glycoprotein having 440 amino acids of 80kDa (123). The protein has a typical folding for the attachment of cytokines which are alpha-helical (124). It constitutes of 3 regions named transmembrane, intracellular, and extracellular domains (125). The intracellular domain is typically used in the transduction of signals, whereas the outer portion has the same functions as that of the receptors of the type 1 family (126). Interaction of receptors with IL-7 induces the signal transduction through JAK1 and JAK3 tyrosine kinases, which ultimately increases BCL-2 by PI3 kinase induction after the dimerization of following transcription factors-STAT5A, STAT3, STAT5B (127). The alpha receptor of IL-7 is present in progenitors of lymphocytes (128). It plays a significant role in the maturation, growth, and differentiation of lymphocytes and dramatically impacts the signaling of IL-7 (129). Hereditary and recessive inactivating mutations on the *IL-7R* genes are found to be responsible for SCID. Several types of mutations are located in the interleukin-7 receptors, specifically in their extracellular region, which mostly includes non-sense, stop, missense, and splicing defects type of mutations (130). Mutations occur on exon-5, exon-2, and exon-4 of the IL-7 receptor alpha gene (131). IL-7Rα is essential for the growth and maturation of T-lymphocytes, homeostasis expression, and for their signaling. When one is affected with autosomal recessive form of IL-7 receptor alpha deficient SCID, he is susceptible to several pathogenic invasions (132). Nowadays, TREC technique is implemented for the diagnosis and early treatment for this type of autosomal recessive abnormalities (133). The possible curative option for this type of abnormality is HSCT (134).

Adenylate Kinase Deficiency/Reticular Dysgenesis (T-negative, B-negative, NK-negative)

Some mutations in the different locus of homologous gene may cause adenylate kinase 2 (AK2) deficiency (135, 136). In 1959, two scientists, De Vaal and Seynhaeve, first diagnosed twin boys who were devoid of granulocytes and lymphocytes in their peripheral blood (137). Their death were suspected to occur due to secondary bacterial infections. They coined this disease as "Reticular dysgenesis", one of the most severe forms of SCID (137). The activities of AK2 are associated with the inner mitochondrial membrane space (138). The function of AK2 is to regulate the following reaction–ATP + AMP ⇌2ADP, which is an example of a typical energy transfer reaction (139). Two types of mRNA are encoded by the *AK2* gene: AK2A (6 exons) and AK2B, which contains an additional seventh exon located in the 3' end of the gene. AK2 A encodes a 239 amino acid, where AK2B encodes a 232 amino acid protein (140). Mutation in the *AK2* gene and a defect in energy metabolism reduce pluripotent bone marrow cells and precursor cells (141). The 7 exons of AK2B are greatly influenced by the following mutations: Splice site, missense, deletion, and non-sense type of mutation (142). Due to the complete absence of granulocytes, patients with reticular dysgenesis are resistant to several pathogens (viruses, fungus, bacteria, and mycobacteria). However, bone marrow cells stop developing in this disease at the promyelocytic step (143), while the spleen, lymph nodes, and thymus show the absence of lymphocytes (144). Newborns having reticular dysgenesis get affected by various pathogens within 24 hrs of delivery hence appropriate precautionary measures should be taken as early as possible to save the newborn's life (145).

Newborn Screening for SCID

Several genetic mutations cause SCID, which profoundly diminishes the working capabilities of the immune system and markedly decreases the number of T-cells that impact the development and maturation of NK-cells and B-lymphocytes (146). Infants with SCID usually have normal development without any symptoms, but after 3 to 4 months, they have recurrent pathogenic invasions associated with secondary organ damage and life threatening infections originated by live vaccines (147).

The condition worsens further if immune reconstitution does not happen in the first few months (2 to 3 months from birth) of life (148). Approximately 60% of children having SCID in Canada died before a successful transplantation due to secondary pathogenic invasions, which were

diagnosed late (around 4.2 months) (149). Due to the urgent need to diagnose and treat SCID patients early, an advanced newborn screening technique named TRECs was founded (150). In 2005, the TREC technique was started and done on neonatal dried blood spots, which are already a part of routine newborn screening (151). HSCT, gene therapy, and ERT are now possible treatment options available for newborns with SCID (152).

Need of Precocious Diagnosis and Its Advantages

As survival rate of SCID patients depends on treatment time and infection status, delayed diagnosis may cause undesirable outcomes (153). From previous studies, it has been found that patients receiving HSCT before the safety margin (3.5 months) have more than 90% chance of survival, even if there is no matched sibling donor (154). However, patients having HSCT after the safety margin have a survival chance of an average of 70% (ranging from 50% to 90%) because the type of donor and infection status of patients have a significant impact (155).

Since patients may appear healthy from the outside until they are affected by an infectious pathogen or suffer from growth retardation (156), there is a possibility that the infant may die due to infections or even from complications due to HSCT, similar to patients who were previously identified (157). Consequences depending on diagnosis timing have enhanced the quest for a biomarker for screening of SCID newborns, which gave birth to the TREC concept (158).

T-cell Receptor Excision Circles (TREC) Based Newborn Screening

Over the years, a considerable rise in interest for screening children with SCID has been observed. The quantitative PCR of DNA, which is responsible for developing TCRs, can identify all SCID forms. The sample of DNA is collected from dried blood spots of neonates (159). The advantages of newborn screening of SCID include protections of infants from respiratory viruses (Parainfluenza and Adenovirus) and respiratory pathogens, including *Pneumocystis jirovecii*, along with early age transplantation which increases the chances of survival (160). TRECs are the by-product of the *TCR* gene, transcribed in developing T-lymphocytes of the thymus (161). To avoid a technical flaw that would decrease the TREC amount, quantitative PCR is performed in conjunction with a control gene screening (162). The biomarker for the development of T-lymphocytes is TREC, as the amount of TREC represents the amount of newly produced T-cells (163). So, the decrease in TREC count is equivalent to decrease in the T-cell amount (164).

TREC count can also indicate Omenn syndrome and maternally engrafted T-lymphocytes because TRECs are reduced in this condition due to oligoclonal expansion of autologous T-lymphocytes (22). SCID is diagnosed and confirmed by immunophenotyping followed by genetic testing. In infants having T-lymphocytes impairment syndromes (like trisomy of 21 chromosomes, DiGeorge syndrome, ataxia-telangiectasia), absence/low TRECs can also be detected (165). Sequential tests like flow cytometry, immunophenotyping, and genetic analysis are also performed to differentiate between SCID and other subforms (166). The diagnostic performance of SCID newborn screening is excellent, with high specificity and sensitivity (167). When the T-lymphocytes count decreases beyond 300 cells/mL, the child is identified as a SCID patient (88). From the cohort study, the specificity of TREC NBS is 99.98% (168). There may be an error in screening due to the condition in which receptors of T-lymphocytes formation are decreased (169).

False-positives in this screening are observed when children with a low number of TREC without any genetic defect or receptor defect are detected (170). T-cells are usually found to be abundant in infants (ranging from 2.500 to 5.500 per microliter) and often varying degrees of T-cell cut-off values are used in different locations (basically 1.500-2.500/mL). The other phenotype of T-cell is also analyzed to determine the infant's fate (171). The abundance of T-cells ranges from 2.500 to 5.500/L in different locations. Hence for the newborn screen for SCID, the T-cell value cut-offs varies from 1.500 to 2.500/L. So, there is a considerable diversity of false-positive incidence among different places. A very low false-positive results might occur (172). Hence, an appropriate T-lymphocyte count and TREC cut-off value is essential for the successful functioning of the screening technique. Till date, a minimum of 20 deleterious mutated genes have been found to be responsible for SCID (173). The TREC test successfully detects most of these mutations; however, some cases where SCID is caused by MHC class II and ZAP70 deficiency are mistakenly undetected (174). In these cases, as there is a developmental defect of T-lymphocytes but not in their receptors, a wide range of consequences of TREC screening is observed (175).

Implementation of Newborn Screening

Though the screening techniques have emerged many years ago, the major challenge faced is with its implementation (176). In 2008, the first state to screen newborns happened to be Wisconsin, and the test has been globally implemented since then (177). Around 0.08% of 100.597 children screened during 2009-2010 years in Massachusetts were found to be affected by SCID (178). Recently, screening is conducted in every state after getting confirmation and financial support from the concerned state government (179).

Till 2013, screening of around 45% of newborns was done in the following states-Mississippi, Colorado, New York, Texas, Delaware, Connecticut, Michigan (180). By analyzing the screening report of California, where 500.000 infants were screened, and an increase in the number of SCID cases was noticed (181). In a report in which 3.030.083 children were screened in Navajo nation and 11 states, about 52 SCID cases were detected (including 1- Omenn syndrome, 9- leaky syndrome and 42 typical), which equals to 1 SCID case per 58.000 newborns (182). From 2008 to 2010, screening in Wisconsin resulted in the identification of 5 affected individuals with SCID out of 207.696 (that is relatively 1 per 42.000). In California, from 2010 to 2017, 3.252.156 children were screened and 50 SCID patients were identified (the incidence is relatively 1 per 65.000).

All American states have implemented SCID newborn screening till the end of 2018 (183). Currently, besides the US, screening for SCID is done in Australia, Germany, Spain, Israel, Switzerland, New Zealand, Canada, Norway, Italy, Taiwan, and Sweden. Active screening programs conducted in Israel from 2015 to 2017 had screened 290.864 infants from which 13 SCID patients were detected, which is relatively 1 per 22.000 (184). Taiwan had conducted a routine screening program from 2010 to 2017, which resulted in the identification of 7 children with SCID out of 920.398 screened infants, (approximately 1 per 131.000 infants) (185).

Conclusion and Future Perspective

By studying the causes, we learn about the different genetic and clinical forms of SCID that can help us better understand the genetic advancements in therapeutic or diagnostic approaches and family counseling. More than 30 identified genetic defects and some novel mutations cause human SCID. SCID in humans is a major concern of the pediatric group of age characterized by a defect in the growth and maturation of granulocytes. To save the life of newborns from secondary pathogenic infections, more emphasis should be given to early diagnosis. Complete blood cell count is also another way for diagnosis.

Early evaluation with Gene therapy, ERT, and HSCT may save the life of SCID patients. Newborn screening provides knowledge about the incidence of SCID along with the various genotypes and phenotypes of SCID. To date, the implementation of newborn screening is practiced worldwide. However, a review of overall data reveals that NBS for SCID has yet to be implemented throughout the country, as many countries do not perform newborn screening for SCID, resulting in death of infants before receiving proper diagnosis. The challenge is to understand the detailed pathogenesis of SCID and develop improved methods for diagnosis and treatment of affected individuals, and to set uniform cut-off values of TRECs.

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