The Genetic and Molecular Basis of Severe Combined Immunodeficiency

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Abstract Severe combined immunodeficiency (SCID) is a life-threatening disease caused by a heterogeneous group of genetic defects. It is characterized by profound defects of T-cell development, also affecting B and NK cells in some cases. Since the first molecular identification of a causal gene for SCID in 1985, 14 more molecular causes have been identified in patients with a classical SCID phenotype, with no T cells. Some genetic defects specifically block lymphocyte ontogeny, whereas others affect T-cell function and a few cause extra-hematopoietic alterations with a rare complex phenotype. Over the last 15 years, several new causal genes have been identified in patients with low T-cell counts and impaired T-cell function. Patients with a clinical SCID phenotype with normal numbers of dysfunctional T cells have also recently been reported. This last condition is described immunologically as combined immunodeficiency. These discoveries have expanded the complexity and difficulties of molecular characterization in patients with a clinical SCID phenotype. Studies of these disorders have increased our understanding of the role of single-gene products in the development, differentiation, and function of the immune system in humans.

Keywords Severe combined immunodeficiency · Primary immunodeficiencies · T-cell lymphopenia · Genetic defect

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Introduction

Human primary immunodeficiencies (PIDs) comprise a broad group of inherited disorders characterized by developmental or functional defects of myeloid or lymphoid hematopoietic cells, and of non-hemapoietic cells involved in protective immunity [1, 2••]. Severe combined immunodeficiency diseases (SCID) are a heterogeneous group of PIDs caused by defects in T-lymphocyte development, with or without alterations to the development of B lymphocytes, NK lymphocytes, or myeloid cells [3]. The first published report of SCID can be traced back to 1950, when this condition was described in two siblings with profound lymphopenia [4]. A second report described thymic dysplasia and hypogammaglobulinemia in other

Table 1 SCID disorders

Disease	Inheritance	Gene	Clinical identif.	Genetic identif.	Pathogenetic mechanism/gene product	Immunol. phenotype	OMIM number
SCID T-B-NK−							
Adenosine deaminase deficiency	AR	*ADA*	1972	1985	Accumulation of toxic metabolites/ adenosine deaminase	T−B−NK−	102700
Reticular dysgenesis, AK2 deficiency	AR	*AK2*	1959	2009	Increase in apoptosis/adenylate kinase 2	T−B±NK− neutropenia	103020
SCID T−B+NK−							
γc deficiency	XL	*IL2RG*	1958	1993	Impairment of interleukin signaling/(IL-2, - 4, -7, -9, -15, -21 receptors)	T−B+NK−	300400
JAK3 deficiency	AR	*JAK3*	1993	1995	Impairment of interleukin signaling/Janus activating kinase 3	T−B+NK−	600173
SCID T-B-NK+							
RAG1 deficiency	AR	*RAG1*	1988	1996	Defective V(D)J recombination/recombinase activating gene 1	T-B-NK+	179615
RAG2 deficiency	AR	*RAG2*	1988	1996	Defective V(D)J recombination/recombinase activating gene 2	T-B-NK+	179616
Artemis deficiency	AR	*ARTEMIS/ DCLRE1C*	1993	2001	Defective V(D)J recombination and DNA double-break repair/Artemis DNA recombinase-repair protein	T-B-NK+	602450
DNA-PKcs deficiency	AR	*PRKDC*	1993	2001	Defective V(D)J recombination and DNA double-break repair/DNA-PKcs recombinase-repair protein	T-B-NK+	600899
Cernunnos deficiency	AR	*CERNUNNOS/ XLF*	1993	2006	Defective V(D)J recombination and DNA double-break repair	T-B-NK+	611290
DNA ligase IV deficiency	AR	*LIG4*	1993	2001	Defective V(D)J recombination and DNA double-break repair	T low B− NK+	601837
SCID T−B+NK+							
IL7Rα deficiency	AR	*IL7RA*	1998	1998	Impairment of interleukin signaling/IL-7 receptor α chain	T−B+NK+	146661
CD3δ deficiency	AR	*CD3D*	2003	2003	Impairment of TCR signaling/CD3δ chain	T-B+NK+	186790
CD3ε deficiency	AR	*CD3E*	1993	1993	Impairment of TCR signaling/CD3ε chain	T-B+NK+	186830
CD3ζ/CD247 deficiency	AR	*CD3Z*	2006	2006	Impairment of TCR signaling/CD3ζ chain	T-B+NK+	186780
CD3γ deficiency	AR	*CD3G*	1986	1992	Impairment of TCR signaling/CD3γ chain	T low B+NK+	186740
CD45 deficiency	AR	*PTPRC*	1997	2000	Impairment of TCR signaling/CD45	T low B+NK+	151460
TCRα deficiency	AR	*TRAC*	2011	2011	Component of the TCR	T low B+NK+	186880
Purine nucleoside phosphorylase def.	AR	*PNP*	1975	1987	Accumulation of toxic metabolites/purine nucleoside phosphorylase	T low B+NK+	164050
SCID with athymia (T-B+NK+)							
Nude FOXN1 deficiency	AR	*FOXN1*	1996	1999	Defective intrathymic cross-link/ transcription factor FOXN1	T−B+NK+	601705
Complete DiGeorge syndrome	AD	*TBX1*	1968	1981	Heterozygous mutation of *TBX1* or haploinsufficiency	T−B+NK+	188400
CID with no functional T cells							

Table 1 continued

Disease	Inheritance	Gene	Clinical identif.	Genetic identif.	Pathogenetic mechanism/gene product	Immunol. phenotype	OMIM number
ORAI1 deficiency	AR	*ORAI1*	1995	2006	ORAI1 is a CRAC modulatory component	Impairment of T-cell activation	610277
STIM1 deficiency	AR	*STIM1*	1995	2009	STIM1, stromal interaction molecule 1, is a CRAC modulatory component	Impairment of T-cell activation	605921
LCK deficiency	AR	*LCK*	2012	2012	A proximal tyrosine kinase interacting with TCR	TCD4− B+NK+	153390
ZAP70 deficiency	AR	*ZAP70*	1994	1994	ZAP70 intracellular signaling kinase, acting downstream from the TCR	TCD8- B+NK+	269840
IKK2 deficiency	AR	*IKBKB*	2013	2013	I-kappa-B proteins, which are essential for NF-κB activation	Impairment of T-cell activation	603258
MALT1 deficiency	AR	*MALT1*	2013	2013	Caspase-like cysteine protease essential for NF-κB activation	Impairment of T-cell activation	604860
CARD11 deficiency	AR	*CARD11*	2013	2013	A scaffold for NF-κB activity in the adaptive immune response/*CARD11*	Impairment of T-cell activation	615206
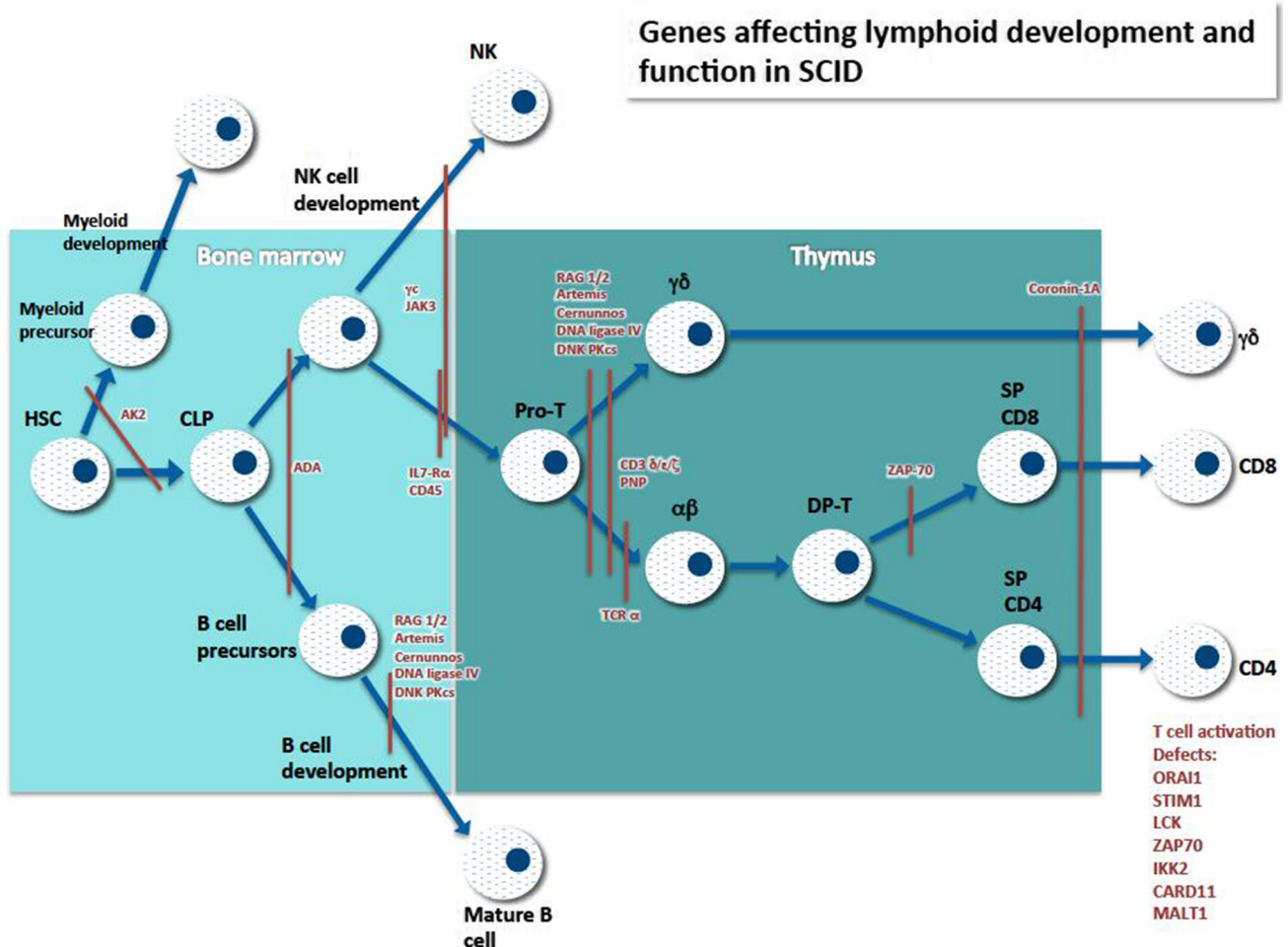
Fig. 1 Genes affecting lymphoid development and function in SCID

SCID patients in 1958 [5]. Patients with SCID present recurrent infections caused by opportunistic microbes (such as *Pneumocystis jiroveci)* viruses, bacteria or fungi, during the first year of life [6]. On physical examination, these patients generally lack lymphatic tissue and no thymus shadow is detectable on chest X-ray [3].

SCID patients generally present profound lymphopenia, with very low T-cell counts, typically below 500/μL, if indeed these cells can be detected at all. The immunophenotyping of T, B, and NK cells can improve the definition of SCID phenotype in patients, guiding subsequent genetic studies [3]. Four groups of SCIDs can be defined on the basis of the presence or absence of various lineages of lymphocytes: (i) SCID with no T, B, and NK cells (T−B−NK−), (ii) SCID with no T and NK cells (T−B+NK−) but with B cells, (iii) SCID with no T and B cells but with NK cells (T−B−NK+) and (iv) SCID with no T cells but with NK and B cells (T−B+NK+) (Table 1). Another variant of SCID is Omenn syndrome which may have early or delayed manifestations, and refers to a subset of infants in whom there is oligoclonal proliferation of dysregulated autologous T cells which lead to generalized erythroderma and desquamation of the skin, lymphadenopathy, splenomegaly, eosinophilia, and elevation of immunoglobulin E. Analyses of thymopoiesis based on T-cell receptor excision circles (TRECs) can be used for diagnostic purposes, particularly for the screening of neonates [7•] (see Kwan et al., review in this series). We review here SCID disorders and their molecular etiologies, mutations in the genes encoding cytokine receptors, key molecules in V(D)J recombination, pre-T-cell receptor (TCR) and TCR compounds, metabolic disorders, or impairment of the thymic development of T cells. Finally, we discuss a group of patients with a clinical SCID phenotype but no significant T-cell lymphopenia (table). These patients have normal numbers of T cells, but these cells are not functional, due to a T-cell activation defect (Fig. 1).

T−B−NK−SCIDs

Adenosine Deaminase Deficiency

The first etiology of autosomal recessive (AR) T−B−NK−SCID was identified by Giblett et al. in 1972, who showed that these patients lacked the adenosine deaminase (ADA) enzyme [8]. The gene encoding ADA was mapped to chromosome 20q13.2–11 and cloned [9]. The ADA deficiency (MIM 102700) caused by mutations of this gene results in the marked accumulation of adenosine, 20-deoxyadenosine and 20-*O*-methyladenosine, and an absence of detectable ADA activity [10] This is accompanied by an intracellular accumulation of dATP. The first homozygous *ADA* mutation was reported in 1985 [11] and the first compound heterozygous mutations were identified in 1986, in one patient with ADA deficiency [12]. Since then, over 50 deleterious mutations have been identified in ADA-deficient patients [10]. Typically, ADA-deficient patients have profound lymphopenia, with mean absolute lymphocyte counts of less than 500/μL and a deficiency of all three types of lymphocytes (T−B−NK−SCID) [10]. ADA-deficient patients also display the skeletal abnormalities of chondro-osseous dysplasia, a flaring of the costochondral junctions and a bone-in-bone abnormality of the vertebral bodies [10] There are also other non-immunological defects, such as cognitive and behavioral abnormalities, deafness, and pulmonary defects. Milder forms of this condition have been reported, potentially delaying the diagnosis of immunodeficiency with CD4 T lymphopenia, even in adult patients [13]. ADA deficiency accounts for 17 % of SCID patients in North America and 4 % of SCID patients in France [3, 14] and the exact incidence is very dependent on the specific population. In patients with T−B−NK−SCID, ADA activity or dATP accumulation can be assessed before genetic testing, in whole-blood cells or isolated leukocytes, if the patient has received a transfusion of red blood cells.

Adenylate Kinase 2 Deficiency

Reticular dysgenesis is an AR SCID characterized by a premature arrest of differentiation in the myeloid lineage and impaired lymphoid maturation, associated with sensorineural deafness. It was first clinically described in 1959 [15]. Patients have a T−B low NK-phenotype and neutropenia [16, 17]. Linkage mapping by two independent groups in 2009 led to the demonstration that mutations of the *AK2* gene were responsible for reticular dysgenesis [18, 19]. The *AK2* gene encodes the mitochondrial energy metabolism enzyme adenylate kinase 2 (AK2). AK2 deficiency (OMIM 103020) causes the premature apoptosis of myeloid progenitor cells [18, 19]. Fifteen deleterious mutations have been identified in AK2-deficient patients. AK2-deficient patients display profound lymphopenia with deficiencies of all three types of lymphocytes, with no T cells (T−B±NK−SCID), neutropenia and, occasionally, anemia, and/or thrombocytopenia [10]. Bone marrow analysis shows a complete absence of mature myeloid cells [10]. This PID highlights the key role of the AK2 protein in hematopoiesis and is the first example of a human PID causally linked to energy metabolism and classified as a mitochondriopathy.

T−B+NK−SCIDs

IL-2 Receptor γc Deficiency

SCID X-linked (XL) was first described in 1958 [5]. Patients with SCID-XL lack circulating T and NK cells, but have normal or higher than normal numbers of B lymphocytes (T−B+NK−). In 1987, the causal gene for SCID-XL was mapped, by linkage analysis, to the proximal long arm of the X chromosome [20]. In 1993, two research groups determined that the *IL2RG* gene, which encodes the common gamma chain (γc) of the IL-2 receptor, was located on the proximal long arm of the X chromosome and identified the first deleterious hemizygous mutation in a boy with SCID-XL (OMIM 300400) [21, 22],. The disease-causing gene encodes the common cytokine receptor γ-chain (γc), which is essential for the response to at least six cytokines: interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [22, 23]. Several hundred amorphic or hypomorphic *IL2RG* mutations have been reported [3, 10, 24, 25]. SCID-XL is the most common form of SCID, accounting for 25–46 % of SCID cases [3, 14, 26, 27]. In male patients with the T−B+NK−SCID phenotype, γc (CD132) expression on B cells should be assessed before genetic testing as the absence of γc expression confirms the diagnosis. After successful allogeneic hematopoietic stem cell transplantation (HSCT), γc-deficient patients have been found to have a higher than normal incidence of skin infections caused by human papilloma virus (HPV) during long-term follow-up, suggesting a role of γc-dependent cytokine receptors in the immunity of epithelial cells [28].

JAK3 Deficiency

AR T−B+NK−SCID, the immunological and clinical features of which are indistinguishable from those associated with γc deficiency, was first reported in 1993 [26]. JAK3 is a protein kinase of the Janus kinase family. As the only signaling molecule known to be required for signal transduction through γc-containing cytokine receptors, it was identified as a strong candidate gene for AR T−B+NK−SCID. Mutations of the *JAK3* gene, encoding an intracellular tyrosine kinase, were first identified in 1995, in patients with AR SCID [29, 30]. More than 30 patients with JAK3 deficiency (OMIM 600173) have since been identified [10, 29–37]. JAK3 deficiency accounts for 10–18 % of all SCID cases [3, 14, 27]. A few patients with milder clinical phenotypes have been reported, with missense mutations or small in-frame deletions of the *JAK3* gene [10, 35, 36]. These patients are able to develop autologous T cells due to residual JAK3 expression. Thus, *JAK3* mutations are associated with AR T−B+NK−SCID, or, more rarely, with combined immunodeficiency (CID).

Following successful allogeneic HSCT, JAK3-deficient patients have a higher frequency of skin HPV infections than the general population during long-term follow-up, as reported for γc-deficient SCID-XL patients, suggesting that NK cells or γc/JAK3-dependent signaling in keratinocytes may play a role in anti-HPV immunity [28].

T−B−NK+SCIDs

RAG1 and RAG2 Deficiencies

AR T−B−NK+SCID patients were first reported in 1988 [38]. A recombinase deficiency was then characterized in these T−B−NK+SCID patients, partly analogous to the defect observed in SCID mice [39]. RAG1 (OMIM 179615) and RAG2 (OMIM 179616) deficiencies were identified by applying a candidate gene strategy once the homologous genes had been cloned in mice, based on the analogous phenotypes of Rag1- and Rag2-knockout mice and some patients with AR T−B−NK+SCID [40]. Recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) encode enzymes required for the initiation of V(D)J recombination in T and B cells [41]. The first six cases of RAG deficiency were detected in single-strand conformation polymorphism assays and direct sequencing then identified homozygous or compound heterozygous mutations in the *RAG1* and *RAG2* genes, respectively [42]. RAG1 and RAG2 deficiencies account for 10 % of all SCID cases in North America and 22 % in Europe [3, 14]. In addition to causing the SCID phenotype, hypomorphic mutations of the *RAG1* and *RAG2* genes lead to partially impaired V(D)J recombination activity, resulting in Omenn's syndrome or a leaky or atypical CID phenotype [10, 43] although Omenn's syndrome can arise from hypomorphic mutations in other SCID causing genes. Flow cytometry analysis of the B-cell precursor compartment in bone marrow can be helpful, because typical SCID patients with RAG1 or RAG2 deficiency display a complete block of B-cell precursor differentiation before the cytoplasmic Igμ-positive pre-B-II cell stage, whereas this blocking of differentiation may be incomplete in patients with hypomorphic *RAG* mutations [44••].

Artemis Deficiency

Some of the AR T−B−NK+SCID patients harbor no mutations of the *RAG1* or *RAG2* genes, suggesting that other factors are involved in V(D)J recombination. Indeed, patients with defects of V(D)J recombination can be classified into two distinct groups: one with normal sensitivity to ionizing radiation (RAG1 and RAG2-deficient patients present no radiosensitivity) and the other with higher sensitivity to ionizing radiation (radiosensitive (RS) SCID) [45,

46]. The molecular mechanism for V(D)J recombination is initiated by the creation of DNA double-strand breaks, which are introduced by the lymphoid lineage-specific RAG1 and RAG2 proteins and are repaired by the ubiquitous nonhomologous end-joining (NHEJ) mechanism [47••]. The first genetic etiology of RS SCID was discovered in 2001. Linkage analysis and a candidate gene approach led to the identification of a novel V(D)J recombination/DNA repair factor belonging to the metallo-β lactamase superfamily [48]. This factor is encoded by a gene on chromosome 10p called *ARTEMIS* (*DCLRE1C*) (OMIM 602450) [49]. ARTEMIS deficiency accounts for 13 % of all SCID cases [14]. ARTEMIS deficiency is the most common form of RS SCID, and has also been called Athabascan SCID, because it is particularly frequent in the Athabascan population of Native Americans [50, 51]. After allogeneic HSCT, ARTEMIS-deficient patients who had received alkylation therapy in the conditioning regimen were found to have a significantly higher than normal frequency of infections during long-term follow-up, abnormalities of dental development, and endocrine disorders [52••].

Other RS T−B−NK+SCID/NHEJ Pathway Deficiencies

Other molecular defects of genes belonging to the NHEJ pathway have been identified in patients with AR RS T± low B−NK+SCID without *ARTEMIS* mutation. Several molecules are involved in NHEJ: Ku70, Ku80, and DNA-PKcs (*PRKDC* gene) together constitute the DNA-dependent protein kinase (DNA-PK), DNA ligase IV (*LIG4* gene), X-ray cross-complementation group 4 (XRCC4) and Cernunnos−XRCC4-like factor (XLF) are responsible for the final ligation step [53]. The etiology of RS SCID in humans include deficiencies of *PRKDC* (OMIM 600899), with the first patient genetically characterized in 2009 [54], *LIG4* (OMIM 601837), identified in 2001 [55] and *CERNUNNOS/ XLF* (OMIN 611290), identified in 2006 [56]. LIG4 deficiency is associated with chromosomal instabilities, pancytopenia, microcephaly, developmental and growth delay [57, 58]. Cernunnos-deficient patients also display growth retardation and microcephaly [56]. Genetic defects of the NHEJ pathway render the skin fibroblasts and bone marrow cells of these patients radiosensitive [10] and cause genomic instability, leading to an increase in susceptibility to cancer [59].

T−B+NK+SCIDs

IL-7 Receptor α Deficiency

Some of the patients with AR T−B+NK+SCID have a defect of the alpha subunit of the IL-7 receptor (IL-7Rα)

[60]. All these patients have abnormally low T-cell counts, but normal B- and NK-cell counts (T−B+NK+). IL-7Rα is one of the two subunits of IL-7 receptor in addition to γc, and is crucial for IL-7 signaling. More than 20 patients with AR IL-7Rα deficiency (MIM 146661) have been reported [3, 10, 60, 61]. By contrast to the findings for patients with γc and JAK3 deficiencies, NK-cell development and activity appear to be normal in IL-7Rα-deficient patients. The characteristics of this disorder indicate that defects of IL-7/IL-7R signaling are responsible for the lack of T cells in γc- and JAK3-deficient patients. Patients with IL-7Rα deficiency and those with NK−SCID have similar clinical phenotypes, indicating that NK cells cannot compensate for the T-cell deficiency. IL-7Rα deficiency accounts for less than 10 % of all SCID cases [3, 14, 27].

Pre TCR and TCR Complex Deficiencies

Immunological reports of AR T−B+NK+SCID patients with CD3 expression deficiency were first published in 1986 and 1988 [62, 63]. The TCR is a heterodimer consisting of four CD3 chains: the γ, δ, ε, and ζ chains. The *CD3G*, *CD3D*, and *CD3E* genes map to chromosome 11q23 and *CD3Z* maps to chromosome 1. The first patients with CD3 deficiency to be described were characterized genetically in 1992, with the identification of homozygous mutations of the *CD3G* gene (OMIN 186740) [64]. AR CD3δ deficiency (OMIM 186790) was subsequently reported, in 2003, in a large Canadian kindred [65]. The first case of AR partial CD3ε deficiency (OMIM 186830) was reported in 1993 [66], and the first case of AR complete CD3ε deficiency was described in 2004 [67]. The first case of AR CD3ζ deficiency (OMIM 186780) was reported in 2006 in a patient with a homozygous germline mutation of *CD3Z* (CD247) and somatic mutations partially correcting the CD3ζ deficiency [68]. Up to 30 patients with CD3 deficiencies have been reported to date [10]. Patients with CD3δ deficiency, complete CD3ε deficiency, and CD3ζ deficiency have a clinical SCID phenotype, with susceptibility to infection and a complete absence of T-cell function. They account for 3 % of SCID cases [10, 68–70]. Patients with a homozygous mutation of the CD3δ gene have a profound deficiency of mature circulating CD3 T cells, no CD4 or CD8 T cells, and a total absence of γ/δ T cells [10, 65]. Complete CD3ε deficiency has been shown to result in a complete absence of mature TCR α/β and γ/δ cells [69]. Patients with CD3 deficiencies have either normal or higher than normal numbers of B cells and normal numbers of NK cells. Their lymphocytes do not respond to mitogens [65]. Hypomorphic mutations of *CD3E* and mutations of *CD3G*, result in a partial arrest of T-cell maturation and, therefore, CID, but not an

immunological phenotype of SCID with a mild decrease of T-cell activation in assays in vitro [69].

Another rare AR cause of human SCID, with a defect in the expression of CD45 at the cell surface of T cells, was reported in 1997 [71]. Compound heterozygous mutations of a gene encoding protein tyrosine phosphatase, receptor type, C (PTPRC gene) (OMIM 151460) were then identified in 2000, and a homozygous mutation was identified in one unrelated patient in 2001 [72, 73]. PTPRC is also known as the CD45 antigen. This hematopoietic cell-specific transmembrane protein tyrosine phosphatase regulates the Src kinases required for T- and B-cell antigen receptor signal transduction [74]. CD45-deficient patients have been reported to display signs of SCID, with very small numbers of T cells, but high numbers of B cells and normal NK-cell counts. The T cells of these patients fail to respond to mitogens. In 2011, patients with TCRα deficiency (OMIM 186880) were reported in two kindreds affected by CID; the affected patients displayed chronic viral infection, candidiasis, and autoimmunity [75]. Genetic linkage studies mapped the disorder to chromosomal region 14q11.2, and a homozygous mutation of the TRAC gene, encoding the α chain of the TCR was identified [75]. T cells from patients displayed profound impairment of the surface expression of the TCRα/β complex. In patients with T− or low B+NK+ immunological phenotypes, a careful study of TCR CD3 and CD45 molecule expression on residual T cells should be performed by flow cytometry before any genetic studies are undertaken.

PNP Deficiency

This last etiology of AR T−B+NK+SCID was identified by Giblett et al. in 1975, with the demonstration of an absence of the purine nucleoside phosphorylase (PNP) enzyme in affected patients [76]. The gene encoding PNP was mapped to chromosome 14q13.1 by somatic cell hybridization techniques and cloned [77, 78]. The first homozygous mutation of this gene was reported in 1987 [79]. Over 25 deleterious mutations have been identified in 60 families affected by PNP deficiency (OMIM 164050) [10]. PNP deficiency is caused by mutations in this gene, resulting in a marked accumulation of deoxyguanosine triphosphate (dGTP) with undetectable PNP enzyme activity in cells [10]. This dGTP accumulation inhibits the growth of T cells. PNP-deficient patients display T lymphopenia, but they have some residual T cells, accounting for their susceptibility to opportunistic infections being lower than that of patients with other forms of SCID [10]. These patients display a profound T-cell deficiency, with T-cell counts decreasing over time [80]. One third of patients develop autoimmune disease (autoimmune cytopenia and systemic lupus erythematosus). More than half

the known PNP-deficient patients also display neurological dysfunctions [80, 81], from spasticity to developmental delay. In patients with T−B+NK+SCID and neurological signs, PNP enzyme activity should be assessed in whole-blood cells if the patients have had a blood transfusion, or in leukocytes, before any genetic tests are carried out.

SCID with Athymia

FOXN1 Deficiency

AR severe T-cell immunodeficiency (T−B+NK+) (OMIM 300400), associated with congenital alopecia of the scalp, eyebrows, and eyelashes, and referred to as Nude/SCID (OMIM 601705), was clinically described in humans in 1996, in two Italian sisters [82]. This phenotype is the human equivalent of the murine phenotype reported by Flanagan in 1966 [83]. The genetic etiology of nude mice was identified in 1994. The causal gene encodes a member of the winged-helix domain family of transcription factors [84]. Initially known as winged-helix nude whn, this gene was subsequently renamed forkhead box n1 (FOXN1) [85]. FOXN1 is tissue-specific, being restricted to the thymus and skin [85]. In 1999, a homozygous R255X nonsense mutation of the FOXN1 gene was identified in the two patients with the Nude/SCID phenotype [86]. Two other unrelated patients were reported in 2011: a Portuguese patient with a homozygous R255X nonsense mutation and a French patient with a homozygous R320W missense mutation [87•]. The absence of the FOXN1 transcription factor results, both in mice and humans, in congenital athymia and hairlessness. This immune defect represents the first example of a SCID phenotype not primarily related to an abnormality intrinsic to hematopoietic cell, instead resulting from a particular change in thymic epithelial cells [88, 89]. In patients with T−B+NK+SCID with alopecia, the FOXN1 gene should to be sequenced.

Complete DiGeorge Syndrome

DiGeorge syndrome (DGS) was first described in the 1960s, in patients with T-cell deficiency (due to thymic hypoplasia), hypoparathyroidism, conotruncal heart defects, and facial dysmorphia [90]. In the 1980s, it was recognized that deletions of the long arm of chromosome 22 at position q.11 were most commonly associated with DGS (OMIM 188400) [91, 92]. DGS has a heterogeneous clinical phenotype, with variable expression of the different clinical features, including the immunodeficiency. DGS may be associated with anything from complete T-cell deficiency (T−B+NK+, complete DGS) to normal T-cell numbers. The T-cell immunodeficiency may be incomplete

(T low B+NK+), in which case the term partial DGS is used. The *TBX1* gene, which is located on chromosome 22q11, is involved in the thymus development impairment observed in DGS patients [93]. TBX1 is a T-box gene with an important role in regulating the expression of transcription factors. Studies of patients with complete DGS have provided insight into thymus development and the mechanisms of thymopoiesis required to generate broad T cell-mediated immunity [93]. In SCID T−B+NK+ patients with cardiac malformations, hypocalcemia, and dysmorphia, fluorescence in situ hybridization should be carried out to identify small deletions of chromosome 22q11.2.

Patients with a Clinical SCID Phenotype Without T-cell Lymphopenia

Several genetic defects have recently been identified in patients with severe recurrent infections, including opportunistic infections in particular, and in some patients with autoimmunity and lymphoproliferative disorders [10]. A first group of patients with severe recurrent infections have Ca^{2+} release-activated Ca^{2+} (CRAC) channelopathies, which were first described in 1995 [94]. These disorders are caused by AR deficiencies of ORAI1 (OMIN 610277) and STIM1 (OMIM 605921), which were identified in 2006 and 2009, respectively [95, 96]. Mutations of the *ORAI1* and *STIM1* genes in human patients are associated with a unique clinical phenotype characterized by severe immunodeficiency, muscular hypotonia, and anhidrotic ectodermal dysplasia [97]. The immunodeficiency in these patients is caused principally by a severe defect of T-cell activation, rather than of T-cell development [97]. The second group of patients have genetic defects impairing components of the TCR cell signaling pathway. AR ZAP70 deficiency (OMIM 269840) was first described in 1994 and LCK deficiency (OMIM 153390) was reported in 2012 [98–100]. ZAP70-deficient patients have profound T CD8 lymphopenia and LCK-deficient patients display profound T CD4 lymphopenia. Patients with ZAP70 or LCK deficiency have a major T-cell dysfunction, as their T cells are unable to respond to mitogens. The third group of patients has genetic defects impairing T- and B-cell activation and the NF-κB signaling pathway. These defects include the recently identified (in 2013) AR CARD11 deficiency (OMIM 615206), AR MALT1 deficiency (OMIM 604860), and AR IKK2 deficiency (OMIM 603258) [101, 102, 103••, 104]. CARD11 belongs to the CARD protein family, the members of which carry a characteristic caspase-associated recruitment domain. This protein forms a complex with BCL10 and MALT1. This complex forms an essential molecular link between the triggering of cell-surface antigen receptors and nuclear factor-kappa B activation [105]. AR CARD11 deficiency is characterized by hypogammaglobulinemia and a defect of memory B and T cells, and patients have no regulatory T cells or NKT cells [101, 102]. AR MALT1 deficiency is characterized by normal T- and B-cell counts, but low levels of T-cell proliferation [104]. I-kappa-B kinase 2 (IKK2, also known as IKKβ) is encoded by the *IKBKB* gene and is a component of the NF-κB pathway. IKK2 deficiency leads to hypogammaglobulinemia or agammaglobulinemia. The B and T cells of patients are almost exclusively of the naive phenotype, and regulatory T cells and Tγ/δ cells are absent [103••]. In cases of a clinical SCID phenotype, extensive immunophenotyping of naive and memory T and B cells, and assays of T-cell proliferation in vitro in response to mitogens (PHA, anti-CD3, and PMA+ionomcyn) should be carried out before genetic analysis.

Conclusion

In conclusion, inherited disorders of SCID can be classified into six groups (Table 1) on the basis of immunophenotyping and functional T-cell activation assays. These disorders are associated with mutations of a total of 27 genes. The first mutations were identified in 1985 (*ADA*). The number of recognized SCID disorders is expanding: in the last 10 years, ten new disease-causing genes have been identified. In pediatric practice, SCID should be suspected in the following situations: (i) children with multiple infections in the first year of life, (ii) children with recurrent respiratory infections, warts, neutropenia, and hypogammaglobulinemia, (iii) a lack of T cells and/or poorly functional T cells. Immunological studies are required before undertaking genetic analyses of SCID patients. Molecular characterization of these patients is an important issue for genetic counseling and the choice of the most appropriate treatment. Several countries are now developing newborn screening programs for the early identification of patients with SCID disorders. However, newborn screening based on TREC detection can only identify patients with few or no naive T cells, and patients with defects of T-cell activation will be missed. A careful clinical and immunological evaluation of patients presenting warning signs for SCID thus remains the cornerstone for diagnosis.

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