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Whole-exome sequencing of T$^-$B$^+$ severe combined immunodeficiency in Egyptian infants, JAK3 predominance and novel variants

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Summary

Severe combined immunodeficiency (SCID) is fatal if not treated with immune reconstitution. In Egypt, T$^-$B$^+$ SCID accounts for 38·5% of SCID diagnoses. An accurate genetic diagnosis is essential for choosing appropriate treatment modalities and for offering genetic counseling to the patient's family. The objectives of this study were to describe the clinical, immunological and molecular characteristics of a cohort of twenty Egyptian patients with T$^-$B$^+$ SCID. The initial diagnosis (based on clinical features and flow cytometry) was followed by molecular investigation (whole-exome sequencing). All patients had the classic clinical picture for SCID, including failure to thrive (n = 20), oral candidiasis (n = 17), persistent diarrhea (n = 14), pneumonia (n = 13), napkin dermatitis (n = 10), skin rash (n = 7), otitis media (n = 3) and meningitis (n = 2). The onset of manifestations was at the age of 2·4 ± 1·6 months and diagnosis at the age of 6·7 ± ·5 months, giving a diagnostic delay of 4·3 months. *JAK3* gene variants were most frequent (n = 12) with three novel variants identified, followed by *IL2Rγ* variants (n = 6) with two novel variants. *IL7Rα* and *CD3ε* variants were found once, with a novel variant each. T$^-$B$^+$NK$^-$ SCID accounted for approximately 90% of the Egyptian patients with T$^-$B$^+$SCID. Of these T$^-$B$^+$NK$^-$ SCID cases, 60% were autosomal recessive syndromes caused by *JAK3* mutations and 30% were X-linked syndromes. It might be useful to sequence the *JAK3* gene (i.e. targeted Sanger sequencing) in all T$^-$B$^+$ SCID patients, especially after X-linked SCID has been ruled out. Hence, no more than 10% of T$^-$B$^+$ SCID patients might require next-generation for a molecular diagnosis.

Keywords: *IL2Rγ*, JAK3, severe combined immunodeficiency, T$^-$B$^+$SCID, whole-exome sequencing

Introduction

Patients with severe combined immunodeficiency (SCID) lack functional T and B lymphocytes. This syndrome is fatal if not treated (with immune reconstitution) early in life [1].

Infants with SCID usually suffer from fatal opportunistic infections caused by bacteria, mycobacteria, cytomegalovirus or fungi. The non-infectious clinical manifestations include graft-*versus*-host disease (GVHD) following the engraftment of maternal lymphocytes or the transfusion of non-irradiated blood products. Some 'leaky' SCID patients present with atypical manifestations, such as immune cytopenia and/or granulomatous lesions [2]. Although more than 30 different mutated genes are involved in SCID phenotypes, the patients may be clinically indistinguishable [3]. Molecular diagnosis is important for selecting the optimal treatment modalities and providing genetic counseling – an essential part of the management of primary immunodeficiencies [4–6].

The Primary Immune Deficiency Treatment Consortium classifies SCID as T$^-$B$^-$NK$^-$, T$^-$B$^-$NK$^+$, T$^-$B$^+$NK$^-$ or T-B$^+$NK$^+$, depending on the B and natural killer (NK) cell counts [3]. During a 3-year period, 50·5% of the SCID cases in our tertiary center were T$^-$B$^-$, 38·5% were T$^-$B$^+$ and 11% corresponded to Omenn syndrome [7].

The tyrosine kinase Janus kinase 3 (*JAK*3) and *IL2RG* defects are the main genetic causes of $T^-B^+NK^-$ SCIDs, whereas complete *CD3D*, *CD3E*, *CD3Z* or *CD3G* defects and *IL-7RA* chain deficiency cause $T^-B^+NK^+$ SCIDs. *RAG1*, *RAG2* and *DCLRE1C* variants are the main genetic causes of $T\text{-}B^-NK^+$ SCIDs, while adenosine deaminase deficiency is the main genetic defect in $T\text{-}B^-NK^-$ SCID [8]. We have recently shown that major histocompatibility complex (MHC)-II-deficient patients may also present with T^-B^-SCIDs [9].

Janus kinase 3 is part of the Janus kinase family, which also includes *JAK1, JAK2* and *TYK2* [10]. It is essential for the downstream signaling of the common gamma chain subunit – a receptor for many cytokines, including interleukin (IL)-2R, IL-7R and IL-15R. Binding produces a conformational change in the receptor, which leads to clustering of the associated JAK proteins, tyrosine transphosphorylation and, ultimately, activation of the JAK kinase domains [11]. If T cell cytokines are unable to provide help, functional B cells do not develop in *JAK3* deficiency patients [MIM:600802], and so immunoglobulin production is reduced [10].

The IL-2 receptor gamma (*IL2Rγ*) is an important signaling component for many cytokines, including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. It is essential for the ontogeny and function of T cells and NK cells. Mutations in the *IL2RG* gene may result in X-linked severe combined immunodeficiency (X-SCID) [MIM:300400] [12]. *IL-7Rα* is one of the two subunits of IL-7 receptor and is crucial for IL-7 signaling. In the literature, more than 20 patients with autosomal recessive (AR) *IL-7RA* deficiency [MIM:146661] have been described. Their NK cells develop and function normally, but cannot compensate for the lack of T cells [13].

Here, we describe the clinical, immunological and molecular features of a cohort of 20 Egyptian patients with T^-B^+ SCID.

Materials and methods

This study included 20 SCID patients with a T^-B^+ phenotype evaluated in a tertiary primary immunodeficiency referral center at Cairo University Children's Hospital (Cairo, Egypt) between 2015 and 2019. The SCID was diagnosed according to the International Union of Immunodeficiencies criteria for primary immunodeficiencies [14] and the European Society for Immunodeficiencies (ESID) criteria [15]. In accordance with the principles of the Declaration of Helsinki, the patients' parents or legal guardians gave their informed consent. The study was approved by the local institutional review board. Details of each patient's personal medical history, clinical and laboratory findings were recorded.

Blood samples (3 ml) were drawn in ethylenediamine tetraacetic acid (EDTA) and plain vacutainers. Peripheral blood lymphocyte subsets were analyzed using flow cytometry, commercial fluorochrome-labeled monoclonal antibodies (mAbs) against CD3, CD4, CD8, CD19 and CD56, and standard staining techniques. All samples were analyzed on an FC500 flow cytometer (Beckman Coulter, Brea, CA, USA). Affected individuals with B cells but lacking peripheral T cells were diagnosed with $T\text{-}B^+$ SCID. Serum immunoglobulin levels were measured using Nephstar nephelometry (Goldsite Diagnostic Inc., Shenzhen, China).

For genetic studies, patients were enrolled in ongoing, institutional review board-approved studies of monogenic diseases (trial registration number: ClinicalTrials.gov. number, NCT02735824). Genomic DNA was extracted using a Qiagen DNAeasy kit (Qiagen, Hilden, Germany) from whole blood. Whole-exome sequencing (WES) using a HiSeq4000 system (Illumina, San Diego, CA, USA) was performed. The resulting high-quality reads sequences were aligned to the human genome (GRCh37) using Bowtie2 (version 2.3.2; Johns Hopkins University, Baltimore, MD, USA). Next, single-nucleotide variants and indels were detected by the use of GATK software version 3.7 (Broad Institute, Cambridge, MA, USA), according to the Broad Institute's best practice guidelines for human data (June 2016). Sequences were compared with the published reference sequence by the National Centre for Biotechnology Information. Sanger sequencing was performed to confirm all mutations detected by WES, as previously described by Lee *et al.* [2]. Patient 6 was diagnosed by Sanger sequencing only. The functional effects of the amino acid substitutions were predicted with the tools Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping version 2 (PolyPhen-2) and Combined Annotation Dependent Depletion (CADD).

Statistical methods

Quantitative variables were described as the mean ± standard deviation (s.d.) or median (range). Categorical variables were described as frequencies.

Results

Epidemiological features

We included 20 SCID patients (15 males and five females, from 19 different families) with a T^-B^+ SCID phenotype. Fifteen of the patients had first-degree consanguineous parents. The mean ± s.d. (range) age at onset of the manifestations was 2·4 ± 1·6 months (10 days–6 months), while the mean ± s.d. (range) age at diagnosis was 6·71 ± 3·5 months (7 days–14 months). This resulted in a mean diagnostic delay of 4·31 months. Only one patient (patient 4) was diagnosed by screening before the onset of any symptoms, due to a positive family medical history (as a sibling of patient 3). Twelve patients had affected

Table 1. Patients' clinical and demographic data

	Gender	Consanguinity	Affected siblings	Age at onset	Age at diagnosis	Presentation	Vaccination history	Outcome
1	♀	Positive	1 ♀ died at 9 months: pneumonia & meningitis	20 days	4 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea, hepatomegaly	BCG, OPV	Died at 4 months
2	♀	Positive	–	4 months	8 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea	BCG, OPV	Died at 8 months
3	♀	Positive	1 ♂ died at 4 months: pneumonia	1 month	3 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea, otitis media, meningitis, skin rash (brownish nodules)	BCG, OPV	Died at 4 months
4	♂	Positive	1 ♂ died at 4 months: pneumonia 1♀ died at 4 months: pneumonia & meningitis	__	7 days	Detected by screening	–	Successful BMT from fully matched sibling for 3.5 years
5	♂	Positive	–	1 month	6 months	Oral thrush, napkin dermatitis, diarrhea, skin rash (hypopigmented macules)	BCG, OPV	Died
6	♀	Positive	–	10 days	7 months	Oral thrush, pneumonia	BCG, OPV	Died
7	♂	Positive	–	4 months	10 months	Oral thrush, napkin dermatitis, pneumonia, skin rash (nodules), alopecia	BCG, OPV	Died at 14 months
8	♂	Positive	1 ♂ died at 3 months: pneumonia 1 ♀ died at 5 months: pneumonia	1 month	4 months	Oral thrush, diarrhea, otitis media, meningitis	–	Died at 14 months
9	♂	Positive	1 ♀ died: pneumonia	2 months	5 months	Pneumonia, diarrhea, hepatosplenomegaly, pancytopenia, skin rash (nodules)	BCG, OPV	Died at 7 months
10	♀	Positive	–	4 months	9 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea	BCG, OPV	Died
11	♂	Positive	–	1 month	7 months	Oral thrush, napkin dermatitis, pneumonia, hepatosplenomegaly, skin rash (hypopigmented macules), thrombocytopenia	BCG, OPV	Died
12	♂	Positive	1 ♂ died: pneumonia, diarrhea	4 months	5 months	Oral thrush, diarrhea, pneumonia, skin rash (brownish macules/papules)	BCG, OPV	Successful BMT from fully matched sibling for 2.5 years
13	♂	Negative	1 ♂ died at 40 days: sepsis	1 month	3 months	Oral thrush, napkin dermatitis	BCG, OPV	Died
14	♂	Negative	1 ♂ died at 3 months	2 months	5 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea	---	Died at 7 months
15	♂	Negative	–	6 months	9 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea, otitis media	BCG, OPV	Died at 10 months

Table 1. (Continued)

	Gender	Consanguinity	Affected siblings	Age at onset	Age at diagnosis	Presentation	Vaccination history	Outcome
16	♂	Negative	1 ♂ died at age of 8 months	4 months	14 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea, skin rash (brownish macules), alopecia, pancytopenia	BCG, OPV	Died
17	♂	Positive	1 ♂ died at 6 months: pneumonia	3 months	8 months	Oral thrush, napkin dermatitis, diarrhea, thrombocytopenia	BCG, OPV	Died
18	♂	Negative	1 ♂ died at 8 months: pneumonia, bleeding per rectum	4 months	11 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea	BCG, OPV	Successful BMT from a fully matched sibling for 2.5 years
19	♂	Positive	–	1 month	7 months	Oral thrush, napkin dermatitis, pneumonia, diarrhea	BCG, OPV	Died
20	♂	Positive	♀ died at 1 year: pneumonia ♂ died at 8 months: BCGiosis, pneumonia	2 months	9 months	Oral thrush, diarrhea, pneumonia	BCG, OPV	Died

M = months, BCG: bacillus Calmette–Guérin; OPV = oral polio vaccine; BMT = bone marrow transplant.

siblings; six males (four born to non-consanguineous parents and two born to consanguineous parents) had a history of male sibling death, and four males (born to consanguineous parents) had a history of both female and male sibling death. The patients' demographic characteristics and family pedigrees are summarized in Table1 and Fig. 1, respectively.

Clinical characteristics

The most common clinical manifestations were failure to thrive (n = 20, 100%; all patients were below the third percentile for age, followed by oral candidiasis (n = 17, 85%), persistent diarrhea (n = 14, 70%), pneumonia (n = 13, 65%), napkin dermatitis (n = 10, 50%), skin rash in the form of nodules or hypopigmented macules (n = 7, 35%) and otitis media (n = 3, 15%). Two patients (10%) presented with meningitis, and one of these cases was complicated by hydrocephalus (Fig. 2).

With regard to the vaccination history, 17 patients had received their scheduled bacillus Calmette–Guérin (BCG) vaccine and oral polio vaccine (OPV) without any adverse events, while three patients had not been vaccinated in view of a family history of SCID.

Laboratory characteristics

Most of the patients had the typical features of SCID according to the ESID diagnostic criteria [15], i.e. fewer than 20% of CD3 T lymphocytes and an absolute lymphocyte count below 3000/mm^3. All patients had low immunoglobulin titers. Two patients had associated pancytopenia, and two other patients had associated thrombocytopenia.

Genetics

A genetic analysis revealed that 12 patients (60%) had variants in the *JAK3* gene, six patients (30%) had *IL2RG* variants, one patient (5%) had a *IL7RA* variant and one patient (5%) had a *CD3E* variant (Fig. 3).

Two patients had a $T^-B^+NK^+$ phenotype (one with an *IL7RA* mutation and the other with a *CD3E* mutation). With the exception of patient 8 (with a normal proportion of NK cells but a low absolute count), all *JAK3*-deficient patients had a $T^-B^+NK^-$ phenotype. All the *IL2RG* SCID patients had a $T^-B^+NK^-$ phenotype, except for patients 13 (who had normal proportion of NK cells and a normal absolute count) and 16 (who had a normal proportion of NK cells but a low absolute count).

Sixteen different gene variants were detected, including six previously reported *JAK3* variants and three novel *JAK3* variants [an intronic splice donor site variant c.1142+1G>A in four patients, an in-frame deletion c.3011_3013delTCT (p.Phe1004del) in two patients and a missense variant c.1027G>C (p.Ala343Pro) in one patient]. There were three previously reported *IL2RG* gene variants and two novel

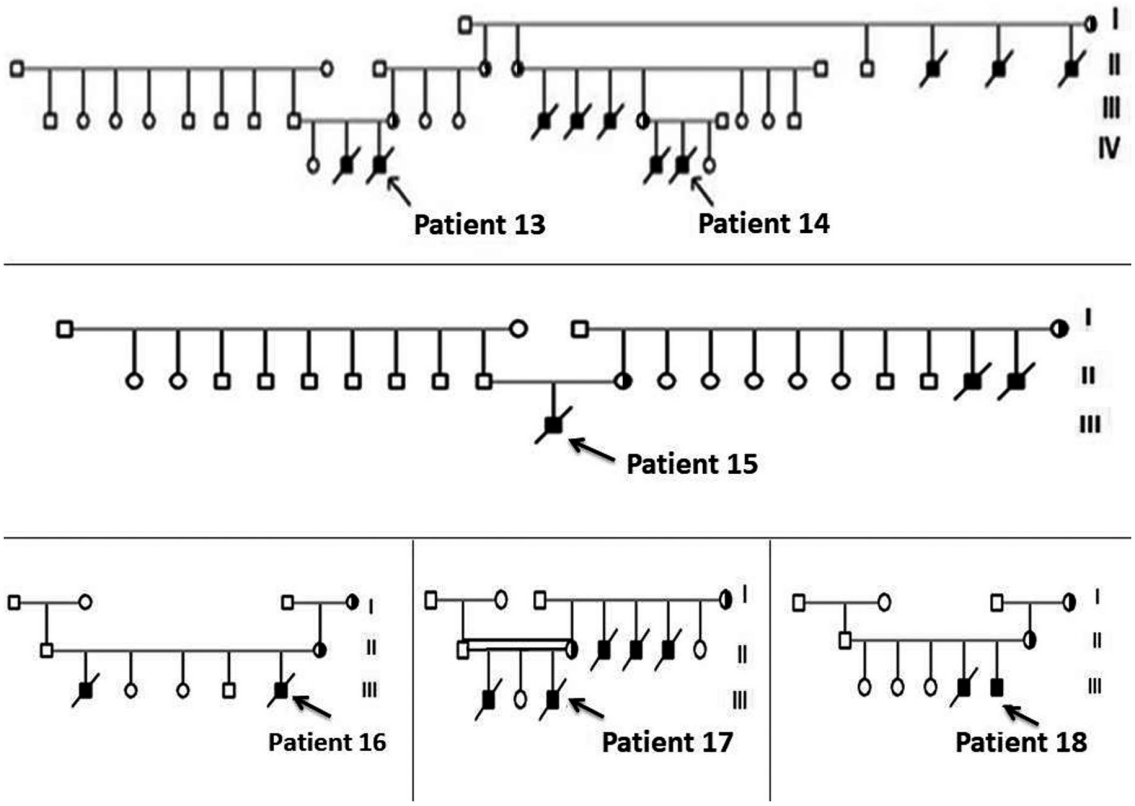

Fig. 1. The family pedigree of the studied X-severe combined immunodeficiency (SCID) patients. Index cases are designated by arrows, double lines indicate consanguinity, shaded squares indicate affected males, half-shaded circles indicate carrier females, inclined lines indicate being deceased.

variants: a missense variant c.115G>C (p.Asp39His) and an intronic splice site variant c.924+2T>G. We also detected a novel frame-shift deletion c.482_483delAA (p.Lys161fs) in an *IL7RA*-deficient patient and a novel non sense variant c.269T>A (p.Leu90Ter) in a *CD3E*-deficient patient. The patients' laboratory findings, immunophenotype data and genetic diagnosis are summarized in Table 2.

During the study period, three patients underwent hematopoietic stem cell transplantation (HSCT) with fully matched donors (female siblings) in the absence of conditioning. Two to 3 years later, all three patients are alive and well. Post-HSCT chimerism was determined with fluorescence *in-situ* hybridization for sex-disparate recipients and donors. Mixed chimerism was defined as the presence of 5–95% of donor-derived cells. The remaining 17 patients (85%) died before suitable fully matched donors could be found.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Discussion

To the best of our knowledge, the present study is the first to provide a comprehensive description of the clinical, laboratory and molecular features of a large series of patients with T^-B^+ SCID in Egypt. Our study revealed that most cases of T^-B^+ SCID had AR causes (mainly *JAK3* gene mutations) related to high rates of consanguinity. These findings contrast with American and European reports, in which X-SCID accounts for 25–46% of SCID cases and *JAK3* deficiency accounts for 10–18% of cases [13]. Similarly, the largest report from China and South East Asia reported that the most common genotype is an *IL2RG* deficiency (n = 19 of 21 males), followed by *JAK3* (n = 2) and *IL7RA* (n = 2) deficiencies [2].

The mean ages at symptom onset and at diagnosis were 2·4 ± 1·6 and 6·71 ± 3·5 months, respectively, and the mean diagnostic delay was 4·31. The lag of diagnosis for several months might be due to (i) non-specific clinical presentations, (ii) the attribution of initial laboratory findings to malnutrition and/or common infections; or (iii) poor access to specialized diagnostic services (notably neonatal T cell receptor excision circle screening programs for SCIDs identification is not applied) [17].

None of the patients having received the BCG vaccine or the OPV developed related complications; this contrasts with Shahbazi *et al.*'s observation [18] of BCG-related complications in 40% of their patients. The latter

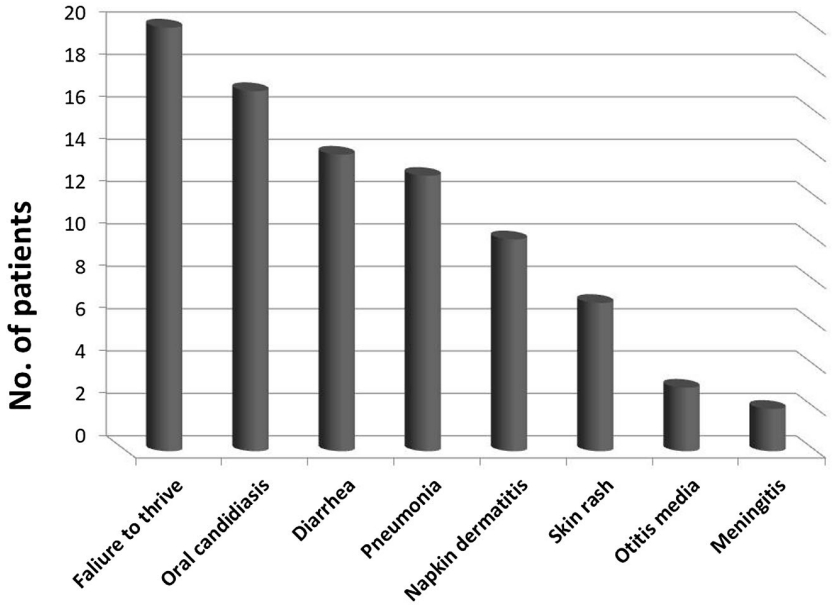

Fig. 2. Bar chart showing the clinical manifestations in the T^-B^+ severe combined immunodeficiency (SCID) patients.

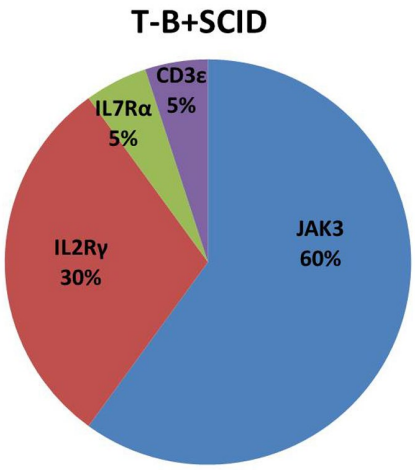

Fig. 3. Pie chart representing the genetic cause of the studied T^-B^+ severe combined immunodeficiency (SCID) patients.

researchers reported that BCGosis was the first symptom in 31·4% of the patients, and that 7% of tested cases shed poliovirus. Similarly, Lee *et al.* [2] found local and disseminated BCG disease in 23·8% of infants with SCID. In an earlier report from Egypt, Galal *et al.*'s study of 130 patients with a primary immunodeficiency [19] found that five confirmed SCID patients excreted vaccine-derived polioviruses in their stools; only one of these cases was $T\text{-}B^+$ SCID, and his genetic defect had not been determined. These conflicting results might be due to the patients' death at a young age, i.e. before the appearance of vaccine-related complications.

Most of the patients in our study had typical clinical manifestations and laboratory findings, including lymphopenia and low immunoglobulin levels.

JAK3 deficiency was the most common cause of autosomal recessive $T\text{-}B^+NK^-$ SCID in our study cohort. Until 2017, only 59 *JAK3*-deficiency patients had been described in the literature [10]. The incidence of *JAK3*-SCID appears to be higher in Egypt than in other countries on the Mediterranean sea; for example, an Italian study reported that only four *JAK3*-SCID patients were detected (using a variety of molecular techniques) during a 10-year period [20]. The majority of reported *JAK3*-SCID patients were compound heterozygotes [20]. However, due to the high rate of consanguinity among Egyptians, 91·6% of the *JAK3* variants described here were homozygous.

JAK3 is comprised of seven domains (JH1 to JH7). The pathogenic variants reported to date are scattered throughout several domains [21]. Patient 1 had a variant Arg103His in the JH7 domain, patient 2 had a variant Ala343Pro in JH5 domain and patients 3, 4, 5 and 6 had an intronic splice donor site variant c.1142+1G>A. The N-terminal JH5, JH6, and JH7 domains are required for binding the gamma chain and for regulating the protein's catalytic activity [11].

Patients 8 and 9 had non-sense variants in JH3, leading to truncation of the protein at amino acid 451 and 458, respectively. Patient 7 had two homozygous variants, including Arg403Cys in the JH4 domain. The functions of the JH3 and JH4 domains have not been determined, although the presence of SH2-like domain homology suggests that they are important for protein–protein interactions [10].

Table 2. Patients' laboratory data

	Lymphocytic count%/ (absolute value)	CD3%/ (absolute value)	CD19%/ (absolute value)	CD56%/ (absolute value)	CD4%/ (absolute value)	CD8%/ (absolute value)	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	Molecular diagnosis[d]	SIFT[e]	PolyPhen[f]	CADD[g]	Previous report
1[a]	4% (788)	0·2% (1·5)	66% (520)	1·5% (12)	0·2% (1·5)	0% (0)	281	42	< 17·9	*JAK3* homozygous Missense c.308G>A; p.Arg103His	0·000	1	15·61	rs774202259
2[b]	38% (4522)	1·3% (58)	63% (2848)	0·9% (41)	0·3% (13·5)	0·1% (4·5)	< 149	< 9·7	< 17·9	*JAK3* homozygous Missense c.1027G>C; p.ALA343Pro	0·003	0·954	19·13	Novel
3[a]	0·5% (100)	0·3% (0·3)	80% (80)	1·6% (1·6)	0·1% (0·1)	0·1% (0·1)	< 149	21	< 17·9	*JAK3* homozygous Intronic (splice site donor variant) c.1142+1 G>A	n.a.	n.a.	18·34	Novel
4[b]	19% (1200)	0·8% (9·6)	81·8% (982)	0·8% (9·6)	0·2% (2·4)	0·1% (1·2)	n.a.	n.a.	n.a.	*JAK3* homozygous Intronic (splice site donor variant) c.1142+1 G>A	n.a.	n.a.	18·34	Novel
5[a]	8% (696)	0·6% (4)	83·1% (578)	2·5% (17)	0·3% (2)	0% (0)	< 149	106	< 17·9	*JAK3* homozygous Intronic (splice site donor variant) c.1142+1 G>A	n.a.	n.a.	18·34	Novel
6[b]	63% (5796)	0·4% (23)	97·7% (5663)	2·1% (122)	0·3% (17·3)	0 (0)	340	25	12	*JAK3* heterozygous Intronic (splice site donor variant) c.1142+1G>A	n.a.	n.a.	18·34	Novel
										JAK3 Heterozygous Missense c.1765G>A, p.Gly589Ser	0·000	1	32	rs886039394
7[b]	17·4% (2000)	6·1% (122)	75·5% (1510)	1·8% (36)	4·8% (96)	1·2% (24)	< 149	23	< 17·9	*JAK3* homozygous Missense c.1207C>T; p.Arg403Cys	0·492	0·108	23·7	rs1257606008
										JAK3 Homozygous Missense c.2164G>A; p.Val722Ile	0·029	0·001	8·424	rs3213409
8[a]	52% (1612)	3·3% (53)	79·7% (1285)	7·7% (124)	1% (16)	1·1% (18)	149	13	5	*JAK3* homozygous Stop gained c.1351C>T; p.Arg451Ter	n.a.	n.a.	35	rs267605358
9[a]	19% (1370)	0·5% (7)	81·5% (1117)	1·6% (22)	0·5% (7)	0% (0)	149	45·3	23	*JAK3* homozygous Stop gained c.1374G>A ; p.Trp458Ter	n.a.	n.a.	35	rs1467541371
10[b]	41% (2378)	3·5% (83)	90·5% (2152)	2·6% (62)	0·2% (5)	0·7% (17)	155	< 9·7	20·4	*JAK3* homozygous Missense c.1765G>A; p.Gly589Ser	0·000	1	32	rs886039394
11[b]	38% (3300)	0·3% (10)	72% (2376)	2·6% (86)	0·1% (3)	0·1% (3)	149	17·2	< 17·9	*JAK3* homozygous In-frame deletion c.3011_3013delTCT; p.Phe1004del	n.a.	n.a.	n.a.	Novel
12[b]	30% (1560)	1·2% (19)	90·8% (1416)	0·2% (4)	0·1% (2)	0·1% (2)	445	18·5	< 17·9	*JAK3* homozygous In-frame deletion c.3011_3013delTCT; p.Phe1004del	n.a.	n.a.	n.a.	Novel

Table 2. (Continued)

	Lymphocytic count%/ (absolute value)	CD3%/ (absolute value)	CD19%/ (absolute value)	CD56%/ (absolute value)	CD4%/ (absolute value)	CD8%/ (absolute value)	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	Molecular diagnosis[d]	SIFT[e]	PolyPhen[f]	CADD[g]	Previous report
13 [a]	51% (6466)	1·5% (97)	80·1% (5179)	3·3% (213)	0·5% (32)	1% (65)	186	18·7	< 17·9	*IL2Rγ* hemizygous Missense c.2T>C; p.Met1Thr	0·000	0·421	21·5	rs886041334
14 [a]	60% (2580)	0·3% (8)	92·7% (2391)	1% (3)	0% (0)	0·3% (8)	< 149	< 9·7	< 17·9	*IL2Rγ* hemizygous Missense c.2T>C; p.Met1Thr	0·000	0·421	21·5	rs886041334
15 [b]	52% (624)	2·2 % (14)	83·3 % (517)	0·6 % (4)	1·8 % (11)	0·4 % (3)	149	n.a.	< 17·9	*IL2Rγ* hemizygous Missense c.115G>C; p.Asp39His	0·009	1	22·4	Novel
16[c]	15% (570)	4·7% (27)	30·5% (173)	5·3% (30)	3% (17)	1·2% (7)	149	24·8	n.a.	*IL2Rγ* hemizygous Missense c.677G>A; p.Arg226His	0·005	1	22·4	rs869320660
17 [b]	19% (1615)	0% (0)	84·5% (1365)	2% (32)	0% (0)	0% (0)	n.a.	n.a.	n.a.	*IL2Rγ* hemizygous stop codon c.865C>T; p.Arg289Ter	n.a.	n.a.	22·4	rs137852508
18 [c]	50% (850)	32·1% (272)	46·1% (392)	1·6% (14)	6·8% (58)	10·2% (87)	149	102	< 17·9	*IL2Rγ* hemizygous intronic c.924+2T>G	n.a.	n.a.	17·25	Novel
19 [b]	19% (893)	0·3% (3)	73 % (652)	11·2 % (100)	0 % (0)	0 % (0)	< 149	< 9·7	17·9	*IL7Rα* homozygous Frameshift deletion c.482_483delAA; p.Lys161serfsTer14	n.a.	n.a.	n.a.	Novel
20 [b]	36% (2484)	2·9% (72)	53·5% (1329)	14% (348)	0% (0)	2·9% (72)	180	36	8	*CD3ε* homozygous stop gained c.269T>A; p.Leu90Ter	n.a.	n.a.	24·6	Novel

SIFT = ; Ig = immunoglobulin; n.a. = not available.

Immunophenotyping reference level according to Shearer *et al.* (16) for [a]age 3–6 months (lymphocytes 3900–9000, CD3 (51–77%)/(2500–5600), CD19 (11–41%)/(430–3000), CD56 (3–14%)/(170–830), CD4 (35–56%)/(1800–4000), CD8 (12–23%)/(590–1600).

[b]Age 6–12 months (lymphocytes 3400–9000, CD3 (49–76%)/(1900–5900), CD19 (14–37%)/(610–2600), CD56 (3–15%)/(160–950), CD4 (31–56%)/(1400–4300), CD8 (12–24%)/(500–1700).

[c]Age 1–2 years (lymphocytes 3600–8900, CD3 (53–75%)/(2100–6200), CD19 (16–35%)/(720–2600), CD56 (3–15%)/(180–920), CD4 (32–51%)/(1300–3400), CD8 (14–30%)/(620–2000).

[d]RefSeq: Janus kinase (JAK)3(NM_000215.4), interleukin (IL)-2RG (NM_000206.3), CD3E (NM_000733.4), IL-7RA (NM_002185.5).

[e]Sorting Intolerant From Tolerant (SIFT) prediction cut-off = 0·05, amino acid substitution is predicted damaging if ≤ 0·05 and tolerated if score > 0·05.

[f]Polymorphism Phenotyping version 2 (PolyPhen-2) score, variants with score of 0 are predicted as benign, values closer to 1 are predicted to be deleterious.

[g]Combined Annotation Dependent Depletion (CADD) score greater or equal 10 indicates the prediction from the 10% most deleterious substitutions, score greater or equal 20 indicates the 1% most deleterious.

A nearby variant (Pro402Leu) was described by Qamar *et al.* [11] in a patient with SCID complicated by vertebral osteomyelitis; although steric clashes with surrounding residues are not predicted, this variant might disrupt JAK3's interaction with other proteins.

Patients 10 and 6 had a variant Gly589Ser in the JH2 domain. This variant was previously reported by Roberts *et al.* 2004 [1]. Also, patient 7 had a variant Val722Ile in the JH2 domain that had been previously reported in a homozygous phenotype in a patient from Iran [18] and in a patient with compound heterozygote phenotype in the United States [1]. The JH2 pseudokinase domain is catalytically inactive, but influences the activity of various JAK proteins. The domain might interact directly with STAT proteins, and modulates JAK3's catalytic activity [10,11,21].

The C-terminal JH1 domain constitutes the kinase domain [11]. Patients 11 and 12 had an in-frame deletion in this domain, leading to deletion of the phenylalanine at position 1004.

With regard to *IL2RG*, approximately 10% of the reported mutations have been linked to atypical phenotypes with a 'milder' immunodeficiency. The IL-2RG comprises eight exons, with nearly half of all documented mutations located in exon 5 (29·4%) or exon 3 (19·9%), whereas mutations in exons 7 and 8 are rare [12]. In contrast to previous reports, we found a high proportion of patients with variants in exon 1, and one patient with a variant in each of exons 2, 3 and 7. Patients 13 and 14 had a missense initiator codon variant c.2T>C in exon 1 (described as pathogenic in the ClinVar database). The variants Asp39His and Arg226His found in patients 15 and 16 affect the extracellular domain responsible for proper protein folding, intracellular transport and extracellular receptor binding [12]. Patient 17 had truncated protein at amino acid 289, and patient 18 had an intronic splice site variant at c.924+2T>G; both these variants affect the receptor's cytoplasmic domains and might affect the JAK3 binding site [12]. The Arg289Ter and Arg226His *IL2R*G variants have already been reported by Puck *et al.* [22]. The latter researchers also reported a nearby variant c.924+1G>A, which was found to destroy the canonical splice donor site in intron 7 and thus led to abnormal gene splicing.

Only patient 19 (with the classic $T^-B^+NK^+$ phenotype) had a small frame-shift deletion variant c.482_483delAA in *IL7R*A. The resulting frame-shift in the amino acid sequence started from position 161, which is located in the receptor's extracellular domain. Previous reports in contrast showed that (i) most IL-7RA variants were base substitution variants, and (ii) small deletions in the coding region were rare and usually associated with another heterozygous missense mutation in the *IL7RA* gene [23,24].

T lymphocyte differentiation in the thymus requires appropriate signals from the pre-T cell receptor (TCR) and/or the TCR (TCR-α/β or TCR-γ/δ) [25]. The pre-TCR, TCR-α/β and TCR-γ/δ bind to three signal-transducing complexes; namely, the CD3δε and CD3γε heterodimers and the disulfide-linked CD3ζζ [26]. The nine-exon gene coding for the T cell antigen receptor epsilon subunit (CD3E) is located at 11q23.3 and is responsible for the autosomal recessive early-onset immunodeficiency [MIM:615615] SCID phenotype [25]. Patient 20 had a novel non-sense variant (c.269T>A) in the CD3E gene, leading to protein truncation at amino acid 90.

Conclusion

The $T^-B^+NK^-$ form accounts for around 90% of the Egyptian cases of T^-B^+ SCID. Approximately 30% of T^-B^+ SCIDs are X-linked, and can be suspected in male patients by analyzing the family's pedigree and medical history. Approximately 60% of T^-B^+ SCIDs are due to AR *JAK3* mutations. In view of the lack of suitable stem cell donors, and thus a high mortality rate, the genotype must be analyzed as early as possible. This enables prenatal diagnosis for families with affected infants and facilitated counseling options – at least until the day when HSCT can be offered to all patients with SCID. *JAK*3 gene mutations are frequent among Egyptian patients with SCID; it might be useful to sequence the *JAK3* gene (i.e. with targeted Sanger sequencing) in all T^-B^+ Egyptian SCID patients, especially after X-linked SCID has been ruled out. Hence, no more than 10% of T^-B^+ SCID patients might require next-generation sequencing or whole-exome sequencing for a molecular diagnosis. Based on a patient's clinical and immunological features, the choice of an appropriate, available genetic test can accelerate the molecular diagnosis in a cost-effective way and thus improve the management of T^-B^+ SCID.

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Disclosures

The authors declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study is available from the corresponding author upon reasonable request.

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