


# Mutational landscape of severe combined immunodeficiency patients from Turkey

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## Abstract

Severe combined immunodeficiency (SCID) has a diverse genetic aetiology, where a clinical phenotype, caused by single and/or multiple gene variants, can give rise to multiple presentations. The advent of next-generation sequencing (NGS) has recently enabled rapid identification of the molecular aetiology of SCID, which is crucial for prognosis and treatment strategies. We sought to identify the genetic aetiology of various phenotypes of SCIDs and assessed both clinical and immunologic characteristics associated with gene variants. An amplicon-based targeted NGS panel, which contained 18 most common SCID-related genes, was contumely made to screen the patients ( $n = 38$ ) with typical SCID, atypical SCID or OMENN syndrome. Allelic segregations were confirmed for the detected gene variants within the families. In total, 24 disease-causing variants (17 known and 7 novel) were identified in 23 patients in 9

different SCID genes: *RAG1* ( $n = 5$ ), *RAG2* ( $n = 2$ ), *ADA* ( $n = 3$ ), *DCLRE1C* ( $n = 2$ ), *NHEJ1* ( $n = 2$ ), *CD3E* ( $n = 2$ ), *IL2RG* ( $n = 3$ ), *JAK3* ( $n = 4$ ) and *IL7R* ( $n = 1$ ). The overall success rate of our custom-made NGS panel was 60% (39.3% for NK+ SCID and 100% for NK-SCID). Incidence of autosomal-recessive inherited genes is more frequently found in our cohort than the previously reported populations probably due to the high consanguineous marriages in Turkey. In conclusion, the custom-made sequencing panel was able to identify and confirm the previously known and novel disease-causing variants with high accuracy.

#### KEYWORDS

Primary immunodeficiency, SCID, targeted next-generation sequencing

## 1 | INTRODUCTION

Primary immunodeficiencies (PIDs) are a group of disorders that can present with a wide spectrum of manifestations, including an undue susceptibility to infections, severe allergies, autoimmunity, malignancy and excessive inflammation (Notarangelo, 2010). Severe combined immunodeficiency (SCID) represents the most severe form of PIDs, which is caused by monogenic (mostly autosomal-recessive or X-linked) defects that impair the development and/or function of lymphocytes (Parvaneh, Casanova, Notarangelo, & Conley, 2013). Typical SCIDs are defined by a complete loss of T lymphocytes with or without accompanying defects of the B and/or NK cells. Occasionally, hypomorphic variants of the genes whose null mutations are responsible from the classical SCID phenotypes can lead to a disorder where T-cell differentiation is partially maintained, referred to as "atypical SCID" (Fischer, Notarangelo, Neven, Cavazzana, & Puck, 2015; Shearer et al., 2014).

If remained untreated, SCID is a deadly disease, with most fatalities occurring within the first year of life. SCID patients can be diagnosed early with detailed clinical evaluation, immunophenotyping and molecular analysis. Early diagnosis is the key to the successful management of SCID, whereas failure to diagnose in time will lead to delay in hematopoietic stem cell transplantation (HSCT).

Incidence of SCID was found to be 1/58,000 in the United States, quite higher than once thought (1/100,000) based on retrospective clinical diagnoses (Kwan et al., 2014). Further, despite the lack of robust epidemiologic data, it is expected that the incidence of SCID would be even higher among countries with high consanguinity rates like Turkey (Kilic et al., 2013; Yorulmaz, Artaç, Kara, Keleş, & Reisli, 2008).

To date, mutations in more than 30 genes are related to SCID pathogenesis, caused by different mechanisms that affect various steps in T-cell development. Apoptosis of hematopoietic progenitor cells is observed in reticular dysgenesis disease (AK2) and in adenosine deaminase (ADA) enzyme deficiency. Defects of *IL2RG*, *JAK-3* and *IL7RA* genes cause impairments in cytokine-induced signalling in T cells and NK progenitor cells. TCR complex defects (*CD45*, *CD3D*, *CD3E* and *CD3Z*) are critical for thymus development and mutations lead to the absence of thymus and T cells. In addition, mutations in V(D)J rearrangement and DNA repair mechanisms (*RAG-1*, *RAG-2*, *NHEJ*, *DCLRE1C* and *PRKDC*) genes block T- and B-cell development.

(Cirillo et al., 2019; Fischer et al., 2015; Notarangelo, 2010; Rivers & Gaspar, 2015; Schumacher & Notarangelo, 2002).

Complex phenotypes and an increasing number of responsible genes make the genetic diagnosis difficult (Picard et al., 2015). Whole exome and genome sequencing (WES and WGS) are powerful tools to identify novel disease-related genes/pathways, whereas targeted sequencing approaches have been used with success in the identification of deleterious gene variants on known genes as well as determining the genotype-phenotype correlation for diagnostic purposes (Fang, Abolhassani, Lim, Zhang, & Hammarstrom, 2016). The diverse presentations of PIDs and variable phenotype-genotype correlations require a multifactorial approach for the accurate and timely diagnosis of these potentially fatal conditions. Moreover, targeted next-generation panels have offered a cost-effective and rapid diagnostic tool for clinical use. Choosing the right enrichment strategies in the custom-designed targeted panels is critical for accurate and high-quality sequencing reads (Stoddard, Niemela, Fleisher, & Rosenzweig, 2014). Since probe-based targeted sequencing has some limitations, mainly due to the inability to avoid pseudogenes and genomic repeats, a combination of PCR and probe enrichment strategies has been developed (Yu et al., 2016). The requirement for expanding target size leading to a harder optimization process and higher primer cost, PCR-based (amplicon) enrichment methods still have the best sequencing specificity (De Wilde et al., 2014; Mamanova et al., 2010).

This study aimed to determine the genetic background of SCID by using a custom-designed amplicon-sequencing panel for SCID patients. In this manuscript by using the custom-made sequencing panel, we were able to identify and confirm the previously known and novel disease-causing variants with high accuracy confirm the familial segregations and evaluated the genotype-phenotype correlations for the novel variants in SCID.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

We studied 40 patients with SCID, 38 of whom were newly diagnosed at the time of study inclusion (21 boys and 17 girls) whereas

two were previously known cases. Patients were classified as typical SCID ( $n = 36$ ), atypical SCID ( $n = 3$ ) or Omenn Syndrome ( $n = 1$ ) based on the diagnostic criteria recommended by the European Society for Immunodeficiencies (ESID, ESID.org, ESID Registry—Working Definitions for Clinical Diagnosis of PID). A diagnosis of typical SCID was made in a child who presents with suggestive clinical findings in the first year of life, plus 2 out of 4 T-cell criteria is satisfied; low or absent CD3 or CD4 or CD8 T cells, reduced naive CD4 and/or CD8 T cells, elevated g/d T cells, reduced or absent proliferation to mitogen or TCR (T-cell receptor) stimulation. Clinical criteria for atypical SCID included identification of a mutation in a SCID-causing gene, plus  $> 100$  T cells/ $\mu\text{l}$ , plus absence of characteristic SCID-associated infections in the first year of life, plus lack of clinical criteria for OMENN syndrome. Omenn syndrome was diagnosed if he/she presents with desquamating erythroderma in the first year of life, plus presence of one or more of the following findings; lymphoproliferation, failure to thrive, chronic diarrhoea, recurrent pneumonia findings plus eosinophilia or elevated IgE, plus T-cell deficiency (low naive cells, reduced proliferation and oligoclonality), plus no maternal engraftment, plus exclusion of HIV infection.

Our cohort included six patients with T<sup>-</sup> B<sup>+</sup> NK<sup>+</sup> phenotype, ten patients with T<sup>-</sup> B<sup>+</sup> NK<sup>+</sup> phenotype, five patients with T<sup>-</sup> B<sup>-</sup> NK<sup>-</sup> phenotype and nineteen T<sup>-</sup>B<sup>-</sup>NK<sup>+</sup> patients. Median age was 6 months (min 1.5 months–max 13 years) except one 30-year-old atypical SCID patient. Median ALC was 1,300 (min 40–max 7,300). Seventy-seven per cent (31 in 40) of the families of SCID cases were consanguineous. Maternal engraftment was checked in all atypical SCID patients, and no maternal engraftment was seen. Development delay was seen in 60% of our patients. 24 patients underwent HSCT, and only four of them deceased.

Detailed clinical features of the patients were given at Table 1. This study was approved by the ethical board of Istanbul Medical Faculty (2015/492), and written informed consents were obtained from all parents or legal representatives.

## 2.2 | Amplicon-based targeted next-generation sequencing

The NGS SCID panel was custom designed by SmartChip-TE technology (WaferGen). It consists of 432 amplicons covering the coding regions, promoters and untranslated region (UTR) sites of 18 most seen SCID-related genes (*IL2RG*, *JAK3*, *IL7RA*, *PTPRC*, *CD3D*, *CD3E*, *CD3Z*, *CORO1A*, *DCLRE1C*, *PRKCD*, *AK2*, *ADA*, *RAG1*, *RAG2*, *XLFI/NHEJ1*, *LIG4*, *PNP* and *ZBTB24*) with a target size of 167,092 base pair. Candidate genes were selected based on the 2014 IUIS (International Union of Immunological Societies) SCID classification (Al-Herz et al., 2014). TLEN (observed Template LENgth) median was 386 with the minimum 250 and maximum 450 base pair. Chip capacity was 12 samples per run. Genomic DNA was isolated from the peripheral blood mononuclear cells of the patients and family members by using QIAmp DNA Blood Mini Kit (Qiagen). Quantity and quality of DNA were determined by using Nanodrop 2000 (Thermo

Fisher Scientific). Enrichment of target regions was performed by Seq-Ready™ TE Multisample NanoDispenser according to manufacturers' protocol. Quantification of all libraries was done by Quant-iT PicoGreen dsDNA Assay Kit with LC480 (Roche Diagnostics). Samples were sequenced with Illumina Miseq reagent V2 kit on Illumina Miseq (Illumina) sequencer. Two SCID patients with four known variants (SC01 with *RAG1* and SC31 with *ADA* compound heterozygosity) were used as control samples to validate our targeted sequencing approach and the pipeline of data analysis. Variant validation in patients and allelic segregation analysis in families were performed using Sanger sequencing.

## 2.3 | Data analysis and functional interpretation

Mapping to a reference genome (hg19), variant calling and annotation were performed by using two different programs; SEQ 2.8.2 (Genomize) and Seqnext (JSI Medical systems GmbH). To predict the severity of variants, open source programs such as SIFT (Kumar, Henikoff, & Ng, 2009), Polyphen (Adzhubei et al., 2010), Variant effect predictor (VEP) (McLaren et al., 2016), Mutation Taster (Schwarz, Cooper, Schuelke, & Seelow, 2014) and CADD (Combined Annotation Dependent Depletion) (Kircher et al., 2014) were used. All the variants were classified by the standards and guidelines for the clinical interpretation of sequence variants with respect to human diseases according to ACMG/AMP 2015 guideline based on 28 criteria (Richards et al., 2015).

Multiple alignments were generated by UCSC. The available public databases including dbSNP database (<https://www.ncbi.nlm.nih.gov/SNP/>), 1,000 Genomes Project samples (<http://www.1000genomes.org/>), the Exome Variant Server (ESP) (<http://evs.gs.washington.edu/EVS/>), The Human Gene Mutation Database (HGMD) (<http://www.hgmd.cf.ac.uk/ac/index.php>) database, the Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>) and GNOMAD (<https://gnomad.broadinstitute.org>) were used for frequency data and to predict the functional impact of the variations. The Rockefeller University in house exome databases (3,000 PID and healthy samples) were screened for novel variants. CLC Main workbench 3.6.1 (Denmark) was used for Sanger Sequencing data analysis.

## 3 | RESULTS

### 3.1 | Target enrichment performance and diagnostic efficiency

All coding regions, 5'- and 3'-UTR parts and flanking intronic regions of 18 genes associated with SCID phenotype were amplified with 432 amplicons in the targeted NGS panel. Although the designed primer pairs were aimed to cover all regions of interest (ROI) parts, the observed mean of targeted regions with at least 20X coverage was 96.35% and for the coding regions observed coverage was

TABLE 1 Clinical findings of SCID patients

Patient	Gene Defect	SCID phenotype	Age (year)	Gen.	ALC (lymphocytes/ $\mu$ l)	Dev. Del.	Cons.	HSCT	Outcome
SC31	ADA	T-B-NK-	3	M	2,200	No	No	Yes	Alive
SC42	ADA	T-B-NK-	1	F	300	Yes	Yes	No	Alive
SC44	ADA	T-B-NK-	6 months	F	40	No	Yes	Yes	Alive
SC38	ADA	T-B-NK-	4 months	M	300	Yes	Yes	Yes	Alive
SC21	CD3E	T-B+NK+	3 months	F	1,450	No	Yes	No	Deceased
SC22	CD3E	T-B+NK+	2 months	M	1,900	Yes	Yes	Yes	Alive
SC04	DCLRE1C	T-B-NK+	4 months	M	1,600	Yes	Yes	Yes	Deceased
SC37	DCLRE1C	T-B-NK+	4 months	F	2,400	Yes	Yes	Yes	Alive
SC17	IL2RG	T-B+NK-	3 months	M	700	No	No	No	Deceased
SC34	IL2RG	Atypical T-B-NK-	30	M	1,200	Yes	No	No	Alive
SC43	IL2RG	T-B+NK-	3 months	M	1,650	Yes	No	Yes	Alive
SC36	IL7R	T-B+NK+	11 months	F	2,400	No	Yes	Yes	Alive
SC09	JAK3	T-B+NK-	6 months	M	700	Yes	Yes	No	Deceased
SC20	JAK3	T-B+NK-	6 months	F	400	Yes	Yes	No	Deceased
SC29	JAK3	T-B+NK-	1	M	2,600	Yes	Yes	Yes	Alive
SC39	JAK3	T-B+NK-	2	F	2,200	No	Yes	Yes	Alive
SC19	NHEJ1	T-B-NK+	18 months	F	2,410	Yes	Yes	Yes	Alive
SC49	NHEJ1	T-B-NK+	5.5	F	400	Yes	Yes	Yes	Alive
SC01	RAG1	Atypical T-B-NK+	13	F	700	Yes	Yes	Yes	Alive
SC13	RAG1	T-B-NK+	3	M	2,850	No	No	No	Deceased
SC15	RAG1	T-B-NK+OMENN	3	F	7,300	Yes	Yes	No	Deceased
SC41	RAG1	T-B-NK+	3 months	F	1,330	No	Yes	Yes	Deceased
SC47	RAG1	T-B-NK+	4 months	F	1,000	Yes	Yes	Yes	Alive
SC16	RAG2	T-B-NK+	3 months	M	700	No	Yes	Yes	Alive
SC24	RAG2	T-B-NK+	9 months	M	1,180	Yes	Yes	Yes	Alive
SC02	Not Detected	Atypical T-B+NK+	1.5	M	2,400	Yes	No	No	Alive
SC03	Not Detected	T-B-NK+	3	M	2,900	Yes	Yes	Yes	Alive
SC05	Not Detected	T-B-NK+	4 months	F	500	Yes	Yes	No	Deceased
SC06	Not Detected	T-B+NK+	1.5 months	F	1,600	Yes	No	No	Alive
SC07	Not Detected	T-B+NK+	1	M	2,700	No	No	No	Alive
SC08	Not Detected	T-B-NK+	1.5 months	M	1,300	Yes	Yes	No	Deceased
SC10	Not Detected	T-B+NK+	1	F	1,220	Yes	Yes	Yes	Deceased
SC14	Not Detected	T-B-NK+	3 months	F	400	Yes	Yes	Yes	Alive
SC18	Not Detected	T-B+NK+	1	M	500	No	No	Yes	Deceased
SC25	Not Detected	T-B-NK+	1	M	500	No	Yes	No	Alive
SC32	Not Detected	T-B+NK+	2 months	M	2,400	Yes	Yes	No	Deceased
SC40	Not Detected	T-B-NK+	2 months	F	1,100	No	Yes	Yes	Alive
SC45	Not Detected	T-B+NK+	10 months	M	1,300	No	Yes	No	Alive
SC46	Not Detected	T-B-NK+	6 months	F	1,540	No	Yes	Yes	Alive
SC48	Not Detected	T-B-NK+	4 months	M	770	No	Yes	Yes	Alive

Abbreviations: ALC, Absolute lymphocyte count; Cons, Consanguinity; Dev. Del, Development Delay; F, Female; Gen, Gender; HSCT, Hematopoietic stem cell transplantation; M, Male; Mo, Month.

97.89%. In 11 out of 18 genes, 100% coverage was achieved for all targeted regions, except *CORO1A* (exons 1, 2 and 11), *ADA* (exon 1), *JAK3* (exon 1) and *IL7R* (exon 7) genes. Although the coverage of

*PRKCD*, *LIG4* and *ZBTB24* genes were below 100%, all the uncovered regions were among the noncoding sequences and coding regions of these genes were fully covered (Table S1).

### 3.2 | Identified genetic variations

The accuracy of detecting variants was evaluated by using two previously diagnosed SCID samples (SC01 and SC13) with four known genetic variants, and all were identified and confirmed successfully. The success rate of our custom-made NGS panel was 60% for the SCID cohort with the detection of 24 disease-causing variants in 23 of 38 SCID patients. The variants (Table 2, Figure S1) were found in *RAG1* ( $n = 5$ , 13%), *RAG2* ( $n = 2$ , 5.2%), *ADA* ( $n = 3$ , 7.8%), *DCLRE1C* ( $n = 2$ , 5.2%), *NHEJ1* ( $n = 2$ , 5.2%), *CD3E* ( $n = 2$ , 5.2%), *IL2RG* ( $n = 3$ , 7.8%), *JAK3* ( $n = 4$ , 10.5%) and *IL7R* ( $n = 1$ , 2.6%). Among these, seventeen variants have been previously reported, whereas seven variants were novel. All the identified variants were confirmed by Sanger sequencing in the patients. We were able to show the allelic segregation by Sanger sequencing in all families except in four (SC04, SC16, SC17 and SC20) patient's families due to the lack of parental DNA.

#### 3.2.1 | Variants in T-B-NK+ patients

Six patients with T-B-NK+ phenotype and OMENN syndrome were found to harbour disease-causing variants in *RAG1* and *RAG2* genes. Five of those variants were previously described (A444V, G392E, R159C, R776W and Y589\*) whereas we identified a novel *RAG2* c.1442A>G p.(H481R) variant in patient SC24 (Figure 1a). H481R variant locates at the highly conserved plant homeodomain (PHD) finger of *RAG2* protein. SC24 is nine-year-old boy had growth retardation and clinically presented with anaemia, lymphopenia, malabsorption and pneumonia.

One patient (SC13) was found compound heterozygous for two different *RAG1* variants, a known variant c.1331C>T (rs199474685) and a novel frameshift variant c.736\_736delG;p.(A246Pfs\*18) that were later confirmed as compound heterozygous in an in vitro fertilization (IVF) centre (data not shown). SC13 is a CMV-positive, T-B-NK+, 3-year-old boy who was suffered from recurrent bronchiolitis, lower respiratory tract infections diarrhoea and oral moniliasis.

Two patients with T-B-NK+ phenotype were found to have homozygous *DCLRE1C* variants; SC37 had a novel frameshift variant c.716\_716delC p.(P239Lfs\*46) and SC04 had a novel nonsense variant c.560T>G, p.(L187\*) (Figure 1b,c). The c.716\_716delC variant is located in exon 9 leading to a frameshift and a premature stop codon, and the other c.560T>G variant is in exon 8 and creates an early stop codon. Both variations are predicted as disease causing and pathogenic. SC37 is a 4-month-old girl suffered from recurrent pneumonia (twice a month), lymphopenia and absence of the thymic shadow. She was transplanted and still alive. SC04 is 4-month-old boy who had similar clinical findings and family history, but he died after transplantation.

#### 3.2.2 | Variants in T-B-NK- patients

In three patients who were presented with a T-B-NK- phenotype, *ADA* variants (R101Q, G20R and IVS5 -2 as) were identified. A novel

splice donor variant c.478+2T>C (IVS5 -2 as) was found in SC38 (Figure 1d). He is 4-month-old and suffered for frequent respiratory infections, conjunctivitis and growth retardation, and no *ADA* enzyme activity was detected. After transplantation, he is still alive. Surprisingly, a 30-year-old male patient (SC34), who is diagnosed as T-B-NK- atypical SCID phenotype, was harboured a promoter variant c.-105C>T on *IL2RG* gene. The onset of clinical symptoms was started at the age of two, with recurrent otitis media, upper respiratory tract infections and severe pneumonia. He underwent right lower lobectomy when he was 13. However, the patient has experienced recurrent pneumonia after surgery. He is still receiving Intravenous Immunoglobulin (IVIG) therapy.

#### 3.2.3 | Variants in T-B+NK- patients

*JAK3* gene variants (E694\*, R775H, R771\* and W623\*) were found in four SCID patients. The c.2080G>T p.(E694\*) was a novel nonsense variant and was predicted as pathogenic/disease causing (Figure 1e). SC20 is a 6-month-old girl presented with bronchiectasis, moniliasis, candidiasis, RSV-positive and growth retardation. She died without transplantation. This variant is in a highly conserved JH2 domain of *JAK3* protein. The second *JAK3* variant c.2324G>A p.(R775H) (Figure 1f) was shown in only one ovarian cancer patient previously but this is the first time for this variant to be shown in an immunodeficiency patient.

In addition, two known hemizygous variants (c.865C>T,p.R289\* and c.115G>A,p.D39N) were detected in the *IL2RG* gene in patients SC17 and SC43 (DiSanto, Dautry-Varsat, Certain, Fischer, & de Saint Basile, 1994).

#### 3.2.4 | Variants in (T-B+NK+) patients

A known nonsense variant c.616C>T p.(R206\*) was found in the *IL7R* gene in SC36. Two-second degree cousins (SC19 and SC49) were found homozygous for a c.532C>T, p.(R178\*) mutation in the *NHEJ1* gene which was previously associated with Cernunnos deficiency.

## 4 | DISCUSSION

The genetic aetiology underlying PIDs are diverse, with the number of responsible genes increasing each year. Early and accurate diagnosis has enabled major advances in the care of infants with PID, in particular SCID, including better outcomes of HSCT or alternative options such as enzyme replacement treatment or gene therapy (Rivers & Gaspar, 2015). Moreover, it provides additional information to improve the prognosis, guide genetic counselling and prenatal diagnosis (Notarangelo, 2010). However, many patients do not receive a molecular diagnosis despite extensive clinical and immunologic studies, mainly due to the large number of genes that can cause

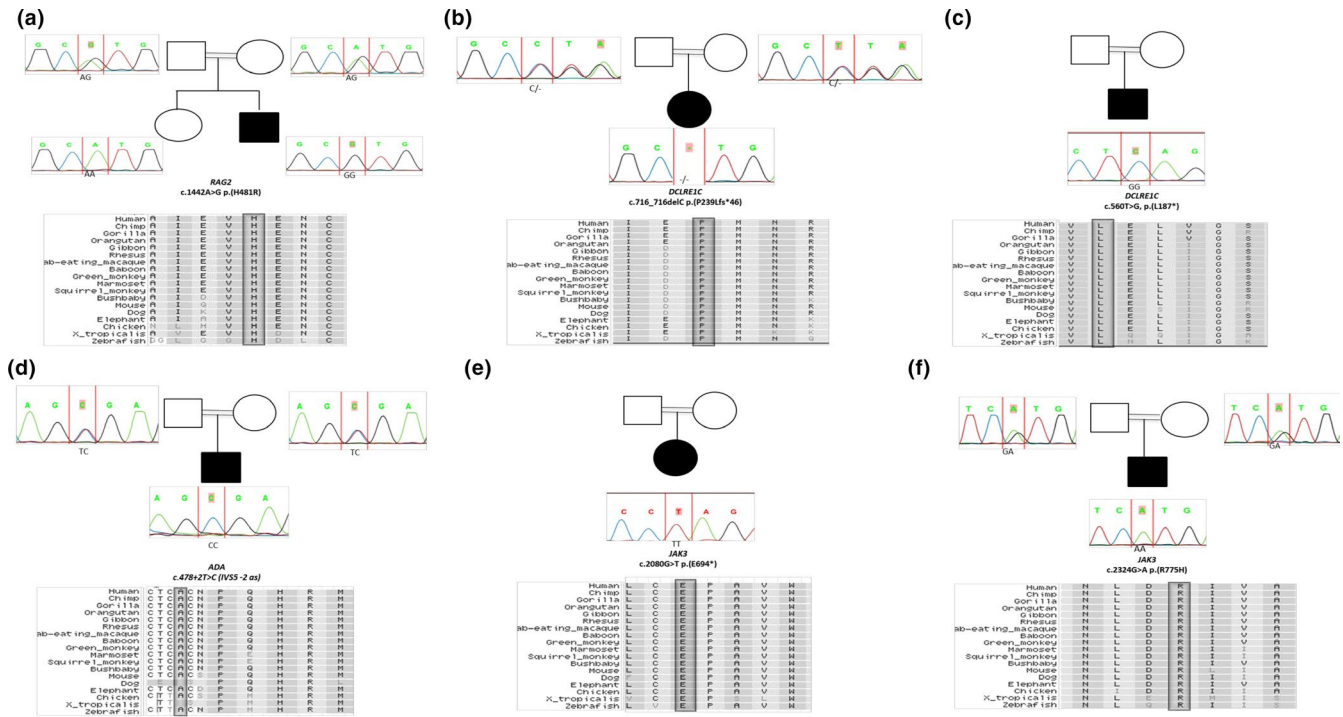
TABLE 2 Disease-causing variants identified in SCID patients

ID	Gene	Genotype	cDNA	Protein	Mutation Type	dbSNP	ACMG/AMP	Mut. Tas.	PolyPhen	SIFT	CADD
SC42	ADA	Hom	c.302G>A	R101Q	Missense	rs121908714	LP	DC	PD	D	31
SC44	ADA	Hom	c.58G>A	G20R	Missense	rs121908724	LP	DC	PD	D	34
SC31	ADA	Com. het	c.704G>A	R235Q	Missense	rs79281338	LP	DC	PD	Del	35
SC31	ADA	Com. het	c.454C>A	L152M	Missense	rs121908728	LP	DC	PD	Del	25,6
SC38	ADA	Hom	c.478+2T>C	IVS5-2 as	Splice donor	Novel	VUS	DC	NA	NA	25,3
SC21	CD3E	Hom	c.173_173delT	L58Hfs*9	Deletion	Firtina et al., 2017	P	DC	NA	NA	1,2
SC22	CD3E	Hom	c.173_173delT	L58Hfs*9	Deletion	Firtina et al., 2017	P	DC	NA	NA	1,2
SC04	DCLRE1C	Hom	c.560T>G	L187*	Stop gained	Novel	P	DC	NA	NA	35
SC37	DCLRE1C	Hom	c.716_716delC	P239Lfs*46	Deletion	Novel	P	DC	NA	NA	33
SC17	IL2RG	Hom	c.865C>T	R289*	Stop gained	rs137852508	P	DC	NA	NA	45
SC34	IL2RG	Hom	c.-105C>T	NA	Promoter	PMID: 26525228	VUS	DC	NA	NA	17,49
SC43	IL2RG	Hom	c.115G>A	D39N	Missense	DiSanto et al., 1994	VUS	DC	B	D	42
SC36	IL7R	Hom	c.616C>T	R206*	Stop gained	rs201559094	P	DC	NA	NA	38
SC20	JAK3	Hom	c.2080G>T	E694*	Stop gained	Novel	P	DC	NA	NA	39
SC29	JAK3	Hom	c.2324G>A	R775H	Missense	COSM6975388	VUS	DC	PD	Del	35
SC09	JAK3	Hom	c.2311C>T	R771*	Stop gained	CM012988	P	DC	NA	NA	42
SC39	JAK3	Hom	c.1868G>A	W623*	Stop gained	rs773308335	P	DC	NA	NA	43
SC19	NHEJ1	Hom	c.532C>T	R178*	Stop gained	rs118204453	P	DC	NA	NA	39
SC49	NHEJ1	Hom	c.532C>T	R178*	Stop gained	rs118204453	P	DC	NA	NA	39
SC01*	RAG1	Com. het	c.1186C>T	R396C	Missense	rs104894289	LP	DC	PD	D	31
SC01*	RAG1	Com. het	c.940C>T	R314W	Missense	rs121918568	LP	DC	PD	D	28,2
SC13*	RAG1	Com. het	c.736_736delG	A246Pfs*18	Deletion	Novel	VUS	DC	NA	NA	9,97
SC13*	RAG1	Com. het	c.1331C>T	A444V	Missense	rs199474685	P	DC	PD	D	25
SC15	RAG1	Hom	c.1175G>A	G392E	Missense	PMID: 17890453	VUS	DC	PD	D	25,5
SC41	RAG1	Hom	c.2326C>T	R776W	Missense	rs121918572	VUS	DC	PD	D	42
SC47	RAG1	Hom	c.1767C>G	Y589*	Stop gained	CM010077	P	DC	NA	NA	35
SC16	RAG2	Hom	c.475C>T	R159C	Missense	rs764485070	VUS	DC	PD	D	27,1
SC24	RAG2	Hom	c.1442A>G	H481R	Missense	Novel	VUS	DC	PD	D	23,8

Abbreviations: ACMG/AMP, The American College of Medical Genetics and Genomics/The Association for Molecular Pathology; CADD, Combined Annotation Dependent Depletion v1.3 (<http://cadd.gs.washington.edu/info>); Com het, Compound Heterozygous; D, Deleterious; dbSNP, Database of Single Nucleotide Polymorphism build ID: 142 (db142), Bethesda MD (<http://www.ncbi.nih.gov/SNP>); DC, Disease Causing; HGMD, The Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac>); HGVSc, Human Genome Variation Society nomenclature; Hom, homozygous; LP, likely Pathogenic; NA, Not Available; P, Pathogenic; PD, Probably Damaging; PolyPhen-2, Polymorphism Phenotyping v2 (<http://genetics.bwh.harvard.edu/pph2/>); SIFT, Sorting Tolerant From Intolerant release 63 (<http://sift.jcvi.org>); VUS, Variant of Unknown Significance.

\*(Asterisk) represents the control samples with previously identified variants to confirm the accuracy of the targeted NGS panel.





**FIGURE 1** Familial segregation of the novel variants in RAG2 (1a), DCLRE1C (b,c), ADA (1d) and JAK3 (e,f) genes in our cohort. Filled black rectangles show affected individuals and double horizontal lines indicate consanguinity. Genotype of individual family members is shown next to the representative symbols in each pedigree (a-f). Evolutionary comparison of the protein sequences for the variations with their orthologous counterparts in 18 vertebrates is shown in the bottom of each pedigree. The altered residues are highlighted by a black rectangle

similar phenotypes. The recent advent of next-generation sequencing technologies and its applications in PID research has accelerated the discovery of novel genes that are associated with PIDs (Cifaldi et al., 2019; Gallo et al., 2016). Targeted NGS panels have been commonly engaged in the clinical laboratories as powerful tools that aid molecular diagnosis by virtue of their capacity to investigate multiple genes simultaneously (Raje et al., 2014), with a reported success rate of 25%–40% among studies (Al-Mousa et al., 2016; Erman et al., 2017; Fang et al., 2016; Nijman et al., 2014; Stoddard et al., 2014; Yu et al., 2016).

In this study, we have developed a SCID-NGS panel for rapid identification of disease-causing variants in patients with a clinical diagnosis of SCID in Turkey and provided a success rate of 60%, with 24 disease-causing variants identified among 23 out of 38 SCID patients. Most common variants were found on the nine SCID-related genes: RAG1, RAG2, ADA, DCLRE1C, NHEJ1, CD3E, IL2RG, JAK3 and IL7R. In addition, twenty-nine per cent (7/24) of identified disease-causing variants were novel in our study. In a previous study from Turkey, Erman *et al* screened 19 SCID patients from Turkey with a targeted NGS panel that contains 356 PID-related gene and showed the genetic alterations in six patients (33%) in RAG1, JAK3 and IL2RG genes (Erman et al., 2017). Here, we generated an NGS panel for SCID, and despite to a small number of genes (18 genes), we had a better success rate in our study. This might be due to the large number of patients, stricter patient enrolment strategies and specific bioinformatics analysis pipelines.

The SCIDX1 patients with IL2RG deficiency are the largest group in the populations with low-consanguinity rates like European or far east countries (Fischer et al., 2015; Luk et al., 2017), but we found RAG1/2 deficiency (47%) which leads to T-B-NK+ phenotype is the most common group in our Turkish SCID cohort. Due to the high consanguineous marriage rates in our country, autosomal-recessive inherited genes might be the most effected genes among the SCID patients in our cohort. Families with more than one child and higher consanguinity rates increase the incidence of monogenic diseases such as SCID in Turkey. Previously, we described a novel frameshift variant in CD3E gene (Firtina et al., 2017) and in two unrelated patients in the same region of Turkey, which showed that consanguineous marriages or isolated populations could cause a high incidence of very rare genotypes in certain regions.

All the T-B-NK- SCID patients were mutated on ADA and IL2RG genes. Among previously known SCID-related variants, a novel splice-site.c.478+2T>C variant on ADA gene (SC38), leading to abrogated enzyme activity, has been identified. ADA deficiency is one of the most prevalent forms of SCID, which together with reticular dysgenesis (caused by AK2 variants) causes a T-B-NK- SCID phenotype in the majority of the patients (Kohn & Gaspar, 2017). The promoter variant detected in the IL2RG gene (c.105C>T) in our cohort (SC34) with an atypical SCID phenotype (T-B-NK-) was previously reported in a case, which had been clinically diagnosed with CVID and NK-cell lymphopenia (Chandra et al., 2016). IL2RG gene is responsible for SCIDX1 and resulted with the absence of T and NK cells with

normal B-cell numbers (Allenspach, Rawlings, & Scharenberg, 1993). However, studies showed that deleterious *IL2RG* variants could lead to variable phenotypes (Mou et al., 2017) as illustrated by this case with no B and T cells (Jones et al., 1997). All patients with T-B+NK-phenotype were found mutated in *JAK3* and *IL2RG* genes. Two novel *JAK3* variants (p.E694\* and p.R775H) were found to be located at the pseudokinase domain, which is essential for *JAK3* function to regulate its catalytic activity. Cheng et al. have previously reported that the patients with mutations in the *JAK3* pseudokinase domain have protein expression but the regulation of the kinase activity is disrupted (Chen et al., 2000). Deleterious mutations in the gene *DCLRE1C* that encodes the ARTEMIS protein have been associated with a wide range of PIDs including the T-B-NK+ SCID, radiosensitive OMENN syndrome or Hyper IgM syndrome (Volk et al., 2015). Here, we found two novel *DCLRE1C* variants (L187\* and P239Lfs\*46) in two infant T-B-NK+ patients. All *DCLRE1C* variants that were detected in this study were novel and are predicted to cause the loss of ARTEMIS protein, which leads to severe combined immunodeficiency phenotype.

Molecular analysis of *DCLRE1C* gene was difficult to define due to homologous recombination of the wild-type *DCLRE1C* gene with a pseudogene located 61.2 kb 5' to the *DCLRE1C* start codon (Pannicke et al., 2010). p.L187\* is located in exon 8 which shows high sequence similarities with the pseudogene of *DCLRE1C*. Single-plex PCR-based methods give flexibility to avoid pseudogenes by designing specific primers and enhance the success of variant detection. Our custom-made panel successfully identified a variant in a region that has high sequence similarities with pseudogenes.

The majority of our cohort was comprised of NK-positive (T-B-NK+;  $n = 18$  and T-B+NK+;  $n = 10$ ) SCID patients and screening the most common 18 SCID-related genes by our custom-designed panel can identify the disease-causing variants in almost 40% of T-B-NK+ patients. On the other hand, all the patients with NK-cell lymphopenia (NK cells negative) were found mutated for the genes in our custom panel. These findings showed that NK-positive patients have a complex background with undiscovered mechanisms/genes or in combination with different syndromes.

NGS-based technologies are the best options for diseases with genetic heterogeneity. An advantage of disease-specific panels, when compared to WES, would be the lack of a need for filtering out unnecessarily high numbers of irrelevant gene variants that is inherent to the latter strategies. On the other hand, immune deficiencies are accompanying to many genetic disorders that can be difficult to diagnose and discriminate in the early years of life and needs larger screening panels. Identified variants were found on the fifty per cent of analysed genes, and later studies showed the mutations of common syndrome genes that were excluded. These findings give the idea that reset of the targeted panels according to the mutation ratios in the individual populations and the most common syndromic genes should also be added. WES or WGS studies with the possible inclusion of the parents and

unaffected siblings could be reserved for the unsolved or more complex cases.

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## CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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