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DNA recombination defects in Kuwait: Clinical, immunologic and genetic profile

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ABSTRACT

Defects in DNA Recombination due to mutations in *RAG*1/2 or *DCLRE1C* result in combined immunodeficiency (CID) with a range of disease severity. We present the clinical, immunologic and molecular characteristics of 21 patients with defects in *RAG1, RAG2* or *DCLRE1C*, who accounted for 24% of combined immune deficiency cases in the Kuwait National Primary Immunodeficiency Disorders Registry. The distribution of the patients was as follow: 8 with *RAG1* deficiency, 6 with *RAG2* deficiency and 7 with *DCLRE1C* deficiency. Nine patients presented with SCID, 6 with OS, 2 with leaky SCID and 4 with CID and granuloma and/or autoimmunity (CID-G/AI). Eight patients [(7 SCID and 1 OS) (38%)] received hematopoietic stem cell transplant (HSCT). The median age of HSCT was 11.5 months and the median time from diagnosis to HSCT was 6 months. Fifty percent of the transplanted patients are alive while only 23% of the untransplanted ones are alive.

1. Introduction

Combined immunodeficiency diseases (CID) comprise a heterogeneous group of genetic conditions characterized by profound deficiencies of T cell (and in some types, B cell and NK cell) numbers and function [1,2]. Typical and atypical forms of severe combined immune deficiency (SCID) represent the most severe form of CID. Based on newborn screening (NBS) results in eleven of the United States of America (USA), typical and atypical SCID were found to affect 1/58000 newborns [3]. However, a recent study from Kuwait showed an estimated occurrence of 1/7500 live births which was attributed to the high rate of consanguineous marriages [4].

V(D)J recombination is crucial for the assembly and expression of T and B lymphocyte antigen receptors and promoting the differentiation of T and B lymphocytes. V(D)J recombination is initiated by binding of the recombination-activating gene products *RAG1* and *RAG2* to the recombination signal sequences (RSSs) flanking the variable (V), diversity (D) and joining (J) coding elements of the B cell receptor (BCR) and T cell receptor (TCR) genes and inducing a DNA double strand break, leaving hairpin structure at coding ends [5]. Upon phosphorylation by the DNA protein kinase catalytic subunit (DNA-PKcs) complex, ARTEMIS, which is encoded by the gene *DCLRE1C* is recruited, and mediates hairpin opening via its endonuclease activity [6]. Joining of the coding ends (as well as of the excised signal ends) is then accomplished by proteins of the non-homologous end-joining pathway. Accordingly, null mutations in *RAG1/2* or *DCLRE1C* affect the development of T and B lymphocytes, causing T^-B^- SCID. However, hypomorphic mutations in the same genes may cause milder phenotypes [5,6].

This study presents the clinical, immunologic and molecular characteristics of 21 consecutive patients from Kuwait who presented with *RAG* or *DCLRE1C* gene defects between the years 2004 and 2016.

2. Methods

2.1. Patients data

The patients' data were retrieved from the Kuwait National Primary Immunodeficiency Disorders Registry (KNPIDR), which prospectively recruited patients since 2004. The project was approved by the Research and Ethics Committee of the Ministry of Health, Kuwait.

2.2. Genetic testing

Genomic DNA was extracted from whole blood. Sanger DNA sequencing was performed according to standard protocols. Targeted next-generation sequencing was performed using the PID v2 panel and Ion Torrent S5 sequencer (ThermoFisher), with an average coverage of 253×. Variant calling was performed using Ion Reporter software (ThermoFisher). For whole exome sequencing, exome capture was

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Table 1
Clinical features of 21 patients with *RAG1/2* and *DCLRE1C* deficiency.

Patient Gene	Phenotype	Reason for testing	Consanguinity	Presentation age[a]	Diagnosis age[a]	Viral infections	Bacterial infections	Fungal Infections	Autoimmunity	Granuloma	Other complications	Outcome	Cause of death	Age of death[a]
A11 *RAG1*	OS	OS	+	0	4	+ Polioviremia	−	+ Candida	−	−	−	D	Multiorgan failure	5
A12 *RAG1*	OS	OS	+	0	2	−	+ Pseudomonas	−	−	−	−	D	Sepsis	2
A13 *RAG1*	OS	OS	+	0	2	−	+ Enterococcus fecalis/Pseudomonas/-Enterobacter cloncae	+ Candida	−	−	IVIG induced renal failure	D	Renal failure	5
A14 *RAG1*	OS	OS	+	0	5	−	+ Pseudomonas	−	−	−	FTT	D	Pneumonia	12
A15 *RAG1*	OS	FH	+	0	0	+ CMV pneumonia	+ Klebseilla pneumonia/E.coli	−	−	−	Urolithiasis/chronic diarrhea/FTT	D	Sepsis	8
A19 *RAG1*	SCID	I	+	3	7	+ Adenoviremia	+ Pneumonia	+ Candida	−	−	−	D	Lung hemorrhage	10
A46 *RAG1*	SCID	FH	+	0	0	+ Rhinovirus pneumonia	−	−	−	−	−	A/W		
A55 *RAG1*	CID-G/I	I/FTT	+	6	7	+ CMVretinitis and viremia EBV viremia HHV-6 viremia	+ Pneumonia and OM Salmonella/E.coli/ Pseudomona/Giardia	+ Candida	−	+ Gut	intermittent neutropenia	D	Sepsis	144
A1 *RAG2*	SCID	AI/FTT/ Chronic diarrhea	+	9	14	+ Corona virus and parainfluenza pneumonia	+ Stenotrophomonas maltophilia	+ Candida	+ AIHA	−	FTT/bronchiectasis neurologic deterioration chronic diarrhea	D	Sepsis	17
A40 *RAG2*	Leaky SCID	FH	+	3	3	−	−	−		−	−	A/c.GvHD		
A63 *RAG2*	CID-G/I	I/AI	+	7	16	−	+ Hemophilus/-Pseudomonas/ Klebsiella	+ Candida	+ AIHA/psoriasis	−	Bronchiectasis/intermittent neutropenia/HSM/FTT	D	Sepsis	120

(continued on next page)

Table 1 (*continued*)

Patient Gene	Phenotype	Reason for testing	Consanguinity	Presentation age[a]	Diagnosis age[a]	Viral infections	Bacterial infections	Fungal Infections	Autoimmunity	Granuloma	Other complications	Outcome	Cause of death	Age of death[a]
A58 *RAG2*	CID-G/I	I	+	12	58	+ EBV viremia and meningitis CMV viremia Adenovirus pneumomia molluscum	+ Skin abscesses/OM	−	+ Polyarthritis	+ Systemic	Primary biliary cirrhosis	D	Sepsis	108
A62 *RAG2*	Leaky SCID	I	+	10	33	+ EBV viremia CMV viremia and pneumonia molluscum	+ Pneumonia/OM/ pneumococcus	−	−	−	−	A/W		
A87 *RAG2*	CID-G/I	I/AI	+	36	144	−	+ Pneumonia/OM/ lymphadenitis	+ Candida	+ Alopecia	−	Bronchiectasis/FTT	A/W		
A22 *DCLRE1C*	SCID	I	+	5	6	+ Parainfluenza pneumonia	+ Pneumonia	+ PJ	−	−	Chronic diarrhea	D	Brain hemorrhage	26
A23 *DCLRE1C*	SCID	I	+	6	7	+ Enteroviremia	+ Klebsiella	+ PJ	−	−	ASD	A/W		
A37 *DCLRE1C*	SCID	I	+	2	8	+ Enteroviremia CMV meningitis	+ OM/Pneumonia/ Pseudomonas	+ Candida	−	−	FTT/colitis	D	Sepsis	44
A47 *DCLRE1C*	OS	OS	−	2	5	+ parainfluenza3 pneumonia norovirus and rotavirus enteritis	−	−	−	−	Chronic diarrhea/FTT	D	Cardiac arrest	15
A48 *DCLRE1C*	SCID	FH	−	0	0	+ sepsis	−	−	−	−	VSD/skeletal anomalies	D	Sepsis	17
A61 *DCLRE1C*	SCID	I	+	2	5	−	+ OM/Pseudomonas/Skin abscess	+ Candida	−	−	−	A/W		
A86 *DCLRE1C*	SCID	FH	+	0	0	+ RSV and rhinovirus pneumonia	−	−	−	−	−	A/W		

SCID: severe combined immunodeficiency; CID: combined immunodeficiency, OS: Omenn syndrome, G/I: granuloma and/or autoimmunity, FH: family history, I: Infections, AIHA: autoimmune hemolytic anemia.
FTT: Failure to thrive, CMV: Cytomegalovirus; EBV: Ebstein-Barr virus; HHV-6: Human herpes virus-6 PJ: Pneumocystis jirovecii, OM: otitis media.
IVIG: intravenous immunoglobulins; ASD: atrial septal defect; VSD: ventricular septal defect, HSM: hepatosplenomegaly.
A: Alive; W: Well; D: Deceased; cGvHD: Chronic graft vs. host disease.
[a] Months.

performed using the SureSelect Human All Exon v4 + UTR kit (Agilent Technologies). A HiSeq 2000 system (Illumina) was used to generate 100 base-pair paired-end reads, with an average on-target coverage of 80×. Reads were aligned to the GRCh37 reference assembly human genome using BWA [7] Single nucleotide variants and indels were detected with GATK using standard hard filtering parameters [8]. Variants with a read coverage <2× and a Phred-scaled SNP quality of ≤20 were eliminated. Whole genome sequencing, read mapping, local de novo assembly, and variant calling and annotation were performed by Complete Genomics, Inc.

2.3. Lymphocyte markers

Peripheral venous blood was drawn using tubes containing EDTA. Blood samples were processed within two hours of collection. Test tubes were prepared with 100 μl of blood, and 10 μl of the CYTO-STAT tetra CHROME CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5, CYTO-STST tetra CHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 murine monoclonal antibody mixture, anti-CD4/CD45RA or CD4/CD45RO (Beckman Coulter, USA) was added. These antibody mixtures allow for the simultaneous identification and quantification of total $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$ and $CD3^-/CD56^+$ lymphocyte subpopulations, and the expression of CD45RA or CD45RO on $CD4^+$ T cells. The samples were incubated in the dark at room temperature for 10 min. After incubation, stabilization and fixation of the stained cells were performed by adding Immunoprep kit reagents (Beckman Coulter, USA). The analysis of the lymphocyte subsets was performed with an EPICS XL-MCL flow cytometer (15 mW) (Beckman Coulter Electronics, FL) equipped with an argon ion laser that was tuned to a wavelength of 488 nm. The values of the lymphocyte subpopulations were determined as a percentage of mononuclear cells. Absolute values of the lymphocyte subsets (counts per μl) were determined via the addition of flow count fluorospheres (Beckman Coulter, USA). We performed a fluorescence gating strategy, using $CD45^+$ vs. side scatter. Internal quality assurance was performed using optical alignment beads, which are compensation reagents that are used to eliminate bleed through fluorescence, and Immunotrol control cells. Data analysis was performed with the Coulter tetraONE SYSTEM software and System II software.

2.4. T-lymphocyte proliferative responses

Peripheral blood was obtained from the subjects by venipuncture, and PBMC were separated by Ficoll-paque (Pharmacia Biotech, Sweden) density gradient centrifugation. The PBMC were suspended in RPMI medium (GIBCO, USA) containing 10% fetal calf serum (GIBCO, USA). The PBMC were aliquoted into 96 well tissue culture plates at a density of 10^5 cells per well and stimulated with one of the following agents: phytohemagglutinin (PHA) (5 μg/ml, Sigma-Aldrich, USA), anti-CD3 antibody (OKT3, 10 μg/ml, Bender MedSystems), candida antigen (2 μg/ml, Greer Laboratories, USA) or purified protein derivative (PPD, 10 μg/ml, CSL Limited, Australia). Cultures were pulsed at 96 h with [^{3}H]thymidine (1 μCi per well) to assess mitogen/antigen-induced proliferation, and the thymidine uptake into DNA was determined 18 h later.

2.5. Serum immunoglobulin and antibodies levels

The quantitation of immunoglobulins (IgM, IgG, IgA) in serum was performed by rate nephelometry using the Beckman specific protein analyzer (Beckman Instruments Inc., CA).

Serum levels of IgG antibodies against tetanus toxoid (TT), diphtheria toxoid (DT) and Haemophilus influenza type b capsular polysaccharide (Hib) were measured using a commercial (ELISA) kit (The Binding site, USA). The antibody concentrations were derived from a standard calibration curve and reported in IU/ml for anti-TT and anti-DT and in mg/L for anti-PCP and anti-Hib.

3. Results

3.1. Patient characteristics and clinical presentations

A total of 21 patients with DNA recombination defects (9 males and 12 females) from 12 families are presented in this report. They represent 7.5% of all patients with PID and 24% of the patients with combined T- and B-cell immunodeficiencies registered in the KNPIDR. The distribution of the patients was as follow: 8 with *RAG1* deficiency, 6 with *RAG2* deficiency and 7 with *DCLRE1C* deficiency. All patients, except 2 siblings (A47 and A48), were born to consanguineous parents. The details of the clinical presentations are shown in Table 1. Based on the case definition developed by the Primary Immune Deficiency Treatment Consortium (PIDTC) (2), 9 patients presented with SCID, 6 with Omenn syndrome (OS), 2 with leaky SCID and 4 with CID with granuloma and/or autoimmunity (CID-G/AI). Five patients were screened by flowcytometry and diagnosed early in life because of family history of the disease while 5 patients were diagnosed due to the typical OS features. Infectious manifestations were the most common, affecting the majority of patients as follow: bacterial (15 patients, 71%), viral (14 patients, 67%) and fungal (11 patients, 52%). Nine patients received the BCG vaccination at birth, and one of them developed localized BCGitis at the time of engraftment after hematopoietic stem cell transplant (HSCT) (patient A23). Four patients (19%) had autoimmune diseases as their initial manifestations.

3.2. Immunologic evaluation

The details of the immunologic evaluations are shown in Table 2. Except patients A11, A14 and A47 with OS and patient A55 with CID-G/AI, all others had $CD3^+$ T cell lymphopenia at the time of diagnosis. The mean $CD3^+$ cell count was 58 cells/μL in the SCID group while it was higher at 1595 cells/μL in the group of CID-G/AI. Patient A55 [11] presented initially with T^+B^- phenotype but re-evaluation at the age of 5 years showed $CD3^+$ T cell lymphopenia at 1150 (1400–3700). Repeat immunologic testing at the age of 11 years showed progressive T-cell lymphopenia (541) with a predominantly $CD45RO^+$ activated phenotype, and nearly absent $CD4^+CD45RA^+CD31^+$ recent thymic emigrants. Two patients (A40 and A61) presented with Leaky SCID and their $CD3^+$ cell counts were 895 and 215 cell/uL, respectively. All patients had $CD19^+$ B cell lymphopenia. Eighteen patients were tested for T lymphocyte proliferation using PHA and all had absent or significantly decreased response except patient A87 with CID-G/AI who had a response >50% of the control.

IgG serum levels were tested in 19 patients and were found to be low in 13 but normal in patients A11, A12. A13, A22 (which may reflect maternal origin), A1 and A87. Ten patients were tested for antibody responses against previous vaccines, and only 3 had good responses.

3.3. Mutations

Fifteen patients were diagnosed by targeted Sanger sequencing, while 4 patients (A63, A58, A62, A61) were diagnosed by Whole Exome Sequencing. Patient A55 was diagnosed by Whole Genome Sequencing and patient A87 was diagnosed by Targeted next-generation sequencing. The details of the mutations and the associated clinical phenotype are shown in Table 3.

3.4. Management and outcome

All patients were treated with intravenous immunoglobulin replacement and prophylactic antibiotics (trimethoprim-sulfamethoxazole). Eight patients [(7 SCID and 1 OS) (38%)] received hematopoietic stem cell transplant (HSCT) (Table 4). The median age of HSCT was 11.5 months (6–24 months) and the median time from diagnosis to HSCT was 6 months (2–18 months). HSCT was from matched related donors in 3

Table 2
Lymphocyte subset count, immunoglobulin concentrations and antibody response prior to substitution therapy and T-lymphocyte proliferation of 21 patients with *RAG1/2* and *DCLRE1C* deficiency.

Patient Gene	CD3$^{+,a}$	CD4$^{+,a}$	CD8$^{+,a}$	CD4^{+}CD45RA^{+} (%)	CD4^{+}CD45R0^{+} (%)	CD19$^{+,a}$	CD16$^{+,a}$	IgG[b]	IgA[b]	IgM[b]	Antibody response	PHA proliferation	Antigen proliferation
A11	3100	950	2017	ND	ND	1	1317	296	<7	7	ND	–	ND
RAG1	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(240–880)	(10–50)	(20 – 100)			
A12	2200	1150	1010	ND	ND	2	1515	251	<7	21	ND	–	ND
RAG1	(2500–5500)	(1600–4000)	(560–1700)			(300 – 2000)	(170–1100)	(210–770)	(5–40)	(15–70)			
A13	1594	325	1267	2	94.2	5	1404	232	<7	<21	ND	–	ND
RAG1	(2500–5500)	(1600–4000)	(560–1700)	(61–94)	(2 – 22)	(300–2000)	(170–1100)	(210–770)	(5–40)	(15–70)			
A14	3746	1827	1738	ND	ND	3	87	<38	56	38	ND	L	ND
RAG1	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(240–880)	(10–50)	(20–100)			
A15	649	572	51	ND	ND	4	1335	240	<6	<15	ND	–	ND
RAG1	(2500–5500)	(1600–4000)	(560–1700)			(300–2000)	(170–1100)	(500–1700)	(1–8)	(50–200)			
A19	11	12	2	ND	ND	0	520	<33	<6	<4	–	–	ND
RAG1	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(300–900)	(15–70)	(40–160)			
A46	88	59	12	11.3	79.1	3	893	220	<6	<6	ND	–	ND
RAG1	(2500–5500)	(1600–4000)	(560–1700)	(61–94)	(2–22)	(300–2000)	(170–1100)	(500–1700)	(1–8)	(50–200)			
A55	4520	3300	1220	ND	ND	2	340	<200	<40	30	ND	–	–
RAG1	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(300–900)	(15–70)	(40–160)			
A1	63	53	9	3.5	98.7	65	121	1270	57	150	+	–	–
RAG2	(2100–6200)	(1300–3400)	(620–2000)	(62–90)	(7–20)	(720–2600)	(180–920)	(310–1380)	(30 – 120)	(50–220)			
A40	895	693	181	62.4	23.7	20	230	156	<6	81.9	ND	–	ND
RAG2	(2500–5500)	(1600–4000)	(560–1700)	(61–94)	(2–22)	(300–2000)	(170–1100)	(210–770)	(5–40)	(15–70)			
A63	454	244	122	1.82	97	79	113	229	75	327	ND	–	–
RAG2	(2100–6200)	(1300–3400)	(620–2000)	(62–90)	(7–20)	(720–2600)	(180–920)	(310–1380)	(30–120)	(50–220)			
A58	614	315	269	6.7	87	59	409	294	7.6	34.5	–	L	L
RAG2	(1400–3700)	(700–2200)	(490–1300)	(50–85)	(9–26)	(390–1400)	(130–720)	(490–1610)	40–200)	50–200)			
A62	1108	338	666	4	94	13	276	88	8	1480	–	L	ND
RAG2	(1400–3700)	(700–2200)	(490–1300)	(50–85)	(9–26)	(390–1400)	(130–720)	(370–1580)	(30 – 130)	(50–220)			
A87	792	401	399	5.9	79.7	109	467	900	103	230	+	N	ND
RAG2	(1200–2600)	(650–1500)	(370–1100)	(42–74)	(13 – 30)	(270–860)	(100–480)	(540–1610)	(70–250)	(50–180)			
A22	59	16	40	35	69	1	103	829	57	19	+	–	ND
DCLRE1C	(2500–5600)	(1800–4000)	(590–1600)	(64–92)	(3–16)	(430–3000)	(170–830)	(240–880)	(10–50)	(20–100)			
A23	28	11	6	ND	ND	1	340	<33	<6	<4	–	ND	ND
DCLRE1C	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(300–900)	(15–70)	(40–160)			
A37	40	14	9	ND	ND	125	328	<33	<6	<4	–	–	ND
DCLRE1C	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(300–900)	(15–70)	(40–160)			
A47	5970	4767	852	ND	ND	0	4800	33	7	9	–	–	ND
DCLRE1C	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(240–880)	(10–50)	(20–100)			
A48	0	0	0	ND	ND	10	840	ND	ND	ND	ND	ND	ND
DCLRE1C	(2500–5500)	(1600–4000)	(560–1700)			(300–2000)	(170–1100)						
A61	215	152	61	ND	ND	40	287	10	7	4	–	–	ND
DCLRE1C	(2500–5600)	(1800–4000)	(590–1600)			(430–3000)	(170–830)	(240–880)	(10–50)	(20–100)			
A86	18	7	6	ND	ND	3	431	ND	ND	ND	ND	ND	ND
DCLRE1C	(2500–5500)	(1600–4000)	(560–1700)			(300–2000)	(170–1100)						

PHA: phytohemagglutinin, N: normal, L: low, ND: not done.
[a] Cells/μL, normal values in parenthesis [9].
[b] mg/dl, before starting IVIG, normal values in parenthesis [10].

Table 3
Spectrum of phenotypes associated with mutations in *RAG1/2* and *DCLRE1C*.

	RAG1	*RAG2*	*DCLRE1C*
Omenn's syndrome	p.Leu454Gln (A11-A15)		del ex 1-9/del ex 1-3 (A47)
SCID	p.Leu454Gln (A19) p.Arg394Trp (A46)	p.Gly35Ala (A1)	p.Gly135Arg (A22, A23, A86) p.Lys157LysfsX13 (A37) del ex 1-9/del ex 1-3 (A48) p.Gly6Glu (A61)
CID-G/I	p.Arg404Gln (A55)	p.Gly35Ala (A58, A63, A87)	
Leaky SCID		p.Gly35Ala (A62, A40)	

OS: Omenn syndrome, SCID: severe combined immunodeficiency, CID-G/AI: CID with granuloma and/or autoimmunity.

patients, matched unrelated donors in 2 patients, and haploidentical donors in the remaining 3 patients. Half of the HSCT were performed without conditioning. Complications related to HSCT affected 62% of the cases and included graft vs. host disease (GvHD) (2 patients), liver failure (2 patients) and ARDS and pulmonary hypertension (1 patient). The 2 patients who had GvHD did not receive conditioning.

The parents of 2 siblings (A58 and A62) have declined treatment with HSCT. The reasons for not performing HSCT in the remaining patients were due to financial issues in 5 patients and high degree of disease severity in 4 patients, including neurologic deterioration (A1), severe bronchiectasis (A55 and A63) and coexisting severe skeletal anomalies (A48). Two patients (A86 and A87) are awaiting HSCT. Only 7 patients (33%) are alive at the time of writing this report. Fifty percent of the transplanted patients are alive while only 23% of the untransplanted ones are alive. The median age of death was 16 months, 20.5 months for the HSCT-treated group and 14.5 months for untreated patients. The median time of death after HSCT was 2 months (1–30 months).

4. Discussion

V(D)J recombination defects due to mutations in *RAG1/2* or *DCLRE1C* are important causes of autosomal recessive CID in Kuwait, where the incidence of consanguinity is relatively high, these defects accounted for a significant proportion (24%) of patients who suffer from combined T- and B-cell immunodeficiencies. Since newborn screening for SCID is not applied in Kuwait, it is likely that a significant number of patients with CID are deceased before diagnosis and many patients with less severe phenotype are misdiagnosed. The frequency of mutations in *RAG1/2* or *DCLRE1C* in patients with combined T- and B-cell immunodeficiencies in the current study is similar to the United States (21%) and the Netherlands (32%), but much less compared to Greece (41%) and Serbia (61%) where a common founder gene defect in RAG1 is likely [3,12–14].

Patients A11, A12, A13, A14, A15, and A46 had the same homozygous missense mutation in *RAG1*, which led to the conversion of thymidine (T) to adenine (A) at position 1361 in the cDNA (c.1361T>A), causing a change of leucine (L) to glutamine (Q) at position 454 (p.L454Q) in the dimerization and DNA binding domain of the protein. This mutation, which is predicted to affect the dimerization of *RAG1* and the DNA-binding ability of the *RAG1-RAG2* complex, has been associated with OS and shown to decrease the enzymatic activity of *RAG1* by >90% [15–17]. While patients A11, A12, A13, A14, and A15, similar to the other patients described in the literature, had features of OS, patient A46 suffered from SCID, highlighting the heterogeneity of the clinical

Table 4
Details of HSCT in 8 patients with *RAG1/2* and *DCLRE1C* deficiency.

Patient	Gene defect	Age of HSCT[a]	Conditioning	GvHD prophylaxis	HSCT complications	Donor	Source of HSC	Time of follow-up after HSCT (years)	Latest count (cells/μL) $CD3^+$ $CD4^+$ $CD19^+$	Overall outcome	Cause of death	Age of death[a]
A19	*RAG1*	9	–	ATG, prednisolone CsA	Adenoviremia liver failure	MRD	BM	–	–	D	Lung hemorrhage	10
A46	*RAG1*	11	Flu + Mel + Cam	CsA	–	Haplo	PB	6	1702 560 113	A/W		
A40	*RAG2*	6	–	CsA	Skin aGvHD and cGvHD Pulmonary cGvHD	MSD	BM	7	953 358 489	A/cGvHD		
A22	*DCLRE1C*	24	BU + FLU + Mel	ATG CsA	Liver failure	MUD	BM	–	–	D	Brain hemorrhage	26
A23	*DCLRE1C*	12	–	CsA	–	MRD	BM	8	644 429 5	A/W		
A37	*DCLRE1C*	14	–	CsA	Skin a/cGvHD gut aGvHD enterobacter sepsis	Haplo	PB	–	–	D	Sepsis	44
A47	*DCLRE1C*	13	Flu + Mel + Ale	CsA	ARDS and Pulmonary hypertension	Haplo	PB	–	–	D	Heart failure	15
A61	*DCLRE1C*	11	Treo + Flu	Tacrolimus	–	MUD	CB	5	3948 2540 1984	A/W		

HSCT: Hematopoietic stem cell transplant.
MSD: matched sibling donor; MRD: matched related donor; MUD: matched unrelated donor, Haplo: Haploidentical.
BM: bone marrow; PB: peripheral blood; CB: cord blood.
Bu: Busulfan; Flu: fludarabine; Mel: melphalan; Cam: Campath; Treo: treosulfan; Ale: Aletuzumab; ATG: antithymocyte globulin; CsA: cyclosporin.
aGvHD: acute Graft vs. Host Disease; cGvHD: chronic Graft vs. Host Disease.
A: Alive; W: Well; D: Deceased.
[a] Months.

manifestations associated with the same mutation in *RAG1*, which likely stems from the stochastic nature of the V(D)J recombination process compounded by environmental factors.

Patients A19 had a homozygous missense mutation in *RAG1*, which led to the conversion of cytidine (C) to T at position 1180 in the cDNA (c.1180C>T), causing a change of arginine (R) to tryptophan (W) at position 394 (p.R394W). This residue is located in the nonamer-binding domain of the protein, and its mutation abolishes protein activity [17]. While it has already been described in three patients with OS [21,22], patient A19 suffers from SCID due to this mutation.

Patient A55 had a homozygous mutation in *RAG1*, which led to the conversion of c.1211G>A G to A at position 1211 in the cDNA (c.1211G>A), causing a change of R to Q at position 404 (p.R404Q) within the homeodomain region of RAG1 [17–20]. In the homozygous form, R404Q has already been associated with SCID [21], and in the heterozygous form, with a termination of translation mutation on the other allele, has been associated with OS [22]. Patient A55 suffered from CID with granuloma and/or autoimmunity, providing further evidence for the vast heterogeneity in the clinical manifestations associated with the same mutation.

Patients A1, A40, A58, A62, A63 and A87 had a homozygous missense mutation in *RAG2*, which led to the conversion of guanine (G) to C at position 104 in the cDNA (c.104G>C), causing a change of glycine (G) to alanine (A) at position 35 (p.G35A) in the core region of *RAG2*, at the interface of the *RAG1-RAG2* interacting domains [15]. As been described previously [23], our patients with G35A presented with milder disease phenotypes (patients A1, A58, A62, A63 and A87 suffered from CID-G/AI, while patient A40 was diagnosed as leaky SCID). In the contrary, mutation of G35 to valine (p.G35 V) has been associated with SCID and OS, and shown to abolish the activity of the *RAG1- RAG2* complex [24,25].

Patients A22, A23 and A86 had a homozygous missense mutation in *DCLRE1C*, leading to the conversion of G to A at position 403 in the cDNA (c. 403G>A), resulting in the change of glycine to arginine at position 135 (G135R) in the metallo-β-lactamase domain. While the G135R mutation has not be previously reported, mutation of G135 to glutamate (G135E) has been associated with SCID and significant reduces the DNA repair activity [6,26]. Likewise, patients A22 and A23 had a SCID phenotype with *pneumocystis jiroveci* infections.

Patient A37 had a homozygous frameshift mutation in *DCLRE1C*, (c.468_469insA), leading to a premature stop codon at position at residue 170 (p.K157KfsX13) in the β-CASP domain. While this mutation has not been previously reported, premature stop codons at positions 191 and 199 have been found in patients with SCID [6,27]. Patient A37 also had SCID, notable for enteroviremia and CMV meningitis.

Patients A47 and A48 had a compound heterozygous deletion of exons 1–3 and 1–9 in *DCLRE1C*. Exons 1–6 encode the metallo-β-lactamase domain, while exons 7–9 encode part of the β-CASP domain. Deletion of exons 1–9 has not been previously reported, but homozygous deletions of exons 1–3 have been associated with SCID and leaky SCID with autoimmune cytopenias [28,29]. Patient A47 had OS, while patient A48 had SCID, thus demonstrating a similar phenotype to patients with deletions of exons 1–3.

Patient A61 had a homozygous missense mutation in *DCLRE1C*, resulting in the conversion of G to A at position 17 in the cDNA (c.17C>A), resulting in the change of glycine to glutamate at position 6 (G6E) in the metallo-β-lactamase domain. This mutation has not been previously reported. Mutations early in the metallo-β-lactamase domain have been reported to cause SCID (A28P, S32F) and OS (M1T, H35D) [30,31]. Similarly, patient A61 had leaky SCID, characterized by abscesses and infections with *Pseudomonas* and *Candida albicans*.

The overall outcome in the presented cohort is disappointing with death occurring in 66% of the patients. Despite the fact that HSCT is currently the only available curative treatment for defects in *RAG1/2* or *DCLRE1C*, only 38% of the patients received such treatment since it is not available in Kuwait for children. Furthermore, only 50% of the patients who were treated with HSCT survived the procedure. It is well known that HSCT for (S)CID before the age of 3.5 months results in a superior outcome [32]. The median age at HSCT in our cohort was 11.5 months (6–24 months) compared to 7 months in patients treated in the United States [33]. The median time from diagnosis to HSCT in our cohort was 6 months (2–18 months) compared to <2 months in the United States [34] and <3 months in the Netherlands [12]. This delay was due to the arrangements needed to transfer the patients to other centers outside Kuwait and has resulted in an increased occurrence of infections and tissue damage which are known to negatively affect HSCT outcome [34]. Schuetz et al. studied complications in transplanted RAG- and ARTEMIS-deficient patients and showed that the latter group had a significantly higher occurrence of infections in long-term follow-up and they also had poor growth, abnormalities in dental development and endocrine late effects especially in association with the use of alkylating agents [35]. This fact documents that the peculiarities of HSCT for patients with V(D)J recombination defects. These peculiarities can only be understood if a large number of patients are studied through international collaboration due to the relative rarity of these defects.

In conclusion, we have presented the molecular characterization, clinical and immunologic presentation and outcome in 21 patients with V(D)J recombination defects registered in the KNPIDR. General pediatricians should be aware of the wide spectrum of CID since early diagnosis and treatment are associated with a better outcome. Given their relative rarity we highlight the need for international collaboration to collect data about HSCT outcome in different genetic defects causing CID.

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Conflicts of interest

None.

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