

Letter to the Editor

Lesson from hypomorphic recombination-activating gene (*RAG*) mutations: Why asymptomatic siblings should also be tested

To the Editor:

An increasing number of patients with combined immunodeficiencies have been found to carry hypomorphic variants of genes that, with null mutations, cause severe combined immunodeficiency (SCID).¹⁻⁴ These patients not only present with recurrent and sometimes life-threatening infections but

also with a plethora of immunodysregulatory symptoms and are a diagnostic challenge.

We report on 2 siblings of consanguineous descent from Saudi Arabia: the clinically affected younger brother and his unaffected older brother who would have qualified as an HLA-identical donor.

The younger brother presented at the age of 9 years with stunted growth, chronic diarrhea, immune complex glomerulonephritis with severe glomerulosclerosis, and arteriosclerosis. He had been under medical care since the age of 2 months; his disease had been classified clinically as immune dysregulation,

TABLE I. Patients' characteristics and immunologic evaluation before HCT

	Subject 1	Subject 2	Subject 3
Genetic defect	<i>RAG1</i>	<i>RAG1</i>	<i>RAG1</i>
Missense <i>RAG</i> mutations	p.[Arg314Trp];[Arg507Trp;Arg737His]	p.[His612Arg];[His612Arg]	p.[Arg778Gln];[Arg975Trp]
Sex	Female	Male	Female
Age at diagnosis (y)	3	7	8
Cardinal sign or symptom	Granuloma	Granuloma	Granuloma
Infectious history	No severe infections	Severe varicella infection, generalized scabies, recurrent bronchitis	Severe varicella infection, vaccine-induced measles, bronchopneumonias
Autoimmunity autoinflammation	Granuloma (skin)	Granuloma (skin), AIHA + ITP	Granuloma (skin, lungs, tongue, adenoids)
Splenomegaly	Yes	Yes	No
Malignancy	EBV-lymphoma		
Other complications before HCT			
Treatment with rituximab before HCT	Yes	Yes	No
Age at HCT (y)	5	7.5	8.5
Outcome	a/w 7 y after HCT, attending school	a/w 4 y after HCT, attending school	Died 1 y after HCT because sequelae of chronic GvHD and absence of thymic reconstitution
Immunologic characterization			
Total lymphocyte counts	320-721 (1700-6900)*	510-1160 (1100-5900)	1083-1456 (1100-5900)
T cells (CD3 ⁺)/μL	120-315 (900-4500)*	245-570 (700-4200)	592-606 (700-4200)
CD3 ⁺ /CD4 ⁺ T cells/μL	52-204 (500-2400)	200-400 (300-2000)	108-184 (300-2000)
CD3 ⁺ /CD8 ⁺ T cells/μL	48-122 (300-1600)	110-190 (300-1800)	360-420 (300-1800)
CD19 ⁺ B cells/μL	0-30 (200-2100)	0-290 (200-1600)	58-132 (200-1600)
CD56 ⁺ /CD3 ⁻ NK cells/μL	130-548 (100-1000)	110-280 (90-900)	370-504 (90-900)
CD3 ⁺ /HLA-DR ⁺	11% to 40% (6% to 10%)	40% (5% to 9.5%)	18% (5% to 9.5%)
CD4 ⁺ /CD45RA ⁺	1% to 12% (53% to 86%)	1% to 5% (46% to 77%)	3% to 8% (46% to 77%)
CD4 ⁺ /CD45RO ⁺	98% to 100% (9% to 26%)	96% to 99% (13% to 30%)	95% (13% to 30%)
Immunoglobulins			
IgG (mg/dL)	92-209 (500-1300)	146 (600-1300)	890 [†] (600-1300)
IgA (mg/dL)	<6 (60-220)	<6 (70-230)	14 (70-230)
IgM (mg/dL)	<5 (40-180)	11.3 (40-160)	67 (40-160)
IgE (IU/mL)	<4.4	<5	ND
T-cell functions			
PHA (2 μg/mL) [‡]	500 (44,000)	44,000 (204,633)	73,000 (367,622)
Tetanus toxoid (1:1,000) [‡]	1,300 (34,200)	261 (41,390)	Negative
T-cell receptor repertoire	Increased expression of Vβ12 and Vβ14	Diverse Vβ repertoire	Diverse Vβ repertoire

The data on patients 1, 3, and 6 were published previously.⁵

AIHA, Autoimmune hemolytic anemia; a/w, alive and well; GvHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; ITP, idiopathic thrombocytopenia; NA, not applicable; ND, not determined; NK, natural killer.

*Values in parentheses reference ranges for the patient's age group.

[†]After intravenous immunoglobulin substitution.

[‡]Counts per minute of incorporated tritiated thymidine and counts per minute of healthy intraday assay controls are shown in parentheses. All patients were vaccinated against tetanus.

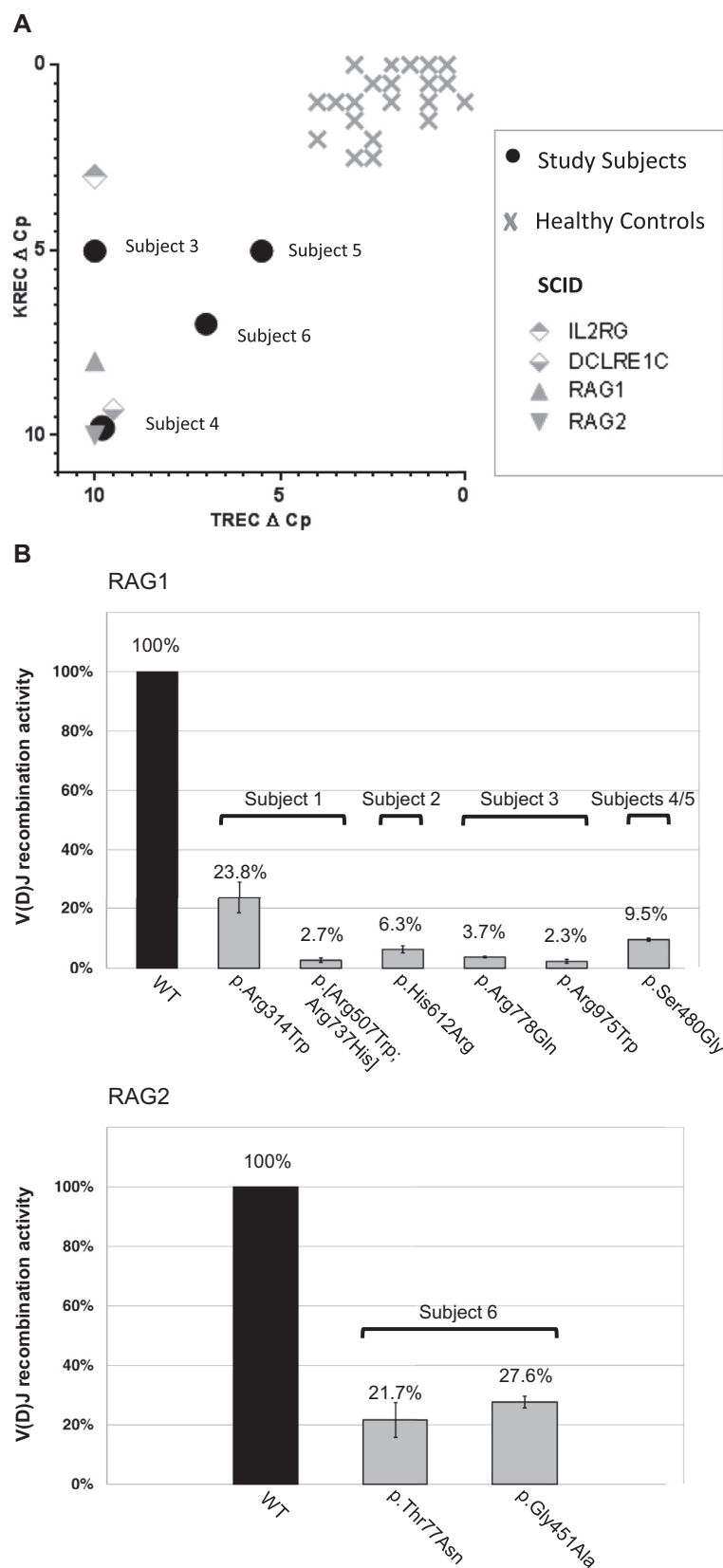
polyendocrinopathy, enteropathy, X-linked–like syndrome. Other findings included recurrent non–life-threatening pulmonary infections with development of bronchiectases, autoimmune hemolytic anemia, tinea capitis, and recurrent urticarial rashes. Immunologically, the patient was lymphopenic, with severely diminished thymus-derived CD4⁺CD45RA⁺ T cells, low T-cell receptor excision circle and kappa-deleting recombination excision circle numbers, and a restricted T-cell receptor repertoire. CD19⁺ B cells and IgG and IgA levels were low, and IgM levels were normal. T-cell function was severely impaired. Table I⁵ and Fig 1, A, show this patient's immunologic evaluations before hematopoietic cell transplantation (subject 4) in comparison with other patients with hypomorphic recombination-activating gene (*RAG*) mutations undergoing

transplantation at our center (patients 1-3 and 6, see Table E1 in this article's Online Repository at www.jacionline.org).

Because of his recurrent infections, enteropathy, autoimmune phenomena, and immunologically combined immunodeficiency, the possibility of a hypomorphic mutation in one of the genes known to cause classical SCID was considered. A homozygous *RAG1* mutation (p.Ser480Gly) was identified, and the parents were found to be heterozygous for this mutation. On analysis of V(D)J recombination efficiency, this variant had a residual function of 10% only compared with wild-type *RAG1*, making it the likely cause of the patient's combined immunodeficiency (Fig 1, B, and see subject 4 in Table E2 in this article's Online Repository at www.jacionline.org).⁶ The decision to offer treatment with hematopoietic cell transplantation was made.

TABLE I. Continued

Subject 4	Subject 5 (brother of subject 4)	Subject 6
<i>RAG1</i>	<i>RAG1</i>	<i>RAG2</i>
p.[Ser480Gly];[Ser480Gly]	p.[Ser480Gly];[Ser480Gly]	p.[Thr77Asn];[Gly451Ala]
Male	Male	Female
9	15	11
Enteropathy		Splenomegaly
Bronchopneumonias	No severe or recurrent infections	Severe varicella infection, bronchopneumonias
Glomerulonephritis, atherosclerosis, AIHA, generalized urticaria	Vitiligo	Granuloma (spleen, lungs), AIHA + ITP, autoimmune neutropenia
Yes	No	Yes
Sclerosing glomerulonephritis		
Yes	NA	Yes
9.5	NA	14
Died 9 mo after HCT due to chronic GvHD, absent thymic reconstitution, and systemic aspergillosis	a/w without any medical care	a/w 3 y after HCT, vitiligo after chronic GvHD, attending school
Immunologic characterization		
312-552 (1100-5900)	1160-1820 (1000-5300)	769-1554 (1100-5900)
234-552 (700-4200)	786-1092 (700-4200)	538-1057 (700-4200)
121 (300-2000)	349-473 (400-2100)	323-668 (400-2100)
126 (300-1800)	386-527 (200-1200)	177-326 (200-1200)
3-143 (200-1600)	237-418 (200-600)	54-202 (200-600)
37-82 (90-900)	212-291 (70-1200)	131-355 (70-1200)
21% (5% to 9.5%)	23% to 27% (5% to 9.5%)	15% to 33% (5% to 9.5%)
2% to 3% (46% to 77%)	22% to 29% (46% to 77%)	9% to 27% (46% to 77%)
95% (13% to 30%)	68% to 72% (13% to 30%)	75% to 96% (13% to 30%)
1250 [†] (600-1300)	1050 (700-1600)	720-1670 [†] (700-1400)
<6 (70-230)	199 (70-400)	7 (70-230)
85 (40-160)	56 (40-230)	9 (40-160)
ND	ND	<2
5,360 (30,469)	50,800 (27,250)	21,000 (59,000)
2,465 (6,566)	5,560 (14,600)	18,000 (2,000)
Increased expression of Vβ8, Vβ13.1, and Vβ14	Increased expression of Vβ11 and Vβ13.1	Diverse Vβ repertoire



The patient's older brother, aged 15 years, was found to be HLA compatible during family screening for a stem cell donor. Unlike the patient, he was in excellent physical condition, without any infectious history. The only clinical finding was generalized vitiligo. Immunologically, he had reduced naive T-cell values of less than 30%, normal CD19⁺ B-cell numbers, low T-cell receptor excision circle and kappa-deleting recombination excision circle numbers, reduced T-cell proliferation to tetanus antigen, and normal immunoglobulin titers (Table I and Fig 1, B, subject 5). Genetic analysis revealed the same homozygous *RAG1* mutation. This unexpected finding made him unsuitable as a stem cell donor for his younger brother.

We provide a comparative overview of clinical findings in 5 patients (some of which have previously been published)⁵ with hypomorphic *RAG1/2* mutations presenting beyond early childhood at our center with autoimmune and autoinflammatory complications, as well as chronic recurrent infections, and in 3 of them there were life-threatening infections (Table I). Subject 5, who is clinically well, will have to be followed for signs and symptoms of immunodysregulation, including renal investigations, because his younger brother had severe glomerulosclerosis after immune complex glomerulonephritis.

Our observation is unique and extends the phenotypic spectrum of hypomorphic *RAG1/2* deficiencies from newborns with Omenn syndrome,⁷ atypical Omenn syndrome,⁸ and classical SCID with predominance of $\gamma\delta$ T lymphocytes⁹ to toddlers and schoolchildren with granulomas^{5,10} to thriving teenagers with a benign autoimmune condition, such as vitiligo. These biallelic *RAG* diseases are in contrast to the recently reported adult with idiopathic T-cell lymphopenia found to have a sole heterozygous *RAG1* mutation¹¹ and in whom an epigenetic or additional genetic factor influencing the phenotype was postulated.

It remains unclear why the above-reported functionally hypomorphic *RAG1* mutation results in 2 diverging phenotypes. In both brothers we were able to exclude compensating mutations in the *RAG1* and *RAG2* genes, which could have modified the onset or severity of the clinical presentation. Also, no compensating mutations were found in the *DCLRE1C* gene. Reversions as alternative modifiers of the phenotype were excluded by sequencing *RAG1* from genomic DNA isolated from sorted CD3⁺, CD20⁺, and CD3[−] cell populations of the older brother. In addition to being due to other possible genetic or epigenetic influences, the immunologic characterization in these brothers highlights that the same *RAG* mutation can result in different lymphocyte repertoires. The stochastic V(D)J recombination process followed by antigen receptor selection in combination with chance infections might contribute to highly variable phenotypes.

Screening for hypomorphic *RAG* mutations should be offered to seemingly unaffected siblings of patients because the knowledge about phenotypic variability is expanding. This applies *a fortiori* for immunologic and genetic testing of potential family donors of hematopoietic stem cells, as is the case for other

primary immunodeficiencies with variable penetrance, such as MHC class II deficiencies or adenosine deaminase deficiencies.¹²

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REFERENCES

- Avila EM, Uzel G, Hsu A, Milner JD, Holland SM. Highly variable clinical phenotypes of hypomorphic *RAG1* mutations. *Pediatrics* 2010;126:1248-52.
- Henderson LA, Frugoni F, Hopkins G, de Boer H, Pai SY, Lee YN, et al. Expanding the spectrum of recombination-activating gene 1 deficiency: a family with early-onset autoimmunity. *J Allergy Clin Immunol* 2013;132:969-71.e2.
- Kuijpers TW, Ijspeert H, van Leeuwen EM, Jansen MH, Hazenberg MD, Weijer KC, et al. Idiopathic CD4⁺ T lymphopenia without autoimmunity or granulomatous disease in the slipstream of RAG. *Blood* 2011;117:5892-6.
- Asai E, Wada T, Sakakibara Y, Toga A, Toma T, Shimizu T, et al. Analysis of mutations and recombination activity in *RAG*-deficient patients. *Clin Immunol* 2011;138:172-7.
- Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with *RAG* mutations and granulomas. *N Engl J Med* 2008;358:2030-8.
- Pannicke U, Ma Y, Hopfner KP, Niewolik D, Lieber MR, Schwarz K. Functional and biochemical dissection of the structure specific nuclease ARTEMIS. *EMBO J* 2004;23:1987-97.
- Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. *J Allergy Clin Immunol* 2008;122:1082-6.
- McCusker C, Hotte S, Le Deist F, Hirschfeld AF, Mitchell D, Nguyen VH, et al. Relative CD4 lymphopenia and a skewed memory phenotype are the main immunologic abnormalities in a child with Omenn syndrome due to

and KRECs were detected by using a customized ready-to-use kit (TIB Molbiol, Berlin, Germany). Two subjects are not plotted because they had received rituximab before testing. **B**, V(D)J recombination assays on patients' mutant *RAG* proteins. Mutant *RAG* proteins found to be encoded by the *RAG1* or *RAG2* genes in immunodeficient patients were tested in human primary dermal fibroblasts, as described previously,⁶ in 3 independent experiments. The percentages of V(D)J recombination activities of the mutant *RAG* proteins compared with *RAG* wild-type (*WT*) proteins are shown. SDs are shown. Negative controls without either *RAG1* or *RAG2* were in the range of 0.9% and 3.1%, depending on the individual experiments (see Table E2).

- homozygous RAG1-C2633T hypomorphic mutation. *Clin Immunol* 2009;131:447-55.
9. Ehl S, Schwarz K, Enders A, Duffner U, Pannicke U, Nikolopoulos E, et al. A variant of SCID with specific immune responses and predominance of gamma delta T cells. *J Clin Invest* 2005;115:3140-8.
 10. De Ravin SS, Cowen EW, Zarembek KA, Whiting-Theobald NL, Kuhns DB, Sandler NG, et al. Hypomorphic Rag mutations can cause destructive midline granulomatous disease. *Blood* 2010;116:1263-71.
 11. Abraham RS, Recher M, Giliani S, Walter JE, Lee YN, Frugoni F, et al. Adult-onset manifestation of idiopathic T-cell lymphopenia due to a heterozygous RAG1 mutation. *J Allergy Clin Immunol* 2013;131:1421-3.
 12. Speckmann C, Neumann C, Borte S, la Marca G, Sass JO, Wiech E, et al. Delayed-onset adenosine deaminase deficiency: strategies for an early diagnosis. *J Allergy Clin Immunol* 2012;130:991-4.

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TABLE E1. Characteristics of patients undergoing hematopoietic stem cell transplantation

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 6
Genetic defect	<i>RAG1</i>	<i>RAG1</i>	<i>RAG1</i>	<i>RAG1</i>	<i>RAG2</i>
Sex	Female	Male	Female	Male	Female
Age at diagnosis (y)	3	7	8	9	11
Donor	MUD*	MUD	MUD	HLA-haploidentical father	MUD
Preconditioning regimen	RIT (BM 20 Gy) Fludarabin, 150 mg/m ² ATG, 10 mg/kg	RIT (BM 17.7 Gy) Treosulfan, 36 g/m ² Fludarabin, 150 mg/m ² ATG, 10 mg/kg	RIT (BM 17 Gy) Fludarabin, 150 mg/m ² Melfhalan, 140 mg/m ² ATG, 10 mg/kg	TBI 12 Gy Fludarabin, 160 mg/m ² ATG, 15 mg/kg	RIT (BM 27 Gy) Treosulfan, 36 g/m ² Fludarabin, 150 mg/m ² Alemtuzumab (MabCampath), 0.5 mg/kg

ATG, Anti-thymocyte globulin; BM, bone marrow; RIT, radioimmunotherapy with yttrium-coupled CD66 antibodies; TBI, total body irradiation.

*Matched unrelated donor 10/10 allelic level match.

TABLE E2. V(D)J recombination assays on patients' mutant RAG proteins

Patient 1 (compound heterozygous <i>RAG1</i> p.Arg314Trp/p.[Arg507Trp;Arg737His])					
	Positive control	–RAG1	+RAG1 WT	+RAG1 p.Arg314Trp	+RAG1 p.[Arg507Trp;Arg737His]
Assay 1	63.60	0.05	3.21	0.96	0.09
Assay 2	53.74	0.06	3.27	0.80	0.06
Assay 3	55.08	0.06	3.13	0.53	0.11
Patient 2 (homozygous <i>RAG1</i> p.His612Arg)					
	Positive control	–RAG1	+RAG1 WT	+RAG1 p.His612Arg	
Assay 1	55.71	0.05	1.63	0.13	
Assay 2	53.74	0.06	3.27	0.19	
Assay 3	55.08	0.06	3.13	0.16	
Patient 3 (compound heterozygous <i>RAG1</i> p.Arg778Gln/p.Arg975Trp)					
	Positive control	–RAG1	+RAG1 WT	+RAG1 p.Arg778Gln	+RAG1 p.Arg975Trp
Assay 1	63.60	0.05	3.21	0.13	0.10
Assay 2	53.74	0.06	3.27	0.12	0.05
Assay 3	55.08	0.06	3.13	0.11	0.07
Patients 4 and 6 (homozygous <i>RAG1</i> p.Ser480Gly)					
	Positive control	–RAG1	+RAG1 WT	+RAG1 p.Ser480Gly	
Assay 1	60.90	0.05	3.43	0.34	
Assay 2	55.26	0.04	3.96	0.35	
Assay 3	57.77	0.05	2.74	0.27	
Patient 5 (compound heterozygous <i>RAG2</i> p.Thr77Asn/p.Gly451Ala)					
	Positive control	–RAG2	+RAG2 WT	+RAG2 p.Thr77Asn	+RAG2 p.Gly451Ala
Assay 1	70.38	0.05	2.47	0.74	0.75
Assay 2	53.74	0.03	3.27	0.57	0.84
Assay 3	55.08	0.06	3.13	0.55	0.84

Mutant RAG proteins found to be encoded by the *RAG1* or *RAG2* genes in immunodeficient patients were tested in human primary dermal fibroblasts in 3 independent experiments, as described previously.⁶ As negative controls, the same cotransfections without the expression plasmids coding for RAG1 (–RAG1) or RAG2 (–RAG2) were performed. As a positive control, a prerecombined substrate vector was transfected. In each assay 5×10^4 fibroblasts were analyzed by using fluorescence-activated cell sorting. The percentages of recombination-positive cells out of the subpopulation of transfected fibroblasts are depicted.