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A novel homozygous mutation in *Recombination Activating Gene 2* in two relatives with different clinical phenotypes: Omenn syndrome and Hyper-IgM syndrome

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To the Editor:

Recombination Activating Genes (*RAG*) 1 and 2 encode proteins necessary for T and B cell antigen receptor rearrangement. Complete deficiency of either *RAG1* or *RAG2* results in classical severe combined immunodeficiency (SCID) lacking T and B cells, since RAG1 mediates DNA binding and cleavage, while RAG2 is an essential cofactor for RAG1 function.[1] Hypomorphic missense mutations that preserve residual RAG activity and allow the development of oligoclonal T cells, but virtually no B cells, result in recurrent infections, erythroderma, hepatomegaly, colitis, and αβ T cell expansion (Omenn syndrome).[2] *RAG1/2* mutations can also cause γδ T cell expansion and immunodeficiency with granulomas.[3]

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Hyper-IgM syndrome is characterized by normal or increased IgM with decreased IgG and IgA levels. It results from defects in class switch recombination caused by mutations in *CD40 ligand, CD40*, *Activation-Induced Deaminase*, *Uracil-DNA glycosylase*, and *Nuclear Factor Kappa B Essential Modifier* (*NEMO*).[4] In addition to defective humoral immunity, T cell function is affected in CD40L, CD40, and NEMO deficiency and is associated with opportunistic infections.[4]

We report a novel homozygous mutation in *RAG2* resulting in two different phenotypes: Omenn syndrome and hyper-IgM syndrome.

Patient 1 was born to first-degree consanguineous parents and presented at 4 months of age with lymphopenia (absolute lymphocyte count of 869 cells/μl), erythroderma, *Pseudomonas aeruginosa* pneumonia, and Coombs' positive hemolytic anemia. She had recurrent pulmonary infections, and developed onchomycosis by 1 year of age. Lymphocyte subset analysis at 16 and 19 months of age revealed lymphopenia with severely decreased T and B cells and normal NK cell numbers (Table I). The majority of her T cells were $CR45RO^+$ activated cells (not shown). HLA typing revealed no evidence of maternal cell engraftment. Lymphocyte proliferation to phytohemagglutinin (PHA) and anti-CD3 mAb was severely decreased (Table I). She had low serum IgG and undetectable serum IgA, while serum IgM was significantly decreased by 19 months of age (Table I). Because her phenotype was consistent with Omenn syndrome, *RAG1* and *RAG2* were sequenced. A previously unreported homozygous missense mutation in *RAG2* (c.1375A>C) was identified, causing a methionine to leucine change at position 459 (M459L) in the protein's plant homeodomain (PHD). Patient 1 subsequently underwent a successful matched sibling bone marrow transplant.

Patient 2 was born to first-degree consanguineous parents. He presented at 4 months of age with recurrent skin abscesses. He subsequently developed recurrent pneumonias, *Pseudomonas aeruginosa* sepsis with disseminated intravascular coagulopathy, colitis, CMV viremia, oral candidiasis, hepatosplenomegaly, autoimmune hemolytic anemia, and antiphospholipid antibodies. Lymphocyte subset analysis at 16 months of age revealed lymphopenia with low T and B cells and normal NK cell numbers (Table I). Serum IgG was very low, serum IgA undetectable, and serum IgM normal. By 5 years 5 months of age, his IgM increased to 1048 mg/dl (Table I), prompting a diagnosis of hyper-IgM syndrome. The patient was referred to us for further investigation. His T cells upregulated CD40L normally following activation with phorbol 12-myristate 13-acetate and ionomycin (not shown). However, he had low numbers of T cells and virtually no B cells (Table I). The majority of his T cells were $CR45RO^+$ activated cells (not shown). T cell proliferation to PHA and anti-CD3 mAb was severely decreased (Table I). These results suggested that Patient 2 had a combined immunodeficiency rather than hyper-IgM syndrome.

A detailed family history revealed that the great-grandparents of Patients 1 and 2 were cousins. This raised the possibility that they might share the same *RAG2* mutation. Patient 2 was indeed homozygous for the *RAG2* mutation found in Patient 1. The healthy sister of Patient 1 has a normal *RAG2* sequence, while the parents of both patients and two healthy brothers of Patient 2 are heterozygous for the *RAG2* mutation.

The M459 residue is highly conserved and located in the zinc-binding loop region of the RAG2 PHD domain. Mutations in this domain impair RAG2 protein stability, translocation to the nucleus, interaction with histones, and recombination capacity, resulting in SCID or Omenn syndrome.[5] The recombination activity of the M459L RAG2 mutant was analyzed using Abelson-transformed $Rag2^{-/-}$ tg.*bcl2* pro-B cells containing an intrachromosomal inverted GFP cassette flanked by recombination signal sequences. Cells were transduced

with retroviral vectors encoding either wild-type or mutant RAG2 and human CD2 as a reporter, then treated with the Abl inhibitor STI-571 to promote cell differentiation and induce RAG activity. The expressed RAG2, in combination with endogenous RAG1, causes inversion of the GFP cassette and the resultant GFP expression correlates with the level of recombination activity.[6,7] The recombination activity of the RAG2(M459L) mutant was 29.7±3.3% of WT RAG2 (Fig 1).

We describe two related patients with a novel mutation in *RAG2* causing different clinical phenotypes. Patient 1 had Omenn syndrome with erythroderma, low T and B cells, and opportunistic infections. Patient 2 lacked erythroderma and had persistently high IgM levels, leading to a presumptive diagnosis of hyper-IgM syndrome. Of note, the phenotype of Patient 2 also evolved over time; the progressive loss of B cells and lack of T cell proliferation to PHA and anti-CD3 seen at five years of age are consistent with a mutation in *RAG2*. Phenotypic heterogeneity due to the same *RAG2* mutation has been attributed to differences in genetic background, epigenetic factors, and environmental exposures.[8,9] The low level recombinase activity *in vitro*, and the presence of residual autologous T and B lymphocytes in the patients, indicate that the M459L RAG2 variant may retain some V(D)J recombination activity *in vivo*. Combined with different antigen exposure, this may contribute to our patients' different phenotypes. Thirty percent recombination activity was also found in a patient with *RAG2* mutations resulting in hypogammaglobulinemia, lymphopenia, and recurrent infections, indicating that this level of reduced recombination activity results in clinically significant disease.[3]

To our knowledge, hyper-IgM has not been previously reported with mutations in *RAG2*. It is therefore important to consider *RAG* mutations in patients with elevated levels of IgM and progressive lymphopenia.

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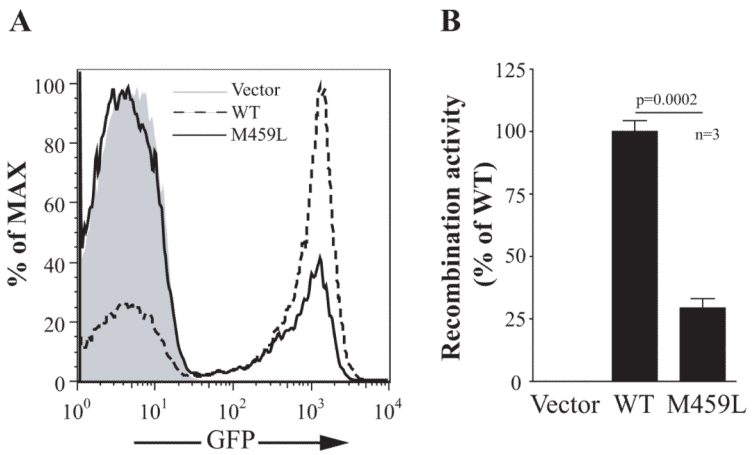

FIG 1.

Recombination activity of RAG2(M459L) mutant. Abelson-transformed *Rag2*$^{-/-}$ tg.*bcl2* pro-B cells containing an inverted GFP cassette were transduced with retroviral vectors encoding wild-type or M459L mutant human RAG2, and human CD2 as a reporter. An empty vector encoding CD2 was used as a negative control and resulted in no GFP expression. **A**, GFP expression on CD2^{+} cells indicate the recombinase activity level. Shown are representative histograms from three independent experiments. **B**, Bar graph represents the average recombination activity from three independent experiments (n=3). The recombination activity is calculated as percent activity of wild-type RAG2.

TABLE I

Immune profiles

	Pt. 1		Pt. 2	
	16 months	**19 months**	**16 months**	**5 years 5 months**
Lymphocyte counts (cells/μl)[1]				
$CD3^+$	149 (1900-6200)	205(1900-6200)	691(1900-6200)	777 (1400-6200)
$CD3^+CD4^+$	85 (1300-3400)	149 (1300-3400)	311 (1300-3400)	443 (700-2200)
$CD3^+CD8^+$	61 (620-2000)	42 (620-2000)	173 (620-2000)	293 (490-1300)
$CD4^+/CD8^+$ ratio	1.4 (1.3-3.9)	3.5 (1.3-3.9)	1.7 (1.3-3.9)	1.5 (0.9-3.7)
$CD19^+$	4 (610-2600)	1 (610-2600)	173 (610-2600)	15 (390-1400)
$CD16^+/CD56^+$	279 (160-1100)	208 (160-1100)	657 (160-1100)	1111 (130-720)
Immunoglobulins (mg/dl)[2]				
IgG	193 (400-1300)	147 (400-1300)	<152 (400-1300)	213 (600-1500)
IgA	Undetectable (20-230)	Undetectable (20-230)	Undetectable (20-230)	1 (50-150)
IgM	54.9 (30-120)	<17.3 (30-120)	171 (30-120)	1048 (22-100)
Proliferation (c.p.m.)				
PHA	ND	16700 (116187)	ND	1980 (57182)
Anti-CD3 mAb	ND	14310 (107265)	ND	12558 (82957)

The values in parentheses represent the normal range for age of lymphocyte counts, immunoglobulin concentrations, and ^{3}H-thymidine incorporation into DNA measured as radioactive counts per minute (c.p.m.). For lymphocyte proliferation studies, a normal healthy control was studied the same day as the patient. Pt, patient; ND, not determined.

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