Supplemental Tables

Table E1. Immunoglobulin levels at different ages for patients with RAC2 deficiency

Age (years)	1	4	5	6	7	8	9	10	11	12	14	15	16	19	20
Proband															
IgM (mg/dl)	180	40	95	110	30↓	42	182	n.a.	n.a.	n.a.	73	40	n.a.	50	n.a.
IgG (mg/dl)	1800↑	800	650↓	940	430↓*	380↓	600↓	n.a.	n.a.	n.a.	780	790	n.a.	700	n.a.
IgA (mg/dl)	0↓	20	0↓	0↓	5↓	5↓	0↓	n.a.	n.a.	n.a.	30↓	40↓	n.a.	0↓	n.a.
IgE (IU/ml)	9	n.a.	n.a.	n.a.	<5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sibling															
IgM (mg/dl)	n.a.	n.a.	n.a.	n.a.	105	190	17↓	28↓	36↓	19↓	0↓	n.a.	21↓	n.a.	58
IgG (mg/dl)	n.a.	n.a.	n.a.	n.a.	900	750	754	640↓*	520↓	600↓	99↓	n.a.	542↓	n.a.	130↓
IgA (mg/dl)	n.a.	n.a.	n.a.	n.a.	0↓	0↓	0↓	19↓	19↓	9↓	0↓	n.a.	0↓	n.a.	10↓
IgE (IU/ml)	n.a.	n.a.	n.a.	n.a.	5.3	n.a.	n.a.	n.a.	n.a.	n.a.	<5	n.a.	n.a.	n.a.	n.a.

*Age-matched reference levels: 1-3 years: IgM, 55-210; IgG, 700-1600; IgA, 19-220; IgE, <100. 4-5 years: IgM, 40-230; IgG, 700-1600; IgA, 48-345. 6-7 years: IgM, 40-230; IgG, 700-1600; IgA, 41-297; IgE, <100. 8-10 years: IgM, 40-230; IgG, 700-1600; IgA, 51-297. 11-13 years: IgM, 40-230; IgG, 700-1600; IgA, 44-395. 14 years and older: IgM, 40-230; IgG, 700-1600; IgA, 70-400; IgE, <100. n.a., not analysed. * Treatment of intravenous immunoglobulin started, with controlled kidney disorder and normal total protein and albumin levels.*

Table E2. IgG subclass levels in the two siblings with RAC2 deficiency

IgG subclass level (mg/dl)	Proband	Sibling
	At age of 4 yrs	*At age of 10 yrs*
IgG1 (reference values)	405 (253-1019)	50 ↓ (289-934)
IgG2 (reference values)	73 (54-435)	<15 ↓ (82-516)
IgG3 (reference values)	172 ↑ (9-102)	12 ↓ (20-103)
IgG4 (reference values)	15 (1-108)	<5 (0.3-111)

*yr, year. *Reference: age-appropriate reference range from healthy Iranian individuals.*

12 **Table E3. Lymphocyte subpopulations in the sibling with RAC2 deficiency**

Laboratory test	Sibling	Reference range
Age at testing	*26 yrs*	
$CD4^+CD25^+FOXP3^+$ regulatory T-cells (%)	1.4 ↓	3.4-5.66
FOXP3 expression in PBMC (%)	1.5 ↓	2.7-4.8
Regulatory T-cells suppressor capacity (%)	18 ↓	30-80
$CD45RA^+$ $CD62L^+CD4^+$ naïve T-cells (%)	17.4 ↓	35-54
$CD31^+CD45RA^+CD62L^+CD4^+$ recent thymic emigrant T-cells (%)	26.4 ↓	32-50
Age at testing	*28 yrs*	
NK-cells (%)	11.7	3-22
$CD19^+$ (%)	0 ↓	6-23
$CD3^+$ (%)	87↑	56-84
$CD4^+$ (%)	26↓	31-52
$CD8^+$ (%)	57 ↑	18-35
CD4:CD8	0.5 ↓	1.0-3.6
NKT-cells (%)	7.7	2.1-13.7
$CD4^-$ $CD8^-$ T-cells (%)	3.8	3-10.2
$CD4^+$ $CD8^-$ T-cells (%)	0.6	0.2-1.4
$CD4^+CD38^+$ T-cells (%)	51	50-79
$CD4^+HLA\text{-}DR^+$ T-cells (%)	30 ↑	5-25
$CD4^+CD38^+HLA\text{-}DR^+$ T-cells (%)	10 ↑	2-6
$CD8^+CD38^+$ T-cells (%)	55	33-80
$CD8^+HLA\text{-}DR^+$ T-cells (%)	66 ↑	5-25
$CD8^+CD38^+HLA\text{-}DR^+$ T-cells (%)	42 ↑	3-18
$CCR7^+CD45RA^+CD45RO^-$ $CD4^+$ naïve T-cells (%)	9.8 ↓	20.2-51.1
Effector memory $CD4^+$ T-cells (%)	55.2	27.5-56.8
T-helper-1 cells (%)	21.0	4.5-25.5
T-helper-2 cells (%)	3.1	1.5-11.3
Central memory $CD4^+$T-cells (%)	10.2	7.7-17.5
T-cells (naïve:effector memory)	0.2 ↓	0.3-3.2
Th17-cells	39.4 ↑	15.1-37.0
Naïve $CD8^+$ T-cells (%)	1.8 ↓	28.4-66.7
Effector memory $CD8^+$ T-cells (%)	67.0 ↑	11.5-42.6
Activated effector memory $CD8^+$ T-cells (%)	1.9	0-22.4
Activated cytolytic effector $CD8^+$ T-cells (%)	0.2	<5.9
Effector $CD8^+$ T-cells (%)	15.9	7.4-24.6
Central memory $CD8^+$ T-cells (%)	1.0	0.5-5.6

13 *yr, year; PBMC, peripheral blood mononuclear cells*

Table E4. Comparison of cases with RAC2 deficiency

Parameters	Proband	Sibling	Colorado Case[7, 8]	Wisconsin case[9]
Gender	Female	Male	Male	Male
Mutation	W56X Hom	W56X Hom	D57N Het	D57N Het
Effect of mutation	Absent expression	Absent expression	Dominant negative	Dominant negative
Age at onset of clinical symptoms	6 months	2 years	5 weeks	26 days
Age at genetic diagnosis	19 years	23 years	Within 10 months of life	Within 5 weeks of life
Clinical features	Recurrent pneumonia, hypogammaglobulinemia, PSGN, factor XI deficiency, urticaria, allergy, bronchiectasis, hypothyroidism, hyperparathyroidism, ESRD	Recurrent sinopulmonary infections, hypogammaglobulinemia, urticaria, PSGN, factor XI deficiency, lymphadenopathy, bronchiectasis, hypothyroidism, GH deficiency	Recurrent perirectal abscess, umbilical stump involution failure, infected urachal cyst, failure of surgical wound healing	Periumbilical erythema, macrocytic anemia, paratracheal abscess
WBC/mm^3	Normal	Normal	High	High
Neutrophils (%)	Normal	Normal	High	High
Lymphocytes (%)	Reduced	Reduced	Low-normal	Reduced/normal
T-cells (%)	Normal	Normal	Low-normal	Reduced/normal
B-cells (%)	Reduced	Normal at age 7 yrs, reduced at age 28 yrs	Low-normal	Reduced/normal
IgM (mg/dl)	Normal then reduced	Normal then reduced	Normal	Reduced
IgG (mg/dl)	Normal then reduced	Normal then reduced	Normal	Normal
IgA (mg/dl)	Reduced	Reduced	Normal	Reduced
Neutrophil chemotaxis	n.a.	Moderately reduced	Markedly reduced	Markedly reduced
Oxidative burst*	Normal	n.a.	Normal	Normal
CD18$^+$ (%)	Normal	n.a.	n.a.	Normal
CD11a$^+$ (%)	Slightly increased	n.a.	n.a.	n.a.
CD11b$^+$ (%)	Slightly increased	n.a.	Normal	Normal
CD11c$^+$ (%)	Increased	n.a.	Normal	n.a.
C3 (mg/dl)	Slightly reduced	Normal	Reduced/normal**	n.a.
C4 (mg/dl)	Normal	Normal	n.a.	n.a.
KRECs	Reduced	Reduced	n.a.	n.a.
TRECs	Reduced	Reduced	n.a.	Reduced
Organisms isolated	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Escherichia coli*, *Enterococcus* species *and Pseudomonas aeruginosa*	*Stenotrophamonas* and *Prevotella* species
Treatment	Antibiotics, IVIG, immunosuppressive therapy, levothyroxine, renal transplant	Antibiotics, IVIG, immunosuppressive therapy, levothyroxine	Antibiotics, prednisolone, granulocyte transfusion, BMT	Surgical debridement, abscess drainage, granulocyte transfusion, HSCT
Outcome	Dead at 21 years from transplant rejection and suspected cerebral hemorrhage	Alive	Cured after BMT	Cured after HSCT

yr, year; Het, heterozygous; Hom, homozygous; PSGN, post-streptococcal glomerulonephritis; ESRD, end-stage renal disease; WBC. white blood cells; GH, growth hormone; n.a., not analyzed; KRECs, kappa-deleting recombination excision circles; TRECs, T-cell receptor excision circles; IVIG, intravenous immunoglobulin; BMT, allogeneic bone-marrow transplantation; HSCT, allogeneic cord-blood hematopoietic stem cell transplantation.

** Oxidative burst tested using phorbol myristate acetate.*

*** Serum complement is mentioned to be reduced and normal in references 8 and 9, respectively.*

N.B. All clinical immunological details of the previously reported cases may be found in references 8 and 9 (Colorado case) and 10 (Wisconsin case).

A Novel *RAC2* Mutation in Two Siblings Associated with Features of Common Variable Immunodeficiency

Alkhairy et al.

Supplemental material

Supplemental Methods

Clinical investigations

Informed consent for this study was obtained from the proband and her relatives in accordance with the principles of the ethics committee of the Tehran University of Medical Sciences.

Immunological analyses

During follow-up, we performed different investigations according to previously published methods including complete blood counts, serum immunoglobulins levels[1], vaccine responses[2], frequency and/or function of regulatory T-cells (Tregs)[3], naïve T-cell[4] and recent thymic emigrant T-cells[4]. Neutrophil chemotaxis assay was performed using zymosan-activated serum as previously described [5].

Measure the serum level of BAFF

The concentration of BAFF was assessed in stored serum samples using the Human BAFF (TNFSF13B) SimpleStep ELISA™ Kit (Abcam, Cambridge, UK) following the manufacturer's instructions. Two healthy controls were included. Statistical analysis (non-parametric analysis [Kruskal-Wallis test]) was performed using the Excel software.

Flow cytometric immunophenotyping

Venous blood was collected in EDTA tubes and the samples were processed within 36 h. Antibody combinations were used for polychromatic 8-color surface staining panels and were optimized as previously published[6].

Whole exome sequencing (WES) and validation by Sanger sequencing

The methods for DNA library preparation, read mapping, variant analysis, and analysis protocol for WES were performed as described previously[1]. Sanger sequencing using specific primers (forward GGAATGACATGGAGCTGGAC and reverse AAGATGGGCACATTGAGGAC) was performed to validate the potential disease-causing variant. The PCR reactions were performed as described previously[1]. The purified PCR products were sequenced by Macrogen Incorporated (South Korea).

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