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Brief Communication

Heterozygous activating mutation in RAC2 causes infantile-onset combined immunodeficiency with susceptibility to viral infections

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ABSTRACT

Here we describe a 10-year-old girl with combined immunodeficiency presenting as recurring chest infections, lung disease and herpetic skin infections. The patient experienced two hematopoietic stem cell transplantations and despite full chimerism, she developed bone marrow aplasia due to adenovirus infection and died at post-transplant day 86. Immunologic investigation revealed low numbers of TRECs/KRECs, a severe reduction of memory B cells, absence of isohemagglutinins, and low IgG levels. Whole exome sequencing (WES) identified a novel heterozygous mutation in *RAC2*(c.275A > C, p.N92 T). Flow cytometric investigation of neutrophil migration demonstrated an absence of chemotaxis to fMLP. Cell lines transfected with RAC2 [N92 T] displayed characteristics of active GTP-bound RAC2 including enhanced NADPH oxidase-derived superoxide production both at rest and in response to PMA.

Our findings broaden the clinical picture of RAC2 dysfunction, showing that some individuals can present with a combined immunodeficiency later in childhood rather than a congenital neutrophil disease.

1. Introduction

Numerous genes and molecules orchestrate the ability of neutrophils to follow a chemotactic gradient after activation and emigrate out of the blood vessel towards tissue injury or an infectious event [1,2]. RAC2, a Rho GTPase, is an essential regulator of neutrophil chemotaxis. It participates in actin polarization, pseudopod assembly, L-selectin capture, and rolling. It is also a regulatory component of the human neutrophil NADPH oxidase controlling the mechanism by which this oxygen radical-generating system is regulated [3]. The involvement of RAC2 in immunity is not limited to cells of the innate arm. Indeed, RAC2-deficiency has also been shown to impact B- and T-cell migration, activation, development [4] Table 1.

Dominant negative inherited RAC2 deficiency was reported for the first time in 2000 in a male infant patient that presented with multiple and progressive soft-tissue infections during his first few weeks of life [5]. The patient displayed abnormalities mainly in phagocytic cells, including defective migration, capture, and rolling on the L-selectin ligand, glycam-1, and reduced superoxide generation in response to some agonists of the phagocytic oxidase pathway [5–7]. Since then,

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Table 1
Demographic, clinical, laboratory and genetic characteristics of all reported Rac2 deficient patients.

Patient_Gender_ Country	Age of onset / age of diagnosis	Rac2 mutation	Clinical presentation	Immunologic abnormalities	Consanguinity/ Family history	Outcome/ Reference
		Type of mutation				
1_Male_ USA	5 week / 4 month	p.D57N AD LOF	Perirectal abscess (4 m) and failure of the umbilical stump to detach	N↑, N migration↓↓, CD11b+, defect in azurophilic granule release in response to PMA, fMLP	No/ No family history of immunodeficiency	[6,7]
2_Male_ USA	26 day/ Screening*	p.D57N AD LOF	Fever and periumbilical erythema (26 d); paratracheal abscess, anemia (56 d)0	N↑, N migration↓↓, CD18+, CD15+, TRECs↓, CD4 + ↓, CD45RO + ↑, fMLP Burst↓, IgA↓, IgM↓	No/ No family history of immunodeficiency	HSCT, alive [9]
3a_Female_Iran	6 months/ 21 years	p.W56X/ p.W56X AR LOF	Recurrent pneumonia, edema, proteinuria and membranous glomerulonephritis, factor XI deficiency, urticaria, erythematous plaques, food allergy, arthralgia, bronchiectasis,hypothyroidism, hyperparathyroidism	IgG↓,CD19 + ↓,CD4 + ↓, TRECs↓, KRECs↓	Yes (first-degree consanguineous)/ Family case	Died, 21 years
3b_Male_ Iran	2 years / 28 years	p.W56X/ p.W56X AR LOF	Recurrent sinopulmonary infections, failure to thrive, urticarial, post-streptococcal glomerulonephritis, lymphadenopathy, bronchiectasis, hypothyroidism, factor XI deficiency, growth hormone deficiency	N migration↓↓,CD18+, CD15+, azurophilic granules in N↓, TRECs↓, KRECs↓, IgG↓, IgM↓, CD19 + ↓, RTE↓, Tregs↓, CD4 + ↓		[8]
4_Male_ European	Childhood/ 39 years	p.P34H AD GOF	Recurrent sinopulmonary infections, bronchiectasis, cutaneous human papillomavirus infections, **basal cell carcinomas**, antiphospholipid syndrome, IgM monoclonal gammopathy, low-grade B-cell **non-Hodgkin lymphoma**	CD19 + ↓, RTE↓, Tregs↓, CD4 + ↓, CD8 + ↓, CD8 + CD57 + ↑	No/family case (father and two daughters)	[11]
4a_Female_ European	Childhood/2 years	p.P34H AD GOF	Recurrent sinopulmonary infections	L↓↓, N↓, CD19 + ↓, RTE↓, Tregs↓, CD4 + ↓, CD8 + ↓, CD8 + CD57 + ↑, IgG↓		
4b_Female_ European	Childhood/7 years	p.P34H AD GOF	Recurrent sinopulmonary infections	L↓↓, N↓, CD19 + ↓, RTE↓, Tregs↓, CD4 + ↓, CD8 + ↓, CD8 + CD57 + ↑, IgG↓		
5_Female_ USA	2 years/ 37 years	p. E62K AD GOF	Recurrent sinusitis, pneumonia, sepsis, lymphadenitis, varicella zoster infection, urinary tract infections, cellulitis, **littoral cell angioma** (22 yr spleen), bronchiectasis,	N migration↓↓,	No/ No family history of immunodeficiency	HSCT, alive 41 yr [10]
6_Female_USA	9 month/ Screening*	p. E62K AD GOF	Acute bronchitis with persistent wheezing	N migration↓↓, TRECs↓, CD3 + ↓	No/ No family history of immunodeficiency	HSCT, alive 2 yr [10]
7_Male_ USA	3 years/ 14 years	p. E62K AD GOF	Recurrent herpes stomatitis, otitis media, chronic rhinitis, cough	N migration↓, IgM↓,	No/ No family history of immunodeficiency	HSCT, alive [10]
8_Female_ Ukraine	4.5 months / 8 years	p.N92 T AD GOF	Recurrent respiratory tract infections, failure to thrive, lymphadenopathy, splenomegaly, repeated herpetic skin infections	N migration↓↓,CD18+, CD15+, CD11a,b,c+, TRECs↓, KRECs↓, IgG↓, Bmem↓, L↓	No/ No family history of immunodeficiency	Died after II HSCT/ Current report

* SCID newborn screening.

another three patients have been described. Two siblings manifested with recessively inherited common variable immunodeficiency with abnormalities in the T cell compartment (8). A third patient with a dominant-negative *RAC2* mutation was identified through newborn screening for severe combined immunodeficiency, and initially presented with lymphopenia (2, 9). All to date identified patients had abnormalities in the guided movement of neutrophils in response to chemical signals (2, 5–9). In 2019, 6 new patients with gain-of-function dominant mutations were described (10, 11).

Here we used whole exome sequencing (WES) and functional assays to immunologically and functionally characterize a patient with a previously unreported activating mutation in *RAC2*.

2. Clinical case

A girl of Ukrainian origin was born to non-consanguineous marriage after uncomplicated pregnancy. From 4.5 months, she suffered from recurrent otitis media (> 10 episodes, with 5 paracentesis procedures, and a spontaneous eardrum perforation at age 4 years). Pulmonary features developed at age 12 months and included recurrent pneumonia and chronic bronchitis that progressed to chronic obstructive pulmonary disease (COPD) at the age of 5. Computer tomography (12) scan demonstrated bilateral pulmonary lymphadenopathy in bronchial roots and mediastinum (Fig. 1), as well as pneumofibrosis and chest deformation (Fig. 1B) at the age of 9 years. Generalized lymphadenopathy and hepatosplenomegaly were repeatedly detected on clinical examination. Additionally, the girl experienced a number of severe viral infections, including chicken pox at age 7, and two episodes of extensive herpetic lesions in the skin (Fig. 1B). Laboratory studies revealed leukopenia (2900–3100 cells/μL NR 5200–10,100 cells/μL) and low IgG (1.1 g/L, 5.04–12.6 g/L) at the age of 4.

Despite regular administration of high-dose intravenous Ig (since the age of 4 years and 7 months) and other prophylactic medications, her weight and height were below the third percentile likely due to repeated infections (20 kg/124 cm, 9 y.o.). She underwent her first hematopoietic stem cell transplantation (HSCT) at the age of 9 years 2 months from a fully identical unrelated donor (FIUR) (6.2×10^6 CD34+ cells/kg, 6.4×10^8 total nucleated cells/kg) with the conditioning regimen fludarabine, treosulfan, anti-thymocyte globulin. Neutrophil and platelet engraftment was achieved on days +20/+23 and 90% donor chimerism was detected on day +20. The post-transplant course was complicated by CMV viremia (+35 day) which responded to ganciclovir and foscarnet combination (donor and recipient were CMV negative before HSCT). Due to the decreased chimerism (+110 day) and increased frequency of viral infections, she underwent second HSCT at the age of 9 years 10 months (FIUR BM, total nucleated cells = 14.5×10^8/kg and CD34 = 9.6×10^6/kg). Conditioning regimen with busulfan, fludarabine and ATG fresenius was used. The

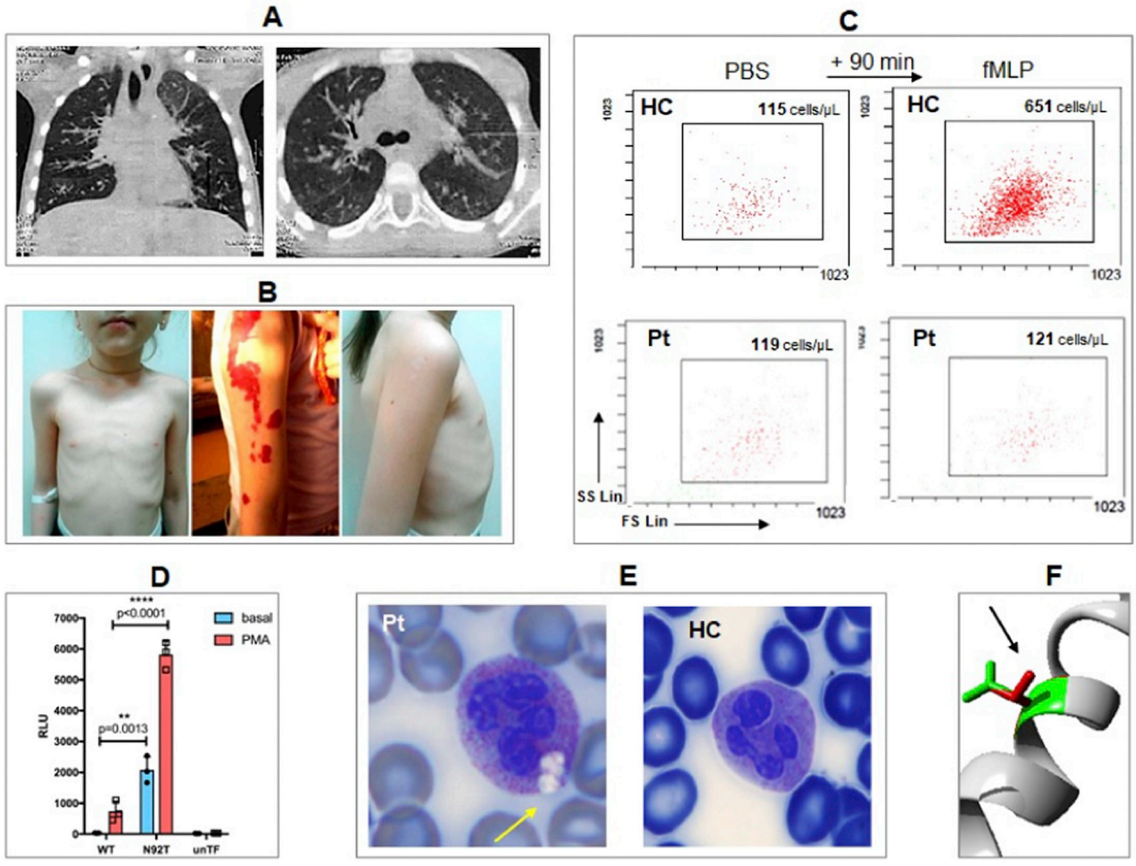

Fig. 1. A. Computed tomography scans of the patient at the age 8 years. Frontal **(1A left)** and axial **(1A right)** image of chest CT demonstrating severe lymphadenopathy and diffuse interstitial lung changes with ground glass opacities in the right lower lobe; B. **(1B left)** The thorax was barrel shaped, with a vascular network on the anterior wall of the abdomen; Massive herpetic lesion of the right hand skin before **(1B middle)** and after antiviral **(1B right)** treatment; C. Neutrophil migration in control **(1C upper left and right)** and patient **(1C lower left and right).** D. Superoxide production from HEK293 cells transfected with components of NADPH oxidase complex and either WT or N92 T RAC2 expression constructs in the presence (red) or absence (blue) of PMA. $P < .0001$ (student *t*-test). E.Light microscopy images of Wright-Giemsa stained blood smears from healthy control (HC) (**1E upper**) and patient Pt (**1E lower**). Large vacuoles are clearly present within the neutrophils of Pt (**yellow arrow**) (Magnification x1000). Fig. 1F. Three-dimensional structural representations of Rac2 protein (HOPE software). 3D homology contains the native Asp-92 residue shown in green and the mutant Trp-92 shown in red (**black arrow).** (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

post-transplant course was complicated by bone marrow aplasia secondary to adenovirus infection in spite of full chimerism. The patient died on day 86 post-HSCT due to disseminated adenovirus infection.

Detailed laboratory evaluations were first performed at 5 years of age. The patient was lymphopenic with normal relative distributions of T and B cells. The memory B cells were reduced, with accompanying absence of isohemagglutinins and low IgG level (Table S1). The persistent absolute lymphopenia, normal T cell receptor Vβ distribution, and low numbers of T and B cell receptor excision circles (TRECs and KRECs) suggested a combined immunodeficiency. Immunological follow-up showed that the total lymphocyte counts and the proportion of memory B cells remained low, and there was a progressive decrease of TRECs and KRECs (Table S1).

3. Genetic and functional analysis

Due to suspicion of inherited combined immunodeficiency, the patient underwent whole exome sequencing. We identified a novel heterozygous missense mutation p.N92T (Chr 22:37627985 G > T) in *RAC2*. The mutation lies within a highly conserved region of the protein (Fig.S1·B) and has a Combined Annotation-Dependent Depletion (CADD) score above 26.2. Multiple *in silico* models predict this *RAC2* mutation to be deleterious (Proven prediction "Deleterious," score 5.67; SIFT prediction "Damaging," score 0.022; PolyPhen2 prediction "Probably damaging," score 0.54, LTR prediction "Damaging", score 0.0, MutationTaster – "Disease causing"). 3D modeling showed that the mutant residue is smaller with a possible loss of external interactions (Fig. 1C). In addition, we found a few heterozygous variants in PID genes that were not estimated as disease causing (Table S2). The variant p.N92 T in *RAC2* was confirmed by Sanger sequencing in the patient's DNA and was absent in the mother; no paternal biological material was available.

As deficiency of RAC2 is associated with neutrophil dysfunction [6,7], we performed functional studies of neutrophils. Expression of CD18, CD11a,b,c and CD15 on rested and activated neutrophils and monocytes was normal (data not shown). PBMC proliferation after PHA stimulation was not impaired, but lymphocyte aggregation in the patient PBMCs was less prominent than in healthy controls (Fig.S2.A). Neutrophils from the proband had reduced chemotaxis towards fMLP compared to neutrophils from control subjects (Fig. 1C). Since RAC2 is the regulatory cytosolic component of the human neutrophil NADPH oxidase [3,4], we evaluated superoxide production (or release) by HEK-293 cells transfected with vectors expressing components of the NADPH oxidase ($gp91^{phox}$, $p67^{phox}$ and $p47^{phox}$) as well as RAC2 wild-type or N92 T. Cells transfected with RAC2-N92 T exhibited increased baseline ROS production, which was further accentuated after addition of PMA (Fig. 1D). Similar to the recently reported RAC2 activating mutation, E62K [10], our patient's neutrophils displayed aberrant macropinocytotic vesicles (Fig. 1E lower) not seen in the healthy control.

Taken together, the abnormal neutrophil migration, excessive ROS production and large macropinocytotic vesicles suggest that the heterozygous RAC2, p.N92 T mutation is an activating mutation leading to the clinical and immunologic abnormalities in the patient.

4. Discussion

Here we report a new heterozygous single base mutation c.275A > C, p.N92 T in *RAC2* as a cause for combined immunodeficiency in a female patient of Slavic origin. This mutation is not present in current online databases (dbSNP, Exac, GnomAD, ClinVar, 1000 genomes) at the time of query (April 2019).

Our patient developed symptoms very early, suffering from recurrent purulent otitis media, sinusitis, bronchitis and pneumonias, with the lung disease progressing to chronic bronchitis and pulmonary fibrosis at the age of 8. Repeated upper and lower respiratory tract infections led to the diagnosis of common variable immunodeficiency (CVID). CVID has previously been reported with *RAC2* mutation (8, 10, 11), and the key finding in those (as well as in our) patients was the absence of severe clinical abnormalities in the neonatal period that are associated with neutrophil dysfunction.

Antibiotic prophylaxis and regular intravenous immunoglobulin replacement (IVIG) therapy were initiated at age 5 but did not improve the clinical disease and the patient continued to suffer from various infections. Dynamic follow-up in the following 3 years revealed constant lymphopenia, decreasing TREC counts accompanied with severe herpetic infections, interstitial pneumonia, and failure to thrive.

RAC2 mutations have been associated with both autosomal dominant and recessive inheritance, with variable phenotypic presentations (2, 5–9). Two of the eleven reported patients harbored the same mutation in *RAC2* gene with a dominant negative effect on protein function (2, 5, 7, 9). One consanguineous family had a homozygous nonsense mutation (8) and 6 patients had gain-of-function (GOF) mutations (10, **11**). With the patient we are presenting here, the cohort of all *RAC2* cases reported worldwide increases to eleven patients from 8 families. Table summarizes the common clinical features in published patients.

Eight described patients displayed abnormalities in neutrophil migration and 5 had low TRECs level at the time of study. TREC level was measured in childhood in our patient and in adulthood in the Iranian siblings (**8**). It is possible that the TREC levels are diminished already at birth, as in the American patients (**9**, 10), and the condition could have been detected in SCID newborn screening [2]. Interestingly, while the D57N loss of function patients showed leukocytosis with neutrophilia (2, 6, 7, 9), the phenomenon was not evident in our patient – one of her main laboratory abnormalities was constant and severe leucopenia from age 4. The studied patient exhibited B cell defects, including the reduction of KREC and B cell counts and low memory B cells (Table S1).

Collectively these findings indicate that RAC2 mutation leads to defects in both myeloid and lymphoid immunity. Besides regulating oxidative phosphorylation, RAC2 participates in signaling and actin cytoskeleton formation, both of which apply broadly to immune cell migration and to the formation of T and B cell immunologic synapses.

5. Conclusion

We report a new case of heterozygous gain-of-function RAC2 mutation, with the clinical presentation of recurrent respiratory infections, lung disease and susceptibility to varicella and herpetic infections. Our report expands the phenotypic spectrum of RAC2 mutations, with presentations of combined immunodeficiency where both myeloid and lymphoid lineages are affected.

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All authors declare no conflicts of financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.05.003.

References

[1] B. Petri, M.J. Sanz, Neutrophil chemotaxis, Cell Tissue Res. 371 (3) (2018) 425–436.

[2] J.M. Routes, W.J. Grossman, J. Verbsky, R.H. Laessig, G.L. Hoffman, C.D. Brokopp, et al., Statewide newborn screening for severe T-cell lymphopenia, Jama 302 (22)

(2009) 2465–2470.
[3] U.G. Knaus, P.G. Heyworth, T. Evans, J.T. Curnutte, G.M. Bokoch, Regulation of phagocyte oxygen radical production by the GTP-binding protein Rac 2, Science 254 (5037) (1991) 1512–1515.
[4] R. Fattouh, C.H. Guo, G.Y. Lam, M.G. Gareau, B.Y. Ngan, M. Glogauer, et al., Rac2-deficiency leads to exacerbated and protracted colitis in response to Citrobacter rodentium infection, PLoS One 8 (4) (2013) e61629.
[5] D.R. Ambruso, C. Knall, A.N. Abell, J. Panepinto, A. Kurkchubasche, G. Thurman, et al., Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation, Proc. Natl. Acad. Sci. U. S. A. 97 (9) (2000) 4654–4659.
[6] A.G. Kurkchubasche, J.A. Panepinto, T.F. Tracy Jr., G.W. Thurman, D.R. Ambruso, Clinical features of a human Rac2 mutation: a complex neutrophil dysfunction disease, J. Pediatr. 139 (1) (2001) 141–147.
[7] D.A. Williams, W. Tao, F. Yang, C. Kim, Y. Gu, P. Mansfield, et al., Dominant negative mutation of the hematopoietic-specific rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency, Blood 96 (5) (2000) 1646–1654.
[8] O.K. Alkhairy, N. Rezaei, R.R. Graham, H. Abolhassani, S. Borte, K. Hultenby, et al., RAC2 loss-of-function mutation in 2 siblings with characteristics of common variable immunodeficiency, The Journal of allergy and clinical immunology. 135 (5) (2015) 1380–4 e1–5.
[9] D. Accetta, G. Syverson, B. Bonacci, S. Reddy, C. Bengtson, J. Surfus, et al., Human phagocyte defect caused by a Rac2 mutation detected by means of neonatal screening for T-cell lymphopenia, The Journal of allergy and clinical immunology. 127 (2) (2011) 535 (8 e1-2).
[10] A.P. Hsu, A. Donko, M.E. Arrington, M. Swamydas, D. Fink, A. Das, et al., Dominant activating RAC2 mutation with lymphopenia, immunodeficiency and cytoskeletal defects, Blood 133 (18) (2019 May 2) 1977–1988.
[11] V. Lougaris, J. Chou, A. Beano, J.G. Wallace, M. Baronio, L. Gazzurelli, et al., A monoallelic activating mutation in RAC2 resulting in a combined immunodeficiency, The Journal of allergy and clinical immunology. 143 (4) (2019) 1649–53 e3.