

DNA double-strand breaks and repair are important for the rearrangement of genomic material, which is crucial for the maturation and diversification of T and B cells. The major mechanism for repair of double-strand breaks in mammalian cells is DNA nonhomologous end-joining, which occurs by the coordinated effect of at least 5 proteins, including Ku70, Ku80, DNA-dependent protein kinase (DNA-PK), Cernunnos, and DNA ligase IV.⁵ Mutations in DNA ligase IV have been previously described in patients ranging in age from 4 to 48 years, and a variety of clinical presentations, including leukemia, lymphoma, bone marrow failure, or developmental delay.^{6,7} A subset of patients with DNA ligase IV mutations and practically absent circulating T and B cells resulting in SCID have been recently reported.^{4,8,9} The patient described here, in addition to SCID, uniquely presented with typical clinical and laboratory features of OS. Unlike mutations that completely abolish ligase IV assisted variable-diversity-joining chain recombination, such as the deletion found on 1 allele in our patient, the H282L mutation found on the other allele probably allowed residual signal-joint formation, resulting in leaky SCID. Indeed, in the 2 previous patients who had the DNA ligase IV H282L mutation, as many as 1074 CD3⁺ T cells/ μ L and wide V β -chain representation were found.⁴

BMT remains the treatment of choice for patients with SCID and OS. Among the few patients with DNA ligase IV-associated SCID who were transplanted, 1 died during conditioning, 2 died from veno-occlusive disease, and 1 died of posttransplant lymphoproliferative disease shortly after BMT.^{4,8,9} In contrast, another patient who received nonmyeloablative conditioning and subsequent matched unrelated donor BMT survived a year after BMT, but his immune reconstitution was not reported.⁴ The patient described here survived full myeloablative conditioning with no persisting long-term acute adverse effects, suggesting tolerance to chemotherapy despite ligase IV-deficient cells' increased sensitivity to ionizing radiation.

In conclusion, we demonstrate here that patients with mutations in DNA ligase IV can present with features of OS. The leaky SCID can be corrected with BMT in spite of the sizable number of circulating T cells, which can be effectively and safely erased with full conditioning.

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Eyal Grunebaum, MD

Andrea Bates, BSc

Chaim M. Roifman, MD, FRCPC

From the Division of Immunology and Allergy, The Hospital for Sick Children, and the University of Toronto, Toronto, Ontario, Canada. E-mail: chaim.roifman@sickkids.ca.

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Association of the lymphoid protein tyrosine phosphatase, R620W variant, with chronic mucocutaneous candidiasis

To the Editor:

Chronic mucocutaneous candidiasis (CMCC) is a heterogeneous group of disorders with a common manifestation of persistent or recurrent candidal and fungal infections of the skin, nails, and mucous membranes. The best delineated entity, autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (APECED), is caused by mutations in the autoimmune regulator (*AIRE*) gene. However, *AIRE* mutations are found in only about a third of cases with CMCC in our institution. In a search for genetic predisposition to CMCC in patients without APECED, we studied the protein tyrosine phosphatase nonreceptor 22 (*PTPN22*), 1858T, a variant of the lymphoid protein tyrosine phosphatase (LYP) R620W.¹

Lymphoid protein tyrosine phosphatase, which was first cloned and sequenced in our laboratory, was found to have a primary role in regulation of T-cell and B-cell antigen receptor signaling.²⁻⁴

An association of a single nucleotide transition in *PTPN22* 1858C>T with a variety of autoimmune diseases including rheumatoid arthritis, type I diabetes, and SLE as well as Graves disease and Addison disease has been established.^{1,5,6} The R620W variant is known to disrupt LYP's interaction with the regulatory kinase, c-src tyrosine kinase (CSK), although the functional consequences of this remain to be determined.¹ We have studied here the clinical manifestations and immunologic laboratory findings of patients with *PTPN22* 1858T.

Fourteen patients with CMCC and autoimmune features (without *AIRE* mutations) were identified. In addition, 5 other patients carrying mutations in the *AIRE* gene were included in this study for comparison purposes. None of the patients in our cohort were members of same kindred, and no autosomal-dominant pattern of inheritance was suggested from family history. The median age of patients with CMCC was 38 years (range, 7-55 years), and the median age at presentation was 3 years. For patients with APECED, the median age was 18 years (range, 3.5-30 years), and median age of presentation was 1.5 years (Table I).

Genomic sequencing of the *PTPN22* gene revealed that 6 of 14 patients, all white, had heterozygous variant allele 1858T, whereas in our control group, matched for sex and ethnicity, 34 of 189

TABLE I. Demographic data on patients with CMCC

	CMCC with no <i>AIRE</i> mutation	APECED
No. of patients	14	5
Sex		
Male	4	2
Female	10	3
Median age of presentation	3 y	1.5 y
Range of age of presentation	1 week to 40 y	0.1-3 y
Current median age	38 y	18 y

carried the variant allele. The allele frequencies were significantly different between the patients and control groups, with a frequency of 0.428 in the patient group and 0.179 in the control group (similar to historic controls) $\chi^2 = 5.095$; $P = .024$; odds ratio, 3.42; 95% CI, 1.11-10.48.

None of the 5 patients with APECED with homozygous mutations in the *AIRE* gene carried the *PTPN22* variant, 1858T.

All 14 patients, regardless of their *PTPN22* alleles, had chronic candidiasis; 86% of the patients had oral candidiasis; 92% had chronic fungal skin infections; and 64% had typical involvement of the nails.

Analysis of the group of patients carrying *PTPN22* 1858T revealed a high prevalence of endocrinopathies, affecting 83% (5/6) of the patients. Hypothyroidism and gonadal failure were the most common, each found in 50% of the patients, whereas only 1 patient had adrenal insufficiency. Gonadal failure was found only in the group with the 1858T allele. This endocrine disorder is also a common feature of APECED, affecting about 60% of the patients.⁷

By comparison, only 3 of the 8 patients with the common *PTPN22* allele, 1858C, had endocrinopathy, all with hypothyroidism. In the distinct APECED group, polyendocrinopathy affected 4 of the 5 patients, most commonly with hypoparathyroidism and Addison disease (Table II).

Other autoimmune disorders were identified more frequently in the group of the common *PTPN22* allele, including arthritis (25%), alopecia (25%), idiopathic thrombocytopenic purpura, polymyositis, and enteropathy. In the *PTPN22* 1858T group, 2 patients had arthritis, and another had chronic enteritis. In the

TABLE II. Clinical features of patients with CMCC

	LYP 1858T (N = 6)	LYP 1858C (N = 8)	APECED (N = 5)
Clinical features			
Oral candidiasis	5	7	5
Skin candidiasis and/or dystrophy	6	8	5
Nails dystrophy, onychomycosis	2	5	5
Enamel defect	None	None	4
Pneumonia	5	2	1
Bronchiectasis	3	1	None
Endocrinopathies			
Hypothyroidism	3	3	1
Gonadal failure	3	None	1
Adrenal insufficiency	1	None	3
Hypoparathyroidism	None	None	4
Other autoimmune manifestations			
ITP	None	1	None
Alopecia	None	2	1
Vitiligo	None	None	3
Arthritis	2	2	None
Polymyositis	None	1	None
Gastrointestinal	2	1	1

ITP, Idiopathic thrombocytopenic purpura.

patients with APECED, vitiligo was the most common autoimmune feature. Conversely, none of the patients with the 1858T allele had vitiligo (Table II).

Remarkably, patients carrying the *PTPN22* 1858T had a higher frequency of infections than patients who carry the common allele or patients with APECED. Eighty-three percent of patients within the *PTPN22* 1858T group had severe pulmonary infections including bronchiectasis, compared with only 25% in the common 1858C allele group. This is likely explained by laboratory studies revealing that 4 of the 6 patients carrying the variant allele had antibody defects, including hypogammaglobulinemia (p1,5) or dysgammaglobulinemia (p2,3), manifested as inadequate responses to protein and/or polysaccharide vaccinations (Table III). The exact mechanism whereby *PTPN22* 1858T predisposes individuals to microbial infections remains unclear.⁸ In part,

TABLE III. Laboratory indices of patients with the LYP variant 1858T

Patients with 1858T	1	2	3	4	5	6	Normal range	Patients with 1858C¶
Age at evaluation (y)	10	20	13	43	35	46		
ALC (cells 10 ⁹ /L)	2.71	1.68	3.2	1.78	1.55	3.19	1.4-3.3	1 of 8
<i>In vitro</i> response to <i>Candida</i> *	1.6	0.6	6	17	25	0.7	>20	Low in 6 of 8
Immunoglobulins								
IgG g/L	4.7	8.7	13.2	12.1	5.2	11.3	7.2-15.8	None
IgA g/L	1.57	1.2	0.9	1.7	3.5	1.5	0.5-3.5	None
IgM g/L	1.45	0.5	0.6	0.7	0.4	2.1	0.2-3.1	None
Tetanus IU/mL†	NA	0.15	0.02	4.0	NA	1.26	>0.04§/>1.00	1 of 8
Pneumococcus mg/L (fold increase)‡	NA	130 (<2)	10.1 (<1)	>270 (>4)	NA	>270 (>4)	>270 (>4 fold increase)	1 of 8

ALC, Absolute lymphocyte count; NA, not available.

*Stimulation index.

†Level post vaccination.

‡Fold increase 4 to 6 weeks postvaccination.

§Protective level.

||Level after tetanus boost.

¶Number of patients with abnormal results.

this may be explained, as recently shown, by the finding that lymphocytes carrying the *PTPN22* variant had decreased responses to stimulation by anti-CD3 and by anti-IgM as well as decreased numbers of memory B cells (CD19⁺CD27⁺ population).⁹ In the future, similar additional abnormalities may eventually evolve in patients with CMCC with the *PTPN22* variant 1858T.

None of the patients had lymphopenia, and lymphocyte markers showed normal numbers of CD3, CD4, CD8, CD19, and CD56-positive cells in 4 patients. One patient had a low CD4 count (P2), and another had a relatively low number of B cells (P6).

In vitro mitogenic responses to phytohemagglutinin or anti-CD3 antibody were comparable to controls, but proliferative responses to *Candida* were low or absent in 4 of 6 patients.

In contrast, only 1 of 8 patients with the common allele showed low responses to tetanus vaccine, and another patient had mild lymphopenia with a count of 1040 cells/ μ L. As expected, the APECED group of patients exhibited only an isolated defect in cellular response to *Candida* antigen (Table III).

In summary, we have identified a group of patients who carry the *PTPN22* variant allele 1858T, who in addition to chronic candidiasis frequently have recurrent microbial infections caused by antibody deficiency as well as gonadal failure and hypothyroidism.

Amit Nahum, MD, PhD
Andrea Bates, BSc
Nigel Sharfe, PhD
Chaim M. Roifman, MD

From the Canadian Centre for Primary Immunodeficiency and the Division of Immunology and Allergy, Department of Pediatrics, The Hospital for Sick Children and the University of Toronto, Toronto, Ontario, Canada. E-mail: chaim.roifman@sickkids.ca.

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Stimulation of human mast cells by activated T cells leads to N-Ras activation through Ras guanine nucleotide releasing protein 1

To the Editor:

We have recently identified and characterized a novel mast cell (MC) activation pathway initiated by contact with activated T cells.¹ It has been reported that this pattern of activation is associated with phosphorylation of the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase and p38.² We showed that the cytokines IL-8 and oncostatin M are released from MCs after contact with activated T cells and that this process is subject to MAPK inhibition.^{3,4} The best characterized upstream regulator of the MAPK system is the small guanosine triphosphate (GTP)-binding protein Ras.⁵ This protein has critical functions in many cell types; however, in MCs its functions are not entirely understood. Therefore, and on the basis of our previous findings, we decided to study the spatio-temporal pattern of Ras activation in MCs stimulated by activated T cells.

Ras is a family of small GTP-binding proteins composed of 3 closely related isoforms: H-Ras, N-Ras, and K-Ras.⁵ These isoforms are expressed in most mammalian cells yet exhibit different biologic outputs: K-Ras mutations are frequently seen in tumors, N-Ras is the central isoform in lymphocytes, and H-Ras regulates angiogenesis.⁵ First, we analyzed the expression levels of the 3 Ras isoforms in different human MC lines. We used LAD2 and HMC-1 MC lines and Jurkat T cells as the control.^{3,6,7} The endogenous levels of the different isoforms were analyzed by means of immunoblotting with specific antibodies. As shown in Fig 1, A, and similar to what has been reported in T cells, both MC lines expressed N-Ras and K-Ras but not H-Ras. The specificity of the antibodies was tested against overexpressed Ras isoforms in LAD2 cells (Fig 1, B).

Ras is a molecular switch and as such can bind either GTP or guanosine diphosphate (GDP). The GTP-binding state activates Ras, whereas the replacement by the nucleotide GDP deactivates it.⁵ We therefore further analyzed whether Ras was activated after contact with T cells. LAD2 cells were incubated with membranes derived from resting or activated (50 ng/mL phorbol 12-myristate 13-acetate [PMA] for 60 minutes followed by extensive washing) T cells or by FCεRI cross-linking.^{4,8} Activated Ras molecules were affinity precipitated with the GST-Ras-binding domain (RBD) pull-down assay.⁷ Total Ras was immunoprecipitated with pan-Ras antibody. As shown in Fig 1, C, the total levels of Ras were similar in all conditions. However, differences were noticed in the fractions of GTP-loaded Ras. In both resting MCs and MCs incubated with membranes from resting T cells, the pool of activated Ras was faintly detected. When MCs were stimulated by FCεRI cross-linking, Ras was found to be activated. Likewise, incubation of MCs with activated T-cell membranes resulted in Ras activation (Fig 1, C).

To further support these findings and to study the localization of activated Ras, we used imaging techniques. LAD2 cells were transiently transfected with cyan fluorescent protein (CFP)-tagged N-Ras and the fluorescent probe for activated Ras, yellow fluorescent protein (YFP)-labeled RBD.⁷ After 48 hours, the cells were treated as indicated in Fig 1, D, and imaged alive with confocal microscopy. As shown in Fig 1, D, we were able to detect mild recruitment of the probe to the Golgi apparatus in all