

Letter to the Editor

The L412F variant of Toll-like receptor 3 (*TLR3*) is associated with cutaneous candidiasis, increased susceptibility to cytomegalovirus, and autoimmunity*To The Editor:*

Chronic mucocutaneous candidiasis (CMCC) is a heterogeneous group of disorders with a common feature of noninvasive, persistent *Candida* species infections of the skin, nails, and mucus membranes. The best studied subgroup of patients with CMCC were found to have mutations in the autoimmune regulator gene (*AIRE*).¹ *AIRE*-deficient patients suffer most commonly from hypoparathyroidism and adrenal insufficiency in addition to chronic fungal skin manifestations. Although common in Finland, as well as among some ethnic groups, such as Sardinians and Iranian Jews, most patients with CMCC in North America do not carry mutations in *AIRE*. Moreover, many of these patients frequently present with clinical features uncommon in patients with *AIRE* deficiency. Polymorphism in the *Dectin-1* gene was recently found to be associated with susceptibility to recurrent *Candida* species infections, yet these patients did not have endocrine abnormalities or other autoimmune manifestations.² Another subgroup of patients with CMCC were found to carry the R620W variant of lymphoid protein tyrosine phosphatase (*LYP*) in a high frequency (PTPN22).³ These patients, unlike *AIRE*-deficient subjects, had mainly hypogonadism and hypothyroidism, as well as susceptibility to microbial infections.

In a search for other genetic abnormalities predisposing to CMCC, we studied Toll-like receptors (TLRs) in a group of patients with CMCC who had no genetic abnormalities in the *AIRE*, *LYP*, or *Dectin-1* genes. TLRs were studied because they play an important role as sensors for viral and fungal pathogens. Ligation of TLRs triggers secretion of proinflammatory cytokines, such as type I interferons, which protect against viruses but also initiate adaptive immune responses.

Susceptibility to *Candida* species was attributed to genetic abnormalities in *TLR2* and *TLR4* receptors.^{4,5} Mice lacking *TLR3* display inadequate responses to murine cytomegalovirus (CMV),⁶ whereas rare mutations in human *TLR3* result in recessive predisposition to encephalitis caused by herpes simplex virus type I.⁷ Moreover, normal activation of *TLR3* is involved in suppression of autoimmune disorders, such as in a murine encephalomyelitis model, making aberrant forms of this receptor a candidate for CMCC with autoimmunity.⁸

Twenty-one patients with CMCC who had no mutations in the *AIRE*, *LYP*, *Dectin-1*, or *IL-2 receptor α* genes were studied. The coding region of *TLR3* was sequenced in all patients. Fourteen (66%) patients were found to carry the *TLR3* L412F mutant. None of these patients were related nor were they products of consanguineous families. Their age ranged from 1 to 30 years, with a median of 5.2 years. Ten were female and 4 were male subjects. More than a third were of Middle Eastern or Asian origin, and the rest were presumed white. One patient was homozygous for this mutation, whereas the rest carried the change on 1 allele only. No other mutations in the *TLR3* gene were identified in these patients, and other candidate genes involved in immune response to *Candida* species, including *TLR1*, *TLR2*, *TLR4*, and *TLR6*, were normal.

TABLE I. Clinical features of patients with the *TLR3* L412F variant and APECED

	<i>TLR3</i> L412F (n = 14), no. (%)	APECED (n = 5), no. (%)
Infections		
Oral thrush	14 (100)	5 (100)
Skin candidiasis	12 (86)	5 (100)
Nail infection/dystrophy	9 (64)	5 (100)
Pneumonia	11 (79)	1 (20)
Bronchiectasis	7 (50)	0
CMV infection	6 (43)	0
Endocrinopathies		
Hypothyroidism	7 (50)	1 (20)
Gonadal failure	1 (7)	1 (20)
Adrenal insufficiency	2 (14)	3 (60)
Hypoparathyroidism	1 (7)	4 (80)
Other autoimmune manifestations		
ITP/pancytopenia	6 (43)	0
Alopecia	3 (21)	1 (20)
Vitiligo	1 (7)	3 (60)
Arthritis	2 (14)	0
Enteritis	8 (57)	1 (20)

ITP, Idiopathic thrombocytopenic purpura; *TLR3* L412F, patients with the variant allele.

The allele frequency of *TLR3* L412F in this group of patients was markedly higher than in control groups. The National Center for Biotechnology Information Single Nucleotide Polymorphism database reports an allele frequency of 0.21 in a mixed population, including white, African American, and Asian subjects, whereas the National Heart, Lung, and Blood Institute refers to frequencies ranging from 0.3 (European) to 0.08 (African), with an overall median frequency of 0.21. The frequency in our population appears significantly higher, regardless of the control population chosen. Patients had a frequency of 0.66 compared with the frequency in a control mixed population of 0.21 ($P = .00008$, Fisher exact probability test, 2-tailed; odds ratio, 4.4; 95% CI, 1.6-10.48).

The patient with the homozygous L412F *TLR3* variant had protracted oral and esophageal candidiasis, as well as a poor response to antifungal treatment since infancy. At 6 years of age, she had severe hepatitis caused by CMV and continued to harbor detectable virion copies, for which she received multiple courses of ganciclovir. The patient had recurrent sinopulmonary infections resulting in chronic lung disease with bronchiectasis. She also had chronic thrombocytopenia since the age of 5 years. She subsequently had hemolytic anemia, as well as neutropenia. At 10 years of age, she had a bout of CMV-induced viremia, progressive pneumonitis, and exacerbation of pancytopenia, which were resistant to treatment with antiviral medications. She eventually had respiratory failure and subsequently died of overwhelming CMV infection.

Even patients who were heterozygous for the *TLR3* L412F mutant had severe chronic infections. Repeated severe pulmonary infections affected 11 (79%) patients in this group, with 7 of them having severe chronic lung disease complicated with bronchiectasis. Furthermore, nearly 60% of them had recurrent sinusitis. We have reviewed the clinical manifestation of patients with CMCC and *AIRE* mutations who did not carry the L412F variant to

TABLE II. Laboratory evaluation of patients with the *TLR3* L412F variant

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Normal range
ALC $\times 10^9/L$	2.6	1.5	4.9	1.8	1.1	0.38	1.2	1.45	3.2	3.7	1.4-3.3
CD3 cells/ μL	2,338	1,324	3,785	1,689	850	549	976	1,131	2,998	2,474	1,000-2,200
CD4 cells/ μL	1,428	706	2,288	1,108	459	93	545	881	1,353	1,953	530-1,300
CD8 cells/ μL	828	570	1,231	497	382	337	377	219	1,379	414	330-920
CD19 cells/ μL	230	164	932	248	32	181	134	194	146	1,273	110-570
NK cells/ μL	26	85	166	143	179		65	83	138	179	70-480
Lymphocyte proliferation*: Mitogenic responses											
PHA	15	65	35	100	100	30	100	100	40	68	>50% of control*
Antigen responses											
<i>Candida</i> species	0	100	0	100	100	10	0	15	0	24	>25% of control*
Tetanus	0	NA	0	100	100	0	0	10	0	NA	
CMV	NA	0	0	18	0	0	0	10	0	16	
Immunoglobulins (g/L)											
IgG	4.7	5.2	13.9	10	4.8	21.3	29.3	11.2	4.8	7.3	7.2-15.8
IgA	1.57	3.5	3.9	1.1	0.7	0.7	4.9	1.8	0.5	2.3	0.5-3.5
IgM	1.45	0.4	1.5	1.2	0.3	0.8	0.4	1.4	0.4	0.7	0.2-3.1
Specific antibody											
Tetanus	NA	NA	0.02	NA	NA	0.13	0.2	0.44	0.08	1.08	>0.04†/>1.00‡
Measles	NA	NA	Negative	Positive	NA	Negative	Negative	Positive	Negative	Positive	
Mumps	NA	NA	Negative	Positive	NA	Negative	Negative	Positive	Negative	Positive	
Rubella	NA	NA	Negative	Positive	NA	Negative	Negative	Negative	Negative	Positive	

ALC, Absolute lymphocyte count; NK, natural killer.

*Expressed as stimulation index percentage of healthy control subjects.

†Protective level.

‡Level after tetanus boost.

highlight the difference between these groups. Indeed, in sharp contrast, but with complete agreement with previous reports, patients with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) in this cohort had far less severe infections, with only 1 episode of pneumonia. Most striking was the high frequency of CMV-related morbidity in the L412F variant group. Six of 14 patients had moderate-to-severe chronic CMV disease. Two had multiorgan failure with evidence of CMV in the peripheral blood and in various organs. All patients had chronic candidiasis of some form, and 64% had typical nail dystrophy (Table I).

Patients who carried the L412F variant also had a high prevalence of hematopoietic autoimmune disorders. Autoimmune cytopenias were identified in 6 of 14 patients, 4 with thrombocytopenia and 2 with pancytopenia. Eight patients had enteritis, whereas arthritis affected 2 patients. One patient had both alopecia and vitiligo. In comparison, among patients with the *AIRE* mutation, 3 patients had vitiligo, 1 had alopecia, and another had enteritis (Table I).

Hypothyroidism was the most prevalent endocrine abnormality in the L412F variant group, affecting 50% of patients. Addison disease was observed in 2 patients, and gonadal failure, type I diabetes mellitus, and hypoparathyroidism each affected 1 patient (Table I). As expected, hypoparathyroidism and adrenal insufficiency were more common in patients with APECED, whereas hypothyroidism and gonadal failure were far less frequent (Table I).

Ten of 14 patients with the L412F variant allele were available for evaluation of the immune system (Table II). Patient 9, who had the homozygous L412F mutation, showed a complete lack of

proliferation in response to antigenic stimulation (*Candida* species, CMV, tetanus, and others). She also had hypogammaglobulinemia with an inadequate response to immunization with diphtheria-tetanus-pertussis (DTPT) or Pneumovax (Merck & Co, Inc, Whitehouse Station, NJ; data not shown).

Two patients had CD3 lymphopenia, one of whom also had low numbers of CD19 cells. Four patients had borderline low numbers of circulating CD8⁺ T cells (Table II).

In vitro mitogenic responses to PHA were reduced in 4 patients (patients 1, 3, 6, and 9), but more frequently, responses to antigens were markedly reduced (Table II). Specifically, 8 of 10 patients had low responses to tetanus toxoid, 7 of 10 had reduced responses to *Candida* species antigen, and all patients had a dramatically decreased response to CMV. Furthermore, 7 patients in this group had antibody deficiency manifested as hypogammaglobulinemia in 4 patients (patients 1, 2, 5, and 9) or antibody deficiency with normal serum IgG levels (dysgammaglobulinemia) in 3 patients (patients 3, 6, and 7) (Table II). In the latter group responses to tetanus toxoid vaccination, as well as other antibody responses to vaccination, were inadequate. The only consistent immune abnormality in the APECED group has been low T-cell proliferative responses to *Candida* species antigen (not shown).

Recent molecular modeling studies by Ranjith-Kumar et al⁹ have suggested that the L412F mutation, with altered affinity to ligands, might affect normal receptor dimerization and signal transduction.

To evaluate the biological significance of the L412F variant, we tested the ability of the receptor to activate a nuclear factor κ B (NF- κ B) response element in an artificial cell system. NF- κ B is a transcription regulator/factor critical for production of

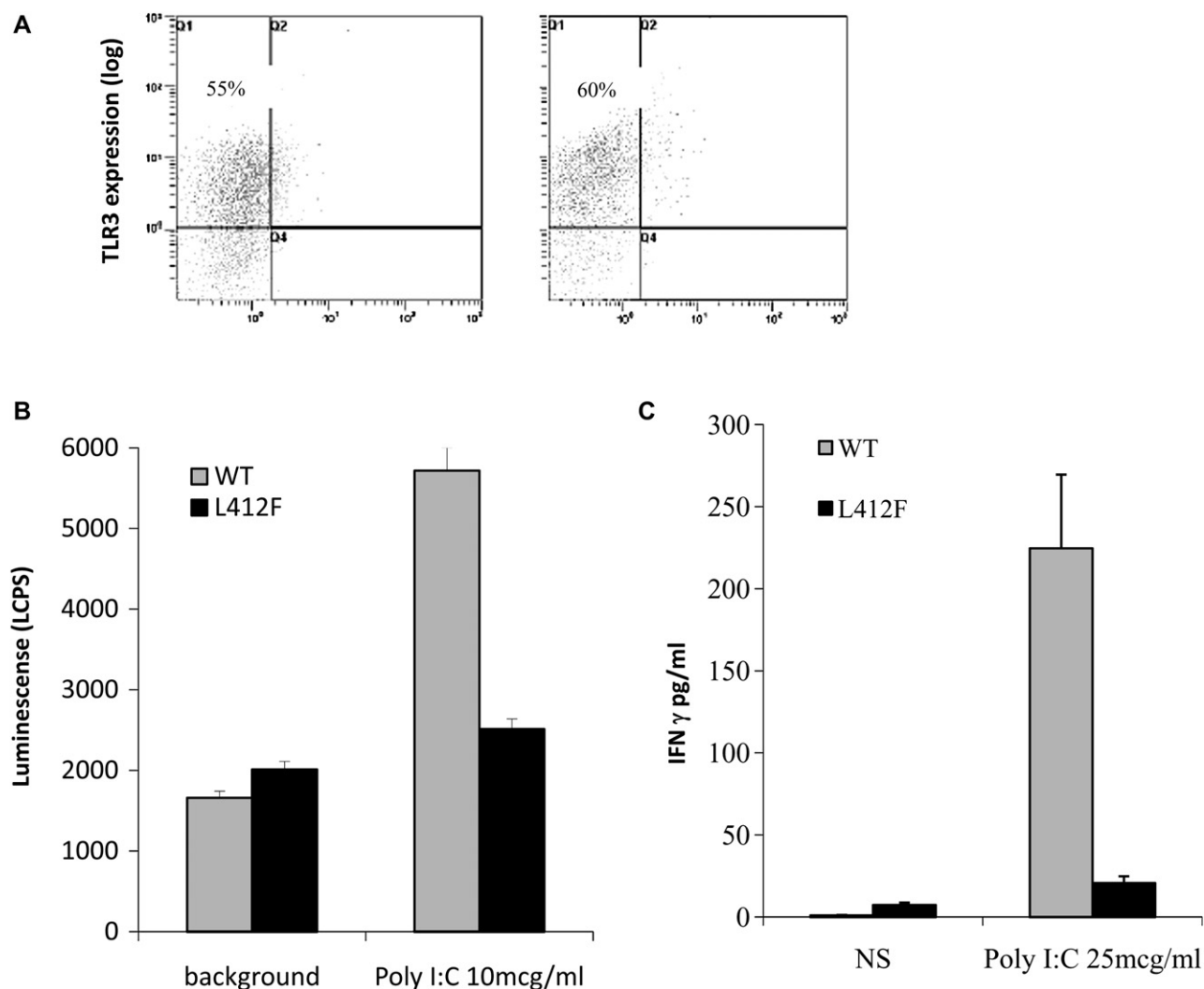


FIG 1. **A**, TLR3 expression on HEK-293 cells transfected with TLR3 WT (left) or TLR3 L412F (right), as measured by means of flow cytometric assay. **B**, NF- κ B response element activation by Poly I:C in HEK293 cells transfected with the WT (shaded bar) or L412F variant (filled bar) TLR3. Results are expressed in luminescence counts per second (LCPS), representing transcription measurement of an alkaline phosphatase secreted to medium and an average of 4 different experiments. **C**, Average production of IFN- γ in PBMCs from 5 healthy control subjects (shaded bars) and 5 patients (filled bars). PBMCs were stimulated with Poly I:C for 24 hours, and supernatants were collected for ELISA. Cells were stained for TLR3 and measured to verify a comparable expression by means of flow cytometry.

inflammatory mediators in response to stimulation of TLR3. We transiently transfected HEK293 cells with either wild-type (WT) or L412F TLR3 and an NF- κ B response element reporter construct encoding secreted serum alkaline phosphatase, as well as positive and negative controls of this reporter system. Expression of the receptor was comparable in the WT and L412F transfected cells as measured by using TLR3 staining with flow cytometry (Fig 1, A). We demonstrated in multiple experiments that in the L412F transfected cells, the induction of the reporter gene after stimulation with 10 μ g/mL polyinosine-polycytidylic acid (Poly I:C), a TLR3 ligand, was only 30% of responses recorded in cells transfected with WT TLR3 receptor (Fig 1, B). These experiments suggested that the L412F variant might indeed interfere with TLR3 function. We have studied cytokine secretion from PBMCs obtained from patients carrying the L412F mutation and healthy subjects with the WT receptor and found a striking

difference in secretion of IFN- γ in response to stimulation with Poly I:C. Patients' cells showed almost 10 times less secretion of IFN- γ compared with cells carrying the WT receptor (Fig 1, C).

We describe here a distinct subgroup of patients with CMCC who carry the L412F mutant of TLR3. These patients, unlike other groups of patients with CMCC, have a high frequency of severe viral infections, especially CMV. This might be explained by the cellular and humoral immune aberrations detected in these patients. We showed that the L412F variant rendered TLR3 dysfunctional, as demonstrated by reduced activation of NF- κ B and reduced secretion of IFN- γ in cells stimulated with the TLR3 ligand Poly I:C. This, together with the high frequency of L412F in this subgroup of patients with CMCC, suggests that this genetic variant might contribute to the phenotype expressed in these patients.

Amit Nahum, MD, PhD
Harjit Dadi, PhD
Andrea Bates, BSc
Chaim M. Roifman, MD

From the Division of Immunology and Allergy, Canadian Centre for Primary Immunodeficiency, 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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