

Letter to the Editor

Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation

To the Editor:

The congenital lymphoproliferative disorder known as B cell Expansion with nuclear factor kappa B (NF- κ B) and T cell Anergy (BENTA) was recently described in 5 patients who all presented with the disease shortly after birth.^{1,2} Salient clinical features of BENTA include splenomegaly and profound polyclonal B-cell lymphocytosis (above 2200/ μ L, 60% to 80% CD19⁺ in peripheral blood), with elevated transitional and mature naive B cells but few circulating class-switched/memory B cells. Patients experience frequent ear and sinopulmonary infections early in life, with poor antibody responses noted to pneumococcal and meningococcal capsular polysaccharides. In addition, T-cell hyporesponsiveness *ex vivo* may be linked to a mild T-cell immunodeficiency in some patients presenting with molluscum contagiosum or chronic EBV infection.

BENTA disease is genetically linked to germline-encoded, gain-of-function mutations in caspase recruitment domain 11 (CARD11), a scaffolding protein largely expressed in lymphocytes and required for antigen receptor (AgR)-induced NF- κ B activation.^{3,4} Like somatic mutations found in approximately 10% of diffuse large B-cell lymphomas, CARD11 mutations in patients with BENTA fall within or immediately adjacent to the coiled-coil (CC) domain.⁵ CC mutations likely abrogate the requirement for AgR-triggered phosphorylation of the inhibitory linker domain, which supports the “open” conformational change necessary for BCL10-MALT1 recruitment and downstream signal transduction via the I κ B kinase complex.⁶

We recently encountered 3 new patients with disease symptoms suggesting BENTA, including moderate, polyclonal B-cell lymphocytosis with a markedly diminished memory B-cell compartment (Table 1). The first patient (patient 1) is an 18-year-old white woman initially evaluated at age 13 months for splenomegaly (10 cm) with splenic hilar adenopathy, leukocytosis (51,000/ μ L), neutropenia (3%), and lymphocytosis (91%). Secondary to recurrent upper respiratory tract (URT) infections, additional immunologic evaluation was completed. Quantitative immunoglobulin levels documented a serum IgG level of 756 mg/dL (normal, 528-2190 mg/dL), a serum IgM level of 75 mg/dL (normal, 48-226 mg/dL), and a serum IgA level of 27 mg/dL (normal, 44-441 mg/dL). Antibody responses to tetanus (0.44 IU/mL) and *Haemophilus influenzae* Type b (0.56 mg/L) vaccine were protective; however, antibody responses to a pneumococcal 13-valent conjugate vaccine were suboptimal (<1.3 μ g/mL for the 11 serotypes that were assessed). Mitogenic stimulation was normal for phytohemagglutinin (stimulation index [SI], 17) and pokeweed mitogen (SI, 9), but low for concanavalin A (SI, 3; laboratory normal SI, >3 for all mitogens). Antigen-specific proliferation was low (tetanus toxoid or Varicella zoster; SI, 2; normal, >3) or even absent (*Candida albicans*). She remains healthy aside from recurrent URT infections, splenomegaly (22 cm) with associated moderate thrombocytopenia (platelet count, 48,000/ μ L; normal, 150-450,000/ μ L) and persistent B-cell lymphocytosis. The second patient (patient 2) is an 18-year-old white man initially

diagnosed at age 2 years as a humoral immunodeficiency patient because of recurrent URT infections, especially otitis media, for which the patient had tympanoplasty. In addition, lower respiratory tract infections with bronchiectasis, splenomegaly (12.5 cm at age 5 years), and peripheral and central lymphadenopathies were reported in childhood. His immunoglobulin serum titers at age 4 years before onset of immunoglobulin replacement therapy were IgG 993 mg/dL (normal, 582-1220 mg/dL), IgM 138 mg/dL (normal, 53-162 mg/dL), and IgA 60 mg/dL (normal, 46-203 mg/dL), and anti-tetanus toxoid antibodies were present (0.22 IU/mL). He has received immunoglobulin substitution since the age of 5 years and is well under this treatment, with no evidence of thrombocytopenia (platelet count, 262,000/ μ L). The third patient (patient 3) is the 51-year-old white mother of patient 2, who is clinically asymptomatic. Childhood medical records were unavailable for this patient; however, she reported several periods of sinusitis in the past. Serum immunoglobulin levels were normal for IgG (1113 mg/dL; normal, 549-1278 mg/dL) and IgA (139 mg/dL; normal, 41-344 mg/dL), but low for IgM (32 mg/dL; normal, 50-209 mg/dL). T-cell responses to phytohemagglutinin were close to normal or normal in patients 2 and 3 (SI, 46 and 82, respectively; laboratory normal SI, >50). Antigen-induced proliferation to tuberculin-purified protein derivative was normal in both patients (SI, 10 and 63, respectively; normal, >10), but responses to *Candida albicans* and tetanus toxoid were either absent in patient 2 (SI, 0; normal, >10) or strongly impaired in patient 3 (SI, 2).

RNA-Seq (patient 1) and whole-exome DNA sequencing (patients 2 and 3) analyses revealed that all 3 patients shared the same heterozygous, missense mutation in exon 3 of *CARD11* (chr7: 2987283 C>T; C49Y), confirmed by traditional Sanger sequencing of DNA from purified B cells and buccal swabs for patient 1 and from total blood for patients 2 and 3 (Fig 1, A). Unlike other BENTA-associated CARD11 mutations described to date, C49Y falls within the N-terminal CARD (Fig 1, B). The CARD domain is critical for BCL10 interaction and downstream TCR signaling, as well as regulatory T-cell development.⁷ This mutation was previously reported in 1 case of diffuse large B-cell lymphoma,⁸ and identified in an unbiased screen for gain-of-function CARD11 mutants capable of activating NF- κ B and promoting human diffuse large B-cell lymphoma tumor growth *in vitro*.⁶ Indeed, we confirmed that transfection of the C49Y CARD11 mutant into the CARD11-deficient Jurkat T-cell line JPM50.6 resulted in spontaneous protein aggregation, colocalization with MALT1 and active I κ B kinase,⁹ and constitutive NF- κ B activation in the absence of AgR stimulation, comparable to other BENTA mutants described (Fig 1, C and D). Similar results were obtained in the BJAB B-cell line (see Fig E1 in this article's Online Repository at www.jacionline.org). Collectively, these results are consistent with biochemical data suggesting that the CARD domain also physically associates with the inhibitory linker domain to keep CARD11 in a closed, inactive conformation.⁶ We can therefore conclude that C49Y is the first germline-encoded, autosomal-dominant gain-of-function mutation reported outside of the LATCH-CC region of the CARD11 protein, manifesting as BENTA disease in 3 patients.

Interestingly, all 3 patients sharing the C49Y mutation presented with milder disease when compared with previous

TABLE I. Phenotype analysis of new patients with BENTA

Marker	Patient 1		Patient 2		Patient 3		BENTA		Normal adult range	
	Percent	Abs no./ μ L	Percent	Abs no./ μ L	Percent	Abs no./ μ L	Percent	Abs no.	Percent	Abs no.
CD19 ⁺ B	82	2801	50	1900	48	912	63.2-87.4	2,253-48,262	3-19	59-329
Immature/transitional B*	3	84	2	38	3	27	27-35	3,150-19,570	2-11	1-36
Memory B	0	0	0.1	5	0.1	3	0.4-1	16-552	0.4-2.3	5-46
CD3 ⁺ T	24	999	44	1672	42	798	10-31.5	1,682-5,522	60-84	714-2,266
CD4 ⁺ T	14	592	29	1102	27	513	4.8-17.9	622-2,651	32-62	359-1,565
CD8 ⁺ T	8	342	11	418	8	152	3.4-17.9	408-1,877	11-35	178-853
NK (CD16 ⁺ CD56 ⁺)	7	283	6	228	10	190	2.3-5.6	226-1,270	6-35	126-729
White blood cell no.		5900		7900		5600		5,750-60,810		4,500-10,000
Lymphocyte no.		4340		3800		1900		4,040-55,220		800-4,800
Sex		Female		Male		Female		1 male, 3 females		
Age at analysis (y)		16 y		18 y		51 y		8-12		

Ranges for BENTA (based on previous pediatric patients identified^{1,2}) versus normal adults (values from the Department of Laboratory Medicine, National Institutes of Health) are listed at right for comparison.

Abs, Antibodies.

*Immature/transitional B cells = CD19⁺CD10⁺ (patient 1) or CD19⁺/CD21⁺CD24⁺⁺ according to institutional flow cytometry guidelines. Memory B cells = CD19⁺CD27⁺.

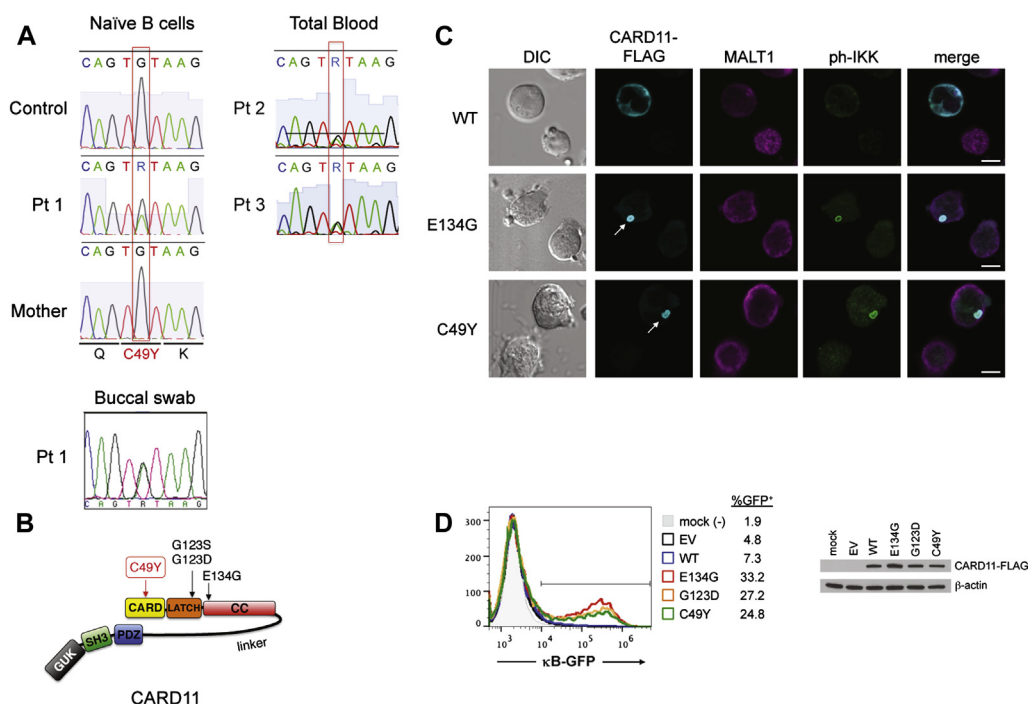


FIG 1. Gain-of-function missense mutation in CARD11 (C49Y) in 3 patients with BENTA. **A**, Sanger gDNA sequencing of patients with BENTA with the C49Y mutation. **B**, Schematic diagram of CARD11 with BENTA-associated mutations. **C**, Fluorescent microscopy of CARD11-deficient JPM50.6 T cells transfected with FLAG-CARD11 constructs. **White arrows** indicate CARD11 aggregates. **Scale bars** = 5 μ m. **D**, Flow cytometric quantitation of κ B-driven GFP reporter and immunoblotting of CARD11-FLAG in transfected JPM50.6 cells. EV, Empty vector; mock, untransfected cells; Pt, patient; WT, wild type.

cases. Much of this may be attributed to a marked decrease in B-cell lymphocytosis noted over time in most pediatric patients with BENTA as they age.² More specifically, reduced output of new transitional B cells from the bone marrow may explain this phenomenon.¹⁰ Consistent with this idea, relative proportions of circulating immature B cells were within normal range in all 3 patients with the C49Y mutation (Table I). However, 2 known patients with BENTA maintained high lymphocyte counts well into adulthood ($\sim 1\text{--}4 \times 10^4$ cells/ μ L), including approximately

70% to 90% CD19⁺ B cells (I. Morison, 2014, personal communication).² However, these 2 patients were also splenectomized in childhood. Indeed, splenectomy appears to be associated with significantly higher peripheral blood B-cell counts in patients with BENTA, and may be a counterproductive clinical measure that predisposes patients to certain infections (eg, encapsulated bacteria) and/or greater risk of B-cell malignancy.^{1,2} We can only speculate that specific CARD11 mutations may be intrinsically linked to mild (C49Y) or severe (G123D) B-cell

lymphocytosis, although comparable gain-of-function activity was measured in various *in vitro* systems.^{1,6} However, the presence of additional genomic modifications as well as environmental factors and infection history may also influence disease severity in individual patients. Additional basic research focusing on mutant CARD11 signal transduction, combined with careful longitudinal analysis of patients with confirmed BENTA, will help clarify those determinants that influence BENTA pathogenesis as new patients continue to be identified.

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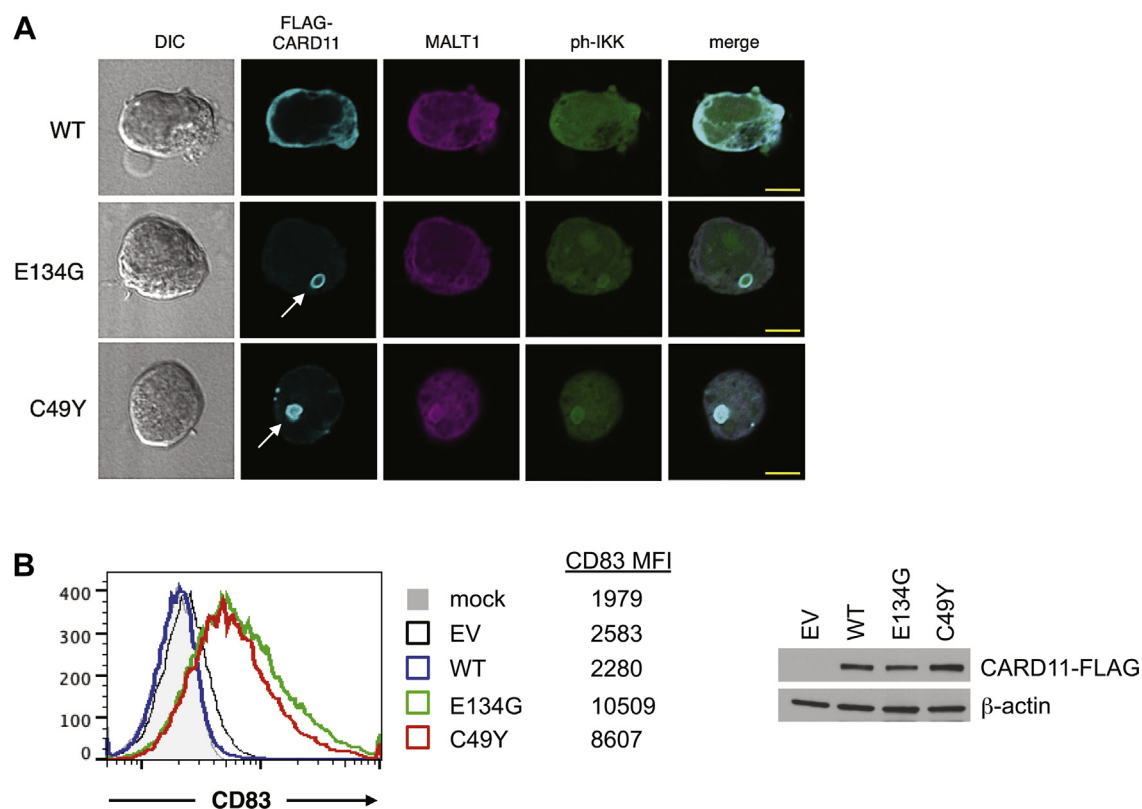


FIG E1. A, BJAB B cells were transfected with FLAG-CARD11 constructs and analyzed by fluorescent confocal microscopy 24 hours after transfection, staining with antibodies against FLAG, MALT1, and phospho-IKK. *White arrows* indicate mutant CARD11 aggregates. *Scale bars* = 5 μ M. **B,** BJAB B cells were transfected as in Fig E1, A and assayed 24 hours later for NF- κ B-dependent CD83 expression by flow cytometry. Mean fluorescence intensity (MFI) values for CD83 are shown, indicative of the relative amount of NF- κ B activation. Immunoblotting of whole-cell lysates is shown at right. *EV*, Empty vector; *IKK*, I κ B kinase; *mock*, untransfected cells; *WT*, wild type.