

Letter

X-linked agammaglobulinemia presenting as polymicrobial pneumonia, including *Pneumocystis jirovecii*

Pneumocystis jirovecii pneumonia is an opportunistic pulmonary infection usually associated with T-cell defects. Clinical and murine studies demonstrate that B cells and *Pneumocystis*-specific antibodies also contribute to effective immune responses.¹ Patients with primary humoral immunodeficiencies, including X-linked agammaglobulinemia (XLA), may develop *P jirovecii* pneumonia despite normal T-cell number and function.^{2,3} XLA arises from BTK gene mutations leading to pre-B-cell differentiation arrest, absence of immunoglobulin expression, and increased susceptibility to bacterial and enteroviral infections.⁴ Although more than 600 BTK mutations have been described, new variations and mutations continue to be identified. We describe an infant with a novel BTK kinase domain variation who presented with polymicrobial pneumonia, including *P jirovecii*.

A 4-month-old, former preterm, male infant, born to non-consanguineous parents, without a family history of primary or secondary immunodeficiencies was evaluated for agammaglobulinemia and polymicrobial pneumonia. Newborn screening was unremarkable, including T-cell receptor excision circle level and human immunodeficiency virus 1. He tolerated 2-month vaccinations. He was admitted at the age of 3.5 months for intermittent increased work of breathing and tachypnea. Chest radiography was normal. He was treated with nebulized albuterol and prednisone. After a short prednisone course, he developed persistent tachypnea and hypoxia and was admitted to the intensive care unit at 4 months of age. On physical examination, lymph node and tonsils were undetectable. Although chest radiography was normal, chest computed tomography revealed diffuse ground glass opacities in the right middle lobe. Bronchoscopy was performed and bronchoalveolar lavage results were positive for *Pneumocystis jirovecii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Serum IgG, IgA, IgM, and IgE were undetectable. Complete blood cell count, as well as C3 and C4 levels were normal. Blood and viral culture results were negative. Immunologic evaluation revealed B-cell lymphopenia, elevated CD4⁺CD45RO⁺, and decreased CD4⁺CD45RA⁺ T-cells (Table 1).⁵ He responded well to piperacillin-tazobactam, trimethoprim-sulfamethoxazole, methylprednisolone, and intravenous immunoglobulin, 0.4 g/kg. He completed 3 weeks of treatment with trimethoprim-sulfamethoxazole followed by prophylactic dosing. After 1 week, he presented with transient tachypnea. Chest radiography revealed bilateral perihilar infiltrates. Silver stain and

Table 1

Summary of clinical laboratory evaluation

Test	Result	Age-Appropriate Reference Range
CD3 ⁺	3,602/ μ L (91%)	2,500–5,600/ μ L (51%–77%)
CD4 ⁺	2,030/ μ L (51%)	1,800–4,000/ μ L (35%–56%)
CD8 ⁺	1,461/ μ L (37%)	590–1,600/ μ L (12%–23%)
CD19 ⁺	12/ μ L (<1%)	430–3,000/ μ L (11–14%)
CD16 ⁺ CD56 ⁺	325/ μ L (8%)	170–830/ μ L (3%–14%)
CD4 ⁺ 45RA ⁺ 45RO ⁺	24%	3%–16% ^a
CD4 ⁺ 45RA ⁺ 45RO [−]	58%	77%–94% ^a
CD8 ⁺ 45RA ⁺ 45RO ⁺	2%	1%–7% ^a
CD8 ⁺ 45RA ⁺ 45RO [−]	95%	85%–98% ^a
Lymphocyte proliferation		
PHA	WNL	WNL
ConA	WNL	WNL
PWM	WNL	WNL
Natural killer cell cytotoxicity	WNL	WNL
IgG	Undetectable	251–906 mg/dL
IgA	Undetectable	4–73 mg/dL
IgM	Undetectable	27–101 mg/dL
IgE	Undetectable	0.18–3.76 IU/mL
C3	97 mg/dL	62–175 mg/dL
C4	27 mg/dL	8.3–38 mg/dL
Sweat test	Negative	Negative
Tetanus toxoid DTH	No wheal or flare	Wheal and flare

Abbreviations: ConA, concanavalin A; DTH, delayed-type hypersensitivity; PHA, phytohemagglutinin; PWM, pokeweed mitogen; WNL, within normal limits.

^aAge-appropriate reference ranges are Shearer et al.⁵

viral and bronchoalveolar lavage culture results were negative. He responded to azithromycin and transitioned to subcutaneous immunoglobulin, 0.1 g/kg weekly. He remains well and infection free, with IgG trough levels greater than 500 mg/dL.

BTK genomic sequencing and concurrent targeted array comparative genomic hybridization analysis by GeneDx revealed a single adenosine deletion in exon 17 within the kinase domain, causing a frameshift and premature stop codon at residue 569 (c.1701delA (p.Val568SerfsX2)). This variation has not been reported previously in BTKbase version 8.52 or Universal Protein Resource (mutant BTK sequence GenBank accession No. KF241986). Two prediction programs, PROVEAN⁶ version 1.1.3 and MutationTaster⁷ predicted significantly impaired protein function. MutationTaster⁷ reported another distinct disease-causing mutation at this position. No BTK gene deletion or duplication was identified. BTK expression in peripheral blood mononuclear cells was evaluated by Western blot (eMethods). Using an anti-amino-terminal antibody recognizing residues 11 to 26, the control expressed the 76-kDa wild-type protein, the patient expressed an approximately 66-kDa truncated mutant, and the mother expressed both wild-type and truncated proteins, consistent with a carrier. The approximately 60-kDa band detected in all samples likely represents nonspecific

Presented in abstract form at the 2013 Annual Meeting of the Federation of Clinical Immunology Societies; June 2013; Boston, Massachusetts.

Disclosures: Dr Jongco is supported by National Institute of Allergy and Infectious Diseases grant T32-AI083223 awarded to Dr Bonagura. Dr Bonagura has received grant support from the National Institutes of Health and CSL-Behring and is on the speaker's bureau for Baxter. The other authors have no relationships to declare.

binding (eFig 1A). Using an anti-carboxyl-terminal antibody recognizing residues 642 to 659, the patient had undetectable protein, whereas the mother expressed diminished wild-type protein compared with control (eFig 1A).

We hypothesized that this expressed truncated protein would be dysfunctional in all BTK-expressing cells. The kinase domain is highly conserved among BTK homologs from diverse organisms, such as zebrafish to primates (data not shown), suggesting that drastic mutations are likely deleterious to proper function. BTK activation begins with transphosphorylation of Y551 in the activation loop (residues 542–559) of the kinase domain by Src-family kinase, followed by autophosphorylation of Y223 in the SH3 domain. X-ray crystal structures of the human BTK kinase domain reveal the importance of Y551 conformation for optimal catalysis and activity.⁸ Molecular dynamics modeling revealed that the truncated protein structurally differs from wild-type, starting from L547 within the activation loop. Furthermore, Y551 conformationally differs from wild-type BTK (eFig 1B). Thus, the altered structure would impair optimal Y551 transphosphorylation and subsequent Y223 autophosphorylation. Western blot of peripheral blood mononuclear cells using phosphorylated Y223-specific antibody revealed that the patient had undetectable phosphorylated Y223, whereas his mother expressed reduced levels compared with control (eFig 1C). These findings suggest that the mutant BTK is inactive.

P jirovecii pneumonia remains an atypical XLA presentation. Although *Pneumocystis*-specific antibodies are detectable in humans and suspected to be protective based on murine studies, their clinical significance is unclear.¹ Prematurity or corticosteroid use may contribute to increased *P jirovecii* susceptibility in XLA patients who rarely develop opportunistic infections.⁹ Because BTK is expressed in all hematopoietic cells except T-cells and plasma cells, BTK deficiency may contribute to antigen-presenting cell (APC) dysfunction, leading to impaired innate and adaptive immunity in XLA patients.¹⁰ Recent data suggest that BTK may be a negative regulator of Toll-like receptor–induced inflammation.¹¹ APC and T-cell function are not universally impaired. XLA patients demonstrate normal APC and T-cell responses to influenza virus^{12,13} and hepatitis B virus¹⁴ but appear unable to develop lasting T-cell memory responses to meningococcus,¹² suggesting heterogeneity in the ability of XLA patients to respond and maintain T-cell responses to different pathogens. Another possibility is that drastic changes, such as kinase domain truncations, may cause more severe phenotypes, but genotype-phenotype correlations are not well established in XLA. Although we did not test APC activation and function in this patient, we suspect that the mutant BTK could cause APC dysfunction. Delayed-type hypersensitivity (DTH) to tetanus toxoid at 16 months of age, more than 6 months after corticosteroid use, was absent in this patient. Decreased DTH, associated with reduced CD4⁺CD45RO⁺ memory T-cells, has been reported previously in XLA patients,¹⁵ but the underlying mechanism is unclear. This finding could reflect differences in (1) local vs circulating APCs, (2) pathogen route of entry or exposure and the efficacy of the ensuing response, (3) severity of the underlying genotype, or (4) altered kinetics of T-cell response in patients vs controls.^{12–15}

B-cell lymphopenia may also perturb proper T-cell maturation and function. Reduced memory T-cells have been reported in XLA patients and common variable immunodeficiency (CVID) patients with less than 2% B-cells,¹⁶ suggesting that B-cell lymphopenia, not BTK deficiency per se, may contribute to impaired CD4⁺ T-cell maturation. Murine studies suggest that clearance of *Pneumocystis* depends on B-cell–mediated activation and expansion of effector and memory T-cells.¹⁷ Current data are conflicting on whether B-cells are dispensable in generating effective T-cell memory in humans.^{12–16} Elevated CD4⁺CD45RO⁺ memory T-cell counts have been reported in CVID patients,¹⁸ but their significance in our

patient or the significance of his absent DTH is unclear. Further research is needed to investigate these possibilities.

In summary, this patient is a reminder that *P jirovecii* pneumonia should be considered in XLA patients who present with respiratory distress. Although BTK is not expressed in T-cells, BTK deficiency may indirectly affect optimal T-cell maturation and activation by modulating APC function. The potential consequences of BTK deficiency in cells other than B-cells should be considered, especially because BTK inhibitors, which irreversibly block BTK activation and function, are being developed for clinical use in malignant tumors and autoimmune diseases.¹⁹

Acknowledgments

We thank Lynda Hatam and Fung Lam for assistance with flow cytometry.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2013.10.008>.

Artemio M. Jongco, MPH, MD, PhD^{*,†}

Jonathan D. Gough, PhD[‡]

Kyle Sarnataro^{*}

David W. Rosenthal, DO, PhD^{*,†}

Joanne Moreau, MD[†]

Punita Ponda, MD[†]

Vincent R. Bonagura, MD^{*,†}

^{*}Laboratory of Host Defense

Center for Immunology and Inflammation

Feinstein Institute for Medical Research

Manhasset, New York

[†]Division of Allergy and Immunology

Hofstra North Shore-LIJ School of Medicine

Great Neck, New York

[‡]Department of Chemistry and Biochemistry

Long Island University

Brooklyn, New York

ajongco@nshs.edu

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eMethods

Western Blot

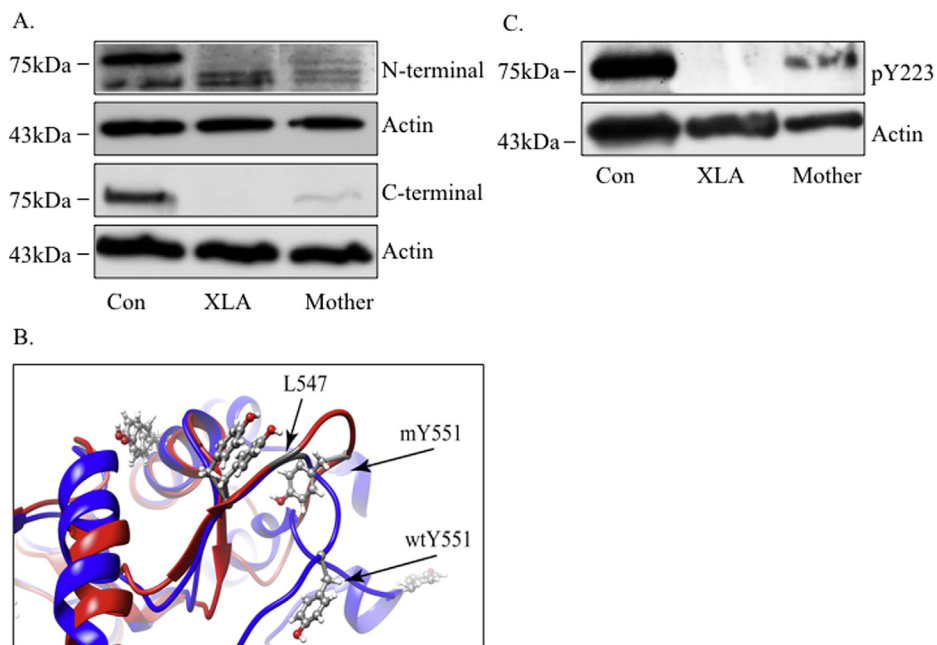
After obtaining informed consent, peripheral blood mononuclear cells from controls, the patient, and the patient's mother were subjected to Ficoll centrifugation. Buffy coat lysate was prepared with Tris-sodium-EDTA buffer using standard protocols. Rabbit anti-BTK amino- (against residues 11–26; B0686) and carboxyl-terminus (against residues 642–659; B0811), as well as antiphosphorylated Y223 (SAB4503801), antibodies were used at 1:250 dilution, and mouse anti- β -actin (A2228) monoclonal antibody was used at 1:10,000 dilution (Sigma-Aldrich, St. Louis, Missouri). Goat anti-rabbit horseradish peroxidase (31460; Thermo Scientific, Rockford, Illinois) was used at 1:2,000 dilution and goat anti-mouse horseradish peroxidase (SC-2031; Santa Cruz Biotech, Dallas, Texas) at 1:5,000 dilution. Pierce ECL Western Blotting Substrate (Thermo Scientific) was used to detect the signal.

Molecular Dynamics Modeling

Mutant and native structures were generated using the X-ray structure (PDB code 1K2P). Simulations were performed using the AMBER suite of software and AMBER.^{1,2} Structures were solvated in explicit solvent (TIP3P), minimized (1,000 steps), heated to 300°K (50 ps), and equilibrated for 100 ns until the root mean square deviation normalized. Visual comparison of lowest energy structures used the VMD software package,³ and figures were generated with the Chimera software package.⁴

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eFigure 1. A, BTK protein expression by control (Con), patient with X-linked agammaglobulinemia (XLA), and patient's mother (mother) using anti-amino-terminal and anti-carboxyl-terminal antibodies. B, Closeup view of the activation loop in wild-type (*blue*) and mutant (*red*) BTK kinase domain using molecular dynamics modeling. C, Phosphorylated BTK expression by control, patient, and patient's mother using antiphosphorylated Y223 antibody. N-terminal indicates amino-terminal; C-terminal, carboxyl-terminal; wtY551, wild-typeY551; and mY551, mutant Y551.