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

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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 Bruton tyrosine kinase (BTK) gene mutations, leading to pre-B-cell differentiation arrest, absence of immunoglobulin expression, and increased susceptibility to bacterial/enteroviral infections.[4] Although more than 600 BTK mutations have been described, new variations/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 pre-term male infant, born to non-consanguineous parents, without family history of primary or secondary immunodeficiencies, was evaluated for agammaglobulinemia and polymicrobial pneumonia. Newborn screening was unremarkable,

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The mutant BTK sequence was submitted to GenBank with accession number KF241986.

Author contributions:
Artemio M. Jongco participated in study conception/design, data generation, data analysis/interpretation, and manuscript preparation/revision. Jonathan D. Gough participated in data generation, data analysis/interpretation, and manuscript preparation/revision. Kyle Sarnataro, is currently an undergraduate student who has worked in the laboratory of Dr. Bonagura, has participated in data generation, data analysis/interpretation, and manuscript preparation/revision. David W. Rosenthal participated in study conception/design, data generation, data analysis/interpretation, and manuscript preparation/revision. Joanne Moreau participated in data generation, data analysis/interpretation, and manuscript preparation/revision. Punita Ponda participated in study conception/design, data analysis/interpretation, and manuscript preparation/revision. Vincent R. Bonagura participated in study conception/design, data analysis/interpretation, and manuscript preparation/revision.

including T-cell receptor excision circle level and HIV-1. He tolerated 2-month vaccinations. He was admitted at age 3.5 months for intermittent increased work of breathing and tachypnea. Chest x-ray (CXR) 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 intensive care at age 4 months. On physical examination, lymph node and tonsils were undetectable. Although CXR was normal, chest computed tomography scan showed diffuse ground glass opacities in the right middle lobe. Bronchoscopy was performed and bronchoalveolar lavage (BAL) was positive for *Pneumocystis jirovecii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Serum IgG, IgA, IgM, and IgE were undetectable. CBC, C3/C4 levels were normal. Blood and viral cultures were negative. Immunologic evaluation revealed B-cell lymphopenia, elevated $CD4^+CD45RO^+$ and decreased $CD4^+CD45RA^+$ T-cells (Table I).[5] He responded well to piperacillin/tazobactam, trimethoprim/sulfamethoxazole (TMP-SMX), methylprednisolone, and intravenous immunoglobulin, 0.4 g/kg. He completed 3 weeks of treatment dose TMP-SMX followed by prophylactic dosing. After 1 week, he presented with transient tachypnea. CXR showed bilateral perihilar infiltrates. Silver stain, viral and BAL cultures 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 >500 mg/dL.

BTK genomic sequencing and concurrent targeted array comparative genomic hybridization analysis by GeneDx showed 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. Two prediction programs, PROVEAN[6] v1.1.3 and MutationTaster[6], predicted significantly impaired protein function. MutationTaster[7] reported another distinct disease-causing mutation at this position. No BTK gene deletion or duplication was identified. BTK expression in peripheral blood mononuclear cells (PBMCs) was evaluated by western blot. Using an anti-amino-terminal antibody recognizing residues 11–26, the control expressed the 76 kiloDalton (kDa) wild type protein, the patient expressed an approximately 66 kDa truncated mutant, while the mother expressed both wild type and truncated proteins, consistent with a BTK carrier. The approximately 60 kDa band detected in all samples likely represents non-specific binding (Supplemental figure 1A). Using an anti-carboxyl-terminal antibody recognizing residues 642-659, the patient had undetectable protein, while the mother expressed diminished wild type protein compared to control (Supplemental figure 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 human BTK kinase domain show 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 (Supplemental figure 1B). Thus, the altered structure would impair optimal Y551 transphosphorylation and subsequent Y223 autophosphorylation. Western blot of PBMCs using phosphorylated Y223-specific antibody revealed the patient had undetectable phospho-Y223, whereas his mother expressed reduced levels compared to control (Supplemental figure 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] Since BTK is expressed in all hematopoietic cells except T- and plasma cells, BTK deficiency may contribute to antigen presenting cell (APC) dysfunction, leading to impaired innate/adaptive immunity in XLA patients.[10] Recent data suggest that BTK may be a negative regulator of TLR-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 virus[14] 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, like 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/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 off any corticosteroids, 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 could reflect differences in: 1) local versus circulating APCs; 2) pathogen route of entry/exposure and the efficacy of the ensuing response; 3) severity of the underlying genotype; or 4) altered kinetics of T-cell response in patients versus controls.[12–15]

B-cell lymphopenia may also perturb proper T-cell maturation/function. Reduced memory T-cells have been reported in XLA patients and common variable immunodeficiency (CVID) patients with <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.[1,17] 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-cells has been reported in CVID patients,[18] but its significance in our patient or 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 presenting with respiratory distress. Although BTK is not expressed in T-cells, BTK deficiency may indirectly affect optimal T-cell maturation/activation by modulating APC function. The potential consequences of BTK deficiency in cells other than B-cells should be considered, especially since BTK inhibitors, which irreversibly block BTK activation and function, are being developed for clinical use in malignancy and autoimmune diseases.[19]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table I

Summary of clinical laboratory evaluation.

Test	Result	Age-appropriate reference range
$CD3^+$	3602 (91%)	2500–5600 cells/μL (51–77%)
$CD4^+$	2030 (51%)	1800–4000 cells/μL (35–56%)
$CD8^+$	1461 (37%)	590–1600 cells/μL (12–23%)
$CD19^+$	12 (<1%)	430–3000 cells/μL (11–14%)
$CD16^+CD56^+$	325 (8%)	170–830 cells/μL (3–14%)
$CD4^+45RA^-45RO^+$	24%	3–16%*
$CD4^+45RA^+45RO^-$	58%	77–94%*
$CD8^+45RA^-45RO^+$	2%	1–7%*
$CD8^+45RA^+45RO^-$	95%	85–98%*
Lymphocyte proliferation	PHA: Wnl ConA: Wnl PWM: Wnl	PHA: Wnl ConA: Wnl PWM: Wnl
Natural Killer Cell cytolysis	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	62–175 mg/dL
C4	27	8.3–38 mg/dL
Sweat test	Negative	Negative
Tetanus toxoid DTH	No wheal or flare	Wheal and flare

ConA, concanavilin A; DTH, delayed type hypersensitivity; PHA, phytohemagglutinin; PWM, pokeweed mitogen; Wnl, within normal limits.

*age appropriate reference ranges from reference 5.