METHODS

Western blot

After obtaining informed consent, peripheral blood mononuclear cells from controls, patient and patient's mother were subjected to Ficoll centrifugation. Buffy coat lysate was prepared with Tris-sodium-EDTA (TNE) buffer using standard protocols. Rabbit anti-BTK amino- (against residues 11-26; B0686) and carboxyl-terminus (against residues 642-659; B0811), as well as anti-phosphorylated Y223 (SAB4503801), antibodies were used at 1:250 dilution, and mouse anti-β actin (A2228) mAb was used at 1:10,000 dilution (Sigma-Aldrich, St. Louis, MO). Goat anti-rabbit-HRP (31460; Thermo Scientific, Rockford, IL) was used at 1:2000 dilution and goat anti-mouse-HRP (SC-2031; Santa. Cruz Biotech, Dallas, TX) at 1:5,000 dilution. Pierce ECL Western Blotting Substrate (Thermo Scientific, Rockford, IL) 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 (1000 steps), heated to 300K (50 ps), and equilibrated for 100 ns until the RMSD normalized. Visual comparison of lowest energy structures used the VMD software package[3] and figures were generated with the Chimera software package.[4]

Supplemental figure legends

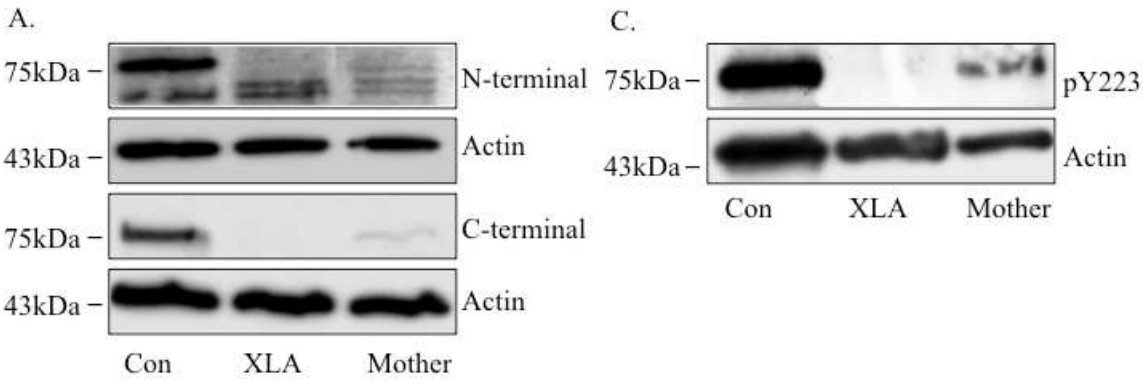
Figure 1. A, BTK protein expression by control (Con), patient (XLA), and patient's mother (Mother) using anti-amino-terminal and anti-carboxyl-terminal antibodies. **B,** Close up view of the activation loop in wild type (blue) and mutant (red) BTK kinase domain using molecular dynamics modeling. **C,** Phosphorylated BTK expression by control, patient, and patient's mother

using anti-phosphoY223 antibody. N-terminal, amino-terminal; C-terminal, carboxyl-terminal; wtY551, wild typeY551; mY551, mutant Y551.

Supplemental references:

1. Case D, Darden T, Cheatham T, et al. AMBER. San Francisco: University of California, San Francisco, 2012.
2. Salomon-Ferrer R, Case D, Walker R. An overview of the Amber biomolecular simulation package. WIREs Comput Mol Sci 2013; 3:198-210.
3. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph 1996; 14:33-8, 27-8.
4. Yang Z, Lasker K, Schneidman-Duhovny D, et al. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. J Struct Biol 2012; 179:269-78.

Supplemental Figure 1



B.