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## *Research Communication*

# Consequences of Two Naturally Occurring Missense Mutations in the Structure and Function of Bruton Agammaglobulinemia Tyrosine Kinase

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***Summary***

**Bruton agammaglobulinemia tyrosine kinase (BTK) is a key protein in the B-cell receptor (BCR) signaling pathway and plays an essential role in the differentiation of B lymphocytes. X-linked agammaglobulinemia (XLA) is a primary humoral immunodeficiency caused by mutations in the gene encoding BTK. Previously, we identified two novel variations, L111P and E605G, in BTK; these are localized within the pleckstrin homology and Src homology 1 domains, respectively. In the present study, we evaluated the potential effects of these variations on the structural conformation and the function of BTK. Using *in silico* methods, we found that the L111P and E650G variations are not located directly in protein–protein interfaces but close to them. They distorted the native structural conformation of the BTK protein, affecting not only its geometry and stability but also its ability for protein recognition and in consequence its functionality. To confirm the results of the *in silico* assays, WT BTK, L111P, and E650G variants were expressed in the BTK-deficient DT40 cell line. The mutant proteins exhibited an absence of catalytic activity, aberrant redistribution after BCR-crosslinking, and deficient intracellular calcium mobilization. This work demonstrates that L111 and E605 residues are fundamental for the activation and function of BTK.** 

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**Keywords** B-cell receptor; Bruton agammaglobulinemia tyrosine kinase; X-linked agammaglobulinemia; missense mutation.

## INTRODUCTION

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency characterized by a dramatic decrease in the frequency of B lymphocytes to less than 1% of peripheral B cells, a severe reduction of all immunoglobulin isotypes and recurrent bacterial, fungal, and enteroviral infections (*1, 2*). XLA was the first primary immunodeficiency identified (*3*), and it is caused by mutations in the gene encoding the Bruton agammaglobulinemia tyrosine kinase (BTK) (*4, 5*). Mutations causing XLA have been found in all BTK domains as well as in the noncoding sequences of the gene. Missense mutations account for 40% of all mutations; 17% are nonsense mutations; 20% are deletions, and 7% are insertions. Splice-site mutations are found in 16% of all cases. Distribution of the mutations follows closely the length of the domains. The vast majority of the substitutions are transitions (69%). C–T transition (29%) is slightly less abundant in XLA patients compared with other immunodeficiencies. Proline (P) is the most common residue in the reported mutations, followed by W, Q, and H. The most frequently substituted residues in XLA patients are G, L, and R, with lower frequency for H and W (*1, 6*). BTK is a member of the Tec family of kinases (*7, 8*). It is essential for the ontogeny of the B cells and is involved in signal transduction pathways that regulate B-cell survival, activation, proliferation, and differentiation (*9*). BTK contains five structural domains: the pleckstrin homology (PH) domain, which is thought to mediate its plasma membrane

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targeting. Mutations in this domain have profound effects on its activity (*7, 8, 10–14*); the Tec homology (TH) domain (*7, 8*); the Src homology 3 domain (*7, 15*); the Src homology 2 domain (*7, 16*), which are important in mediating either intramolecular interactions or interactions with other proteins (*7, 8, 14*) and the catalytic domain Src homology 1 (SH1) (*7, 17*).

In a previous study, we identified and reported a novel variation (HGVS nomenclature), E605G, in a classical XLA patient (*18*); we also reported another novel variation, L111P, in a different patient. An *in silico* analysis showed that these variations introduced alterations in the protein conformation of BTK. We, therefore, pursued a more detailed study to ascertain the potential effects of the mutations on the structural conformation and function of BTK. In this work, we evaluated the functionality of BTK variants in the DT40 cell line. The results showed that the E605G and L111P variations affected BTK function as evidenced by loss of kinase activity; caused low calcium influx, and altered recruitment of the protein to the plasma membrane after B-cell activation; for this reason, we referred these two variations as mutations. In summary, our results showed that L111 and E605 are important for BTK functionality and that alterations in these residues have a causative role in XLA.

## MATERIALS AND METHODS

### *Patients*

The three patients evaluated presented with typical XLA. They had severe, recurrent infections, low levels of all immunoglobulin isotypes, and less than one percent of B lymphocytes in the peripheral blood. This study was performed with the written consent of patients and their parents. The ethics committee at the Centro Médico "La Raza" Mexican Institute for Social Security approved the study protocol.

### *In Silico* Analysis (Mutation Effects)

Structures of L111P and E605G mutants were constructed using molecular operating environment (www.chemcomp.com) version 2010.10. These mutations are located in different domains, L111P in the PH domain, and E605G in the SH1, and there is no a single crystallographic structure of a homolog containing both domains; thus, we used as templates two different crystallographic structures from the Protein Data Bank (www.pdb.org): 1BTKB (*13*) and 1K2PB (*17*), respectively. Both files contain homodimeric structures, which are reported to be functional. As mutations are located far from the protein–protein interface, we used the dimeric structure as template and modeled the dimeric mutant, thus allowing even higher variability during the modeling process. All calculations were performed with CHarMM 27 force field, parameterized for protein structures, hydrogen atoms were initially added to the template structures, all partial atomic charges were assigned according to parameters of the force field, and the positions of hydrogen atoms were optimized by energy minimizations with the rest of the atoms fixed, including zinc and sodium ions and water molecules present in the crystal. One hundred different models were constructed for each dimeric mutant, differing only in the starting point of the side chains of the template closer to the mutation. All intermediate models were refined by molecular minimization until a root mean square (RMS) gradient of 0.5 kcal/mol-A was achieved, in the presence again of ions and water molecules. The best intermediate structure was finally refined to achieve a 0.1-kcal/mol-A gradient as the final structure. Structural analysis was performed in both subunits of the final dimeric models, and also in both subunits, the database of intermediate structures yielded identical qualitative results. For comparative purposes, the wild-type template was submitted to a minimization procedure identical to that used for the mutants.

### *BTK Mutation Analysis and BTK Expression*

Total RNA was extracted from $5 \times 10^6$ peripheral blood mononuclear cells (PBMCs), cDNA was synthesized by reverse transcription polymerase chain reaction (RT-PCR) and BTK from the samples was amplified using specific primers that recognize the whole BTK coding sequence (*18*). All BTK products were analyzed for their electrophoretic mobility, and the products were cloned into the TOPO TA Cloning vector (Invitrogen) and sequenced. All sequences were analyzed, and mutations were determined using the Vector NTI software and by comparing the sequences with the reference sequence (NM-000061.2). The expression of BTK in PBMCs was verified by Western blot. Protein extracts were obtained by treating the cells with lysis buffer containing 1% NP-40, 150 mM NaCl, 20 mM NaF, 500 $\mu$M $NaVO_3$, 10 mM Tris-base, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 $\mu$g/mL aprotinin, and 5 $\mu$g/mL leupeptin (Sigma-Aldrich, St. Louis, MO). For electrophoresis, 40 $\mu$g of total protein was loaded onto 12% polyacrylamide gels and transferred onto nitrocellulose membranes (Hybond, GE Healthcare). For detection, murine IgG2a monoclonal anti-human BTK (Pharmingen, San Jose, CA) and goat anti-mouse IgG coupled to horseradish peroxidase (Zymed, San Francisco, CA) were used. BTK was visualized by chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA).

### *Expression of BTK Mutants and BTK-Specific Phosphorylation Studies*

Wild-type (WT) and BTK-deficient chicken DT40 cells (kindly obtained from Tomohiro Kurosaki) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 5% chicken serum (Gibco, Carlsbad, CA), penicillin, streptomycin, and glutamine (Sigma-Aldrich, St. Louis, MO) at 37°C. Total RNA was extracted from $5 \times 10^6$ peripheral blood mononuclear cells, cDNA was synthesized by RT-PCR and WT, or patient-derived BTK was amplified using specific primers (forward: 5′-CTACGCTAGCATGGCCGCAGTGATTCTGG-3′, reverse: 5′-CATGCCGCGGCCGCTCAGGATTCTTCATCCATGAC-3′). Site-directed mutagenesis was performed with QuikChange II XL (Stratagene, La Jolla, CA) in the plasmid pAp-

**Table 1**
Clinical and molecular data from the patients were examined

| Patient | Age at diagnosis (years) | IgG (486–1211 mg/dL)[a] | IgM (45–211 mg/dL)[a] | IgA (30–182 mg/dL)[a] | B cells (%) | Mutation in protein | Mutation in cDNA | Dom[b] |
|---|---|---|---|---|---|---|---|---|
| P1 | 18 | 36.5 | 60.4 | 9.5 | 0 | L111P[c] | c.497T>C | PH |
| P2 | 16 | 397 | 23 | 17 | 0.04 | E605G[c] | c.1977A>G | SH1 |
| P3 | 18 | 24 | 18 | 23 | 0.65 | E605G[d] | c.1977A>G | SH1 |

[a]Reference values for each immunoglobulin isotype.
[b]Btk domain affected by mutation: PH, pleckstrin homology; SH1, Src homology 1.
[c]Previously reported mutations.
[d]Unrelated patients.

uro-Btk and transfection into BTK-deficient cells was performed by electroporation at 250 volts and 900 $\mu$F (Gene Pulser, Bio-Rad Laboratories, Hercules, CA). Clones were selected with 0.5 mg/mL puromycin (Invitrogen) 24 h after electroporation. The presence of BTK was verified by Western blot. The phosphorylation of the tyrosine 223 (pY223) was determined by Western blot in DT40 cells transfected with WT BTK or BTK from the patients; the cells were stimulated with 5 $\mu$g/mL of mouse anti-chicken IgM Clone M4 (Southern Biotechnology, Birmingham, AL). Protein extracts from $5 \times 10^6$ DT40 cells were obtained and lysed as described before. For detection of BTK phosphorylation, anti-pY223-BTK (Biosource International, Camarillo, CA), peroxidase-conjugated goat anti-rabbit IgG (Zymed) and the enhanced chemiluminescence system (Santa Cruz Biotechnology) were used.

### *Calcium Mobilization*

$BTK^{-/-}$ DT40 B cell line transfected with the indicated constructs were loaded with Calcium Green-1 (Molecular Probes-Invitrogen, Eugene, OR) and incubated for 30 min at 37°C with 5% $CO_2$. The cells were stimulated with 5 $\mu$g/mL of mouse anti-chicken IgM clone M4 or with 1 ng/$\mu$L ionomycin (Sigma). The fluorescence assays were performed by flow cytometry (FACSCalibur) using the green channel (FL1), and the data were analyzed using FlowJo software (Tree Star, Ashland, OR).

### *Distribution of PH Domains Fused to the Enhanced Green Fluorescent Protein*

WT or mutant (L111P) PH domains were fused to green fluorescent protein (GFP) as described previously (*12*). Confocal microscopy of living cells was carried out as follows: A20 lymphoma cells were transiently transfected with the plasmid constructs by electroporation at 300 volts and 970 $\mu$F, the cells were grown in chamber glass slides (Labtek II, Nalge Nunc International, Rochester, NY) with RPMI 1640 medium supplemented with 10% FCS, antibiotics, and glutamine. A20 cells were stimulated with 20 $\mu$g/mL of sheep anti-mouse IgG (Cappel, West Chester, PA) for 10 min and fixed with 4% paraformaldehyde (PFA). Cells were examined in a confocal microscope (Olympus IX71, Center Valley, PA) at 60× magnification. Confocal images were analyzed with ImageJ software (http://rsbweb.nih.gov/ij/). The fluorescence intensity changes across the white lines were plotted as line intensity histograms.

## RESULTS

### *The E605G Mutation in an Unrelated Patient*

In a previous study, our group analyzed 22 XLA patients and characterized two new missense mutations in the gene encoding BTK (L111P and E605G) (*18*). In this study, we identified the E605G mutation in a new patient. The patient presented with a typical XLA phenotype characterized by hypogammaglobulinemia and low B-cells counts (Table 1). To confirm the diagnosis of XLA, the patient's BTK was analyzed by molecular techniques. We evaluated the expression of BTK by Western blot; the 77 kDa BTK protein was detected in PBMC lysates from a healthy donor, but no protein was seen in lysates from the patient (Fig. 1A). Previous results suggest that novel missense mutations affects protein expression because low amounts of protein were detected; these mutations have effects in the stability, solubility, activity, and binding properties of the BTK (*19*). When BTK was amplified using PCR, normal sized transcripts were detected in the patient with the mutation. Sequence analysis of the PCR product identified the E605G mutation (Table 1). This mutation in the BTK gene might contribute to the lack of BTK expression similar to other missense mutations. With these results in mind, we studied the impact of this mutation on the structural conformation and function of BTK (see Materials and Methods). E605G mutation is located in alpha helix 9 and seems to reduce the stability of this region of the structure as it introduces an entropic challenge to folding due to the high mobility of the G residue in the unfolded state, and also because of the loss of a negatively charged residue which favorably interacts with the positive side of the backbone helix dipole. Also, E605 must be functionally important as judged by the analysis of multiple alignments of homolog sequences which shows that this residue is highly conserved among different animal species (Fig. 1B). The E605G mutation affects the salt-bridge interaction of E605 with H609 also affecting helix stability. When we analyzed the conformation of this region of the SH1 domain, we observed that this helix forms part of a complex of alpha helices that interacts

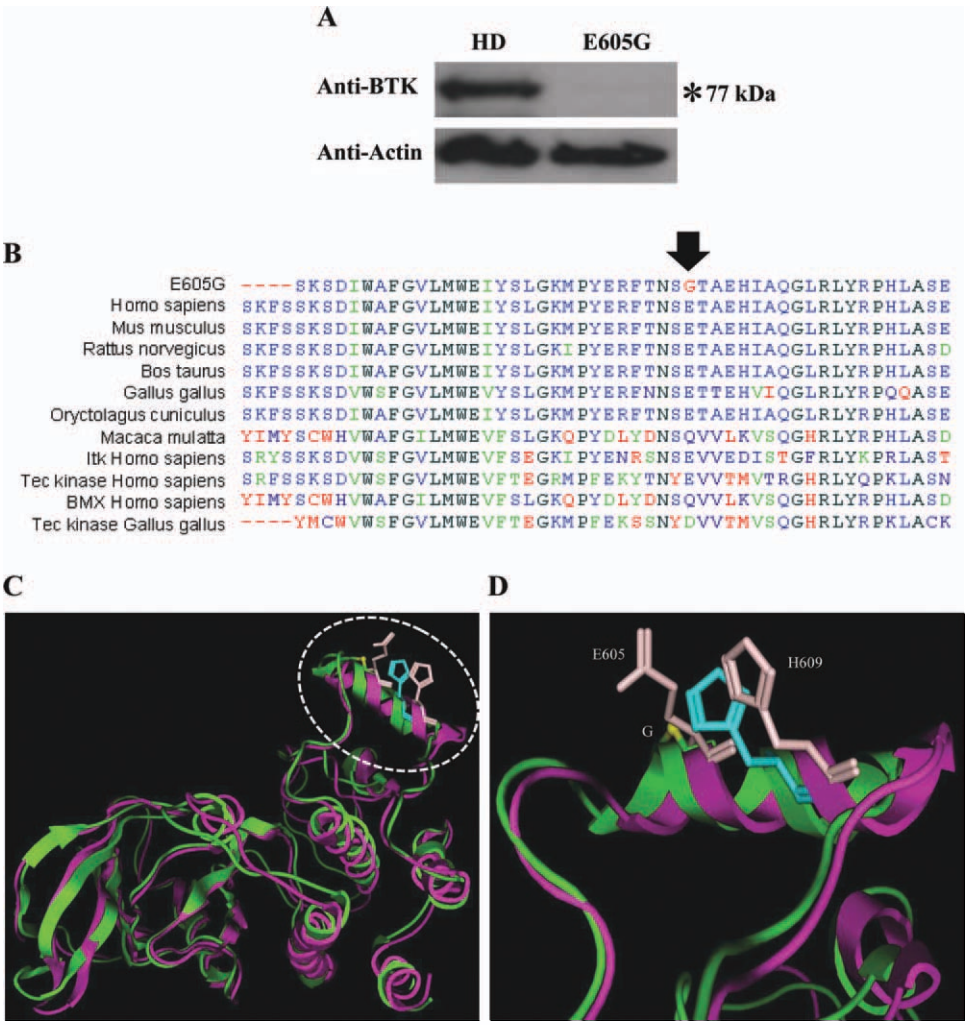


**Figure 1.** E605G mutation. (A) BTK expression in PBMC from patient with XLA was assessed by Western blot. HD, healthy donor. (B) Glutamic acid at position 605 is highly conserved among different species, replaced by glutamine or aspartic acid but never with glycine in all sequences analyzed; the black arrow indicates the identified mutation. Extensive multiple alignment was generated with the Vector NTI software, this figure schematically shows only some of the sequences. (C) Superposition of wild-type structure (magenta) and E605G mutant model (green) of BTK SH1 domain. Residue E605 and its hydrophobic interactions with H609 are highlighted within the dotted white circle. (D) Alpha carbon of glycine residue is shown in yellow and H609 in mutant is shown in blue. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with other residues and forms a hydrophobic core (Figs. 1C and 1D). Mutation causes a skew in position of helix 9. The lack of such interactions is expected to decrease the stability of the hydrophobic core of the SH1 domain and to present partial unfolding leading to aggregation or to degradation by cellular reparation systems. Moreover, a previous report indicates that mutation of residue T606 affects the interaction with Y598 and affects local structure stability (*17*).

As the E605G mutation is distant from the K430, E445, R544, and Y551 residues, important in the activation of BTK, this mutation probably does not directly affect the active site of the enzyme. BTK is associated to the plasma membrane and transphosphorylated in the Y551 into the SH1 or catalytic domain, this domain is organized into two lobes, the N-terminal lobe and the C-terminal lobe. The two lobes in the BTK structure adopt a closed conformation due to unphosphorylated Y551 in the A loop of the SH1 domain interacts with R544, the transphosphorylation of Y551 can lead to BTK activation by triggering an exchange of hydrogen-bonded pairs from E445/R544 to E445/K430 and subsequent relocation of helix $\alpha$C of the terminal lobe. This structural change facilitates the autophosphorylation of Y223 and a total of BTK activation. Finally, these structural changes allow the protein to be close to its substrate. According to our analysis, we can suggest that this mutation may destabilize the C-terminal lobe of the kinase domain by altering its interactions with neighboring residues.

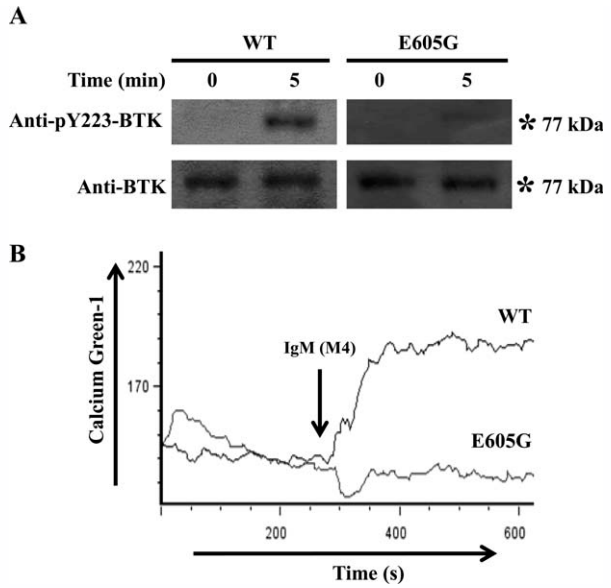


**Figure 2.** Functional analysis in DT40 lymphoma cells transfected with the E605G mutant. (A) Kinase activity was analyzed by immunoblot with anti-phospho-Y223-BTK in $BTK^{-/-}$ DT40 cells reconstituted with WT and E605G mutant. The time points after M4 stimulation (5 $\mu$g/mL) are indicated. (B) Calcium mobilization was assessed by flow cytometry on BCR engagement in DT40 $BTK^{-/-}$ cells transfected with the indicated constructs. The cells were stimulated with 5 $\mu$g/mL of mouse anti-chicken IgM (M4) at the time indicated.

### *Functionality of the E605G Mutant*

Bruton's tyrosine kinase participates in the signal transduction pathways of the B-cell receptor (BCR): on BCR engagement, BTK is recruited to the plasma membrane by interaction of the PH domain with PtdIns(3,4,5)$P_3$, a product of PI3K (*10–14*). Lyn then phosphorylates Y551-BTK (*14, 20, 21*), and then, BTK is autophosphorylated at Y223 (pY223) to achieve full activation (*14, 20–23*). Activated BTK phosphorylates tyrosine residues of PLC$\gamma$2 resulting in its full activation and this leads to the generation of the second messengers inositol triphosphate ($IP_3$) and diacylglycerol. $IP_3$ binds to the $IP_3$ receptor to mobilize calcium from the endoplasmic reticulum (*14, 21, 24, 25*). Phosphorylation has been shown to be a critical regulatory mechanism controlling BTK function, making the phosphorylation of pY223 a good indicator of BTK kinase activity (*22*). For this reason, we determined BTK kinase activity by measuring the amount of phospho-Y223 (pY223-BTK) by immunoblot of protein extracts from DT40 $BTK^{-/-}$ cells transfected with WT BTK and the E605G mutant. These cells have previously been shown to be suitable for reconstitution with human BTK. Anti-IgM stimulation of DT40 cells transfected with E605G mutant showed minimal kinase activity when compared to WT BTK (Fig. 2A). These results show that the E605G mutation indeed affects the kinase activity of BTK. The E605G mutation is located in the catalytic domain (SH1), which is important for BTK activity. We determined whether this mutation affects the activation of PLC$\gamma$2 and therefore intracellular calcium mobilization. Upon BCR stimulation, BTK-deficient cells expressing the reconstituted WT BTK evoked vigorous calcium mobilization. In contrast, transfectants expressing the E605G mutant showed a much lower calcium response after BCR ligation (Fig. 2B). These experiments show that calcium flux in response to BCR engagement is absent in DT40 $BTK^{-/-}$ cells transfected with the E605G mutant in comparison with DT40 $BTK^{-/-}$ cells transfected with WT BTK.

### *The Mutation L111P in BTK Affects the Ability of the Protein to Localize to the Membrane*

Several mutations for XLA are located within the PH domain of BTK and impair its membrane association (*10*). In a previous study, we reported a novel missense mutation in patient with XLA that affected the L111 residue of the PH domain of the BTK protein (Fig. 3A, Table 1). Mutations of L111 are expected to affect BTK function as it is a highly conserved amino acid (Fig. 3B). The L111P mutation is located in beta strand 7. Proline produced a shortened beta strand and retraction of the loop in beta 6-loop-beta 7 hairpin structure, preventing critical hydrophobic interactions between L111 and V104 (Fig. 3C). The mutation affects interactions among residues which stabilize contacts between PH and BTK motifs of the proteins, also disturbing structure around the zinc center (Fig. 3D), BTK is a metalloprotein enzyme requiring $Zn^{2+}$ for optimal activity and stability. Binding and coordination of BTK to the $Zn^{2+}$ ion is mediated by a highly conserved zinc finger motif, also called the BTK motif, located in the TH domain. Mutations affecting $Zn^{2+}$ binding lead to the generation of extremely unstable protein. Similar to the E605G mutant, we evaluated the BTK kinase activity of BTK with the L111P mutation. We measured the amount of phospho-Y223 (pY223-BTK) by immunoblot using protein extracts from DT40 $BTK^{-/-}$ cells transfected with WT BTK and the L111P mutant. Anti-IgM stimulation of DT40 cells transfected with L111P yielded minimal kinase activity when compared to the stimulation of cells transfected with WT BTK (Fig. 4A). As the PH domain serves to recruit BTK to the plasma membrane, it was not surprising that mutations in this domain had profound effects on the kinase activity. Because the L111P mutation is located in the PH domain, we next examined whether this mutation affects the distribution of BTK to the plasma membrane after B-cell activation. Previous studies in HeLa, COS-7, and NIH 3T3 cells have shown that BTK's PH domain (studied in isolation) interacts with PtdIns(3,4,5)$P_3$ in the plasma membranes (*10, 12*), this interaction is fundamental for the BTK function. Previous report of our group showed that A20 lymphoma B cells can be a suitable model for reconstitution with these and others fused proteins (*26*). The PH domains of either WT or mutated BTK (amino

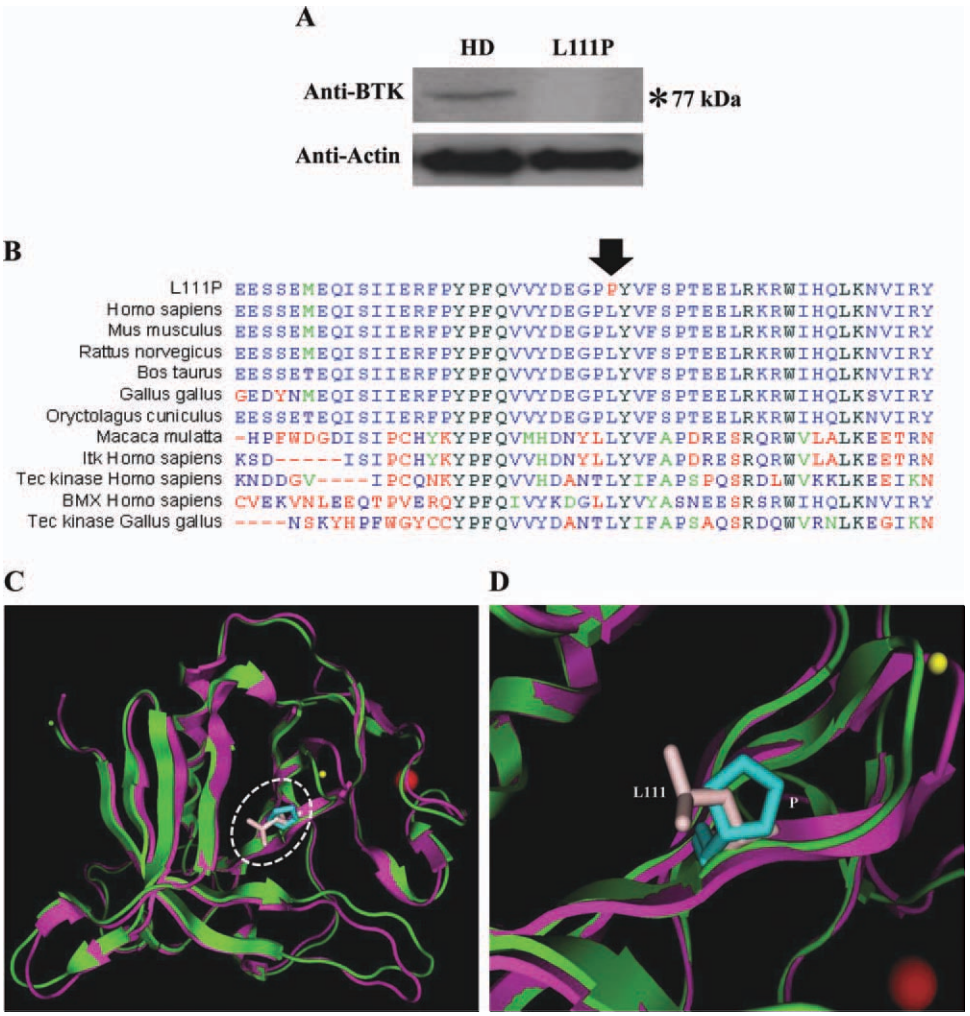


**Figure 3.** L111P mutation. (A) BTK expression was assessed by Western blot. (B) Leucine at position 111 is completely conserved among different species; the residue is shown below the black arrow. Extensive multiple alignment was generated with the Vector NTI software, this figure schematically shows only some of the sequences. (C) Superposition of wild-type structure (magenta) and L111P mutant model (green) of BTK PH domain. Modification introduced by the proline substitution into the PH domain, as shown in the dotted white circle. (D) Loop distorted by mutation is highlighted with a yellow sphere. Btk domain is located at right side surrounding the zinc ion (red), wild-type leucine 111 (red) and mutation to proline (P, blue) are also shown. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

acids 1–177) were fused to GFP and expressed in A20 lymphoma cells. After BCR crosslinking, we observed that the redistribution of the L111P mutant to the plasma membrane was reduced compared with the WT (Fig. 4B). When comparing a larger number of cells, the result remained the same (Fig. 4C). This result shows that the L111P mutation affects BTK recruitment to the membrane, altering its distribution after BCR activation and its catalytic function.

## DISCUSSION

In this study, we report biochemical evidence that supports a relationship between missense mutations in BTK and the XLA phenotype.

The susbstitution of the leucine 111 (L111) by proline alters the structure, function, and activity of BTK. L111 is located in beta sheets 7 of the PH domain, which is important for BTK redistribution from the cytosol to the plasma membrane. When the L111P mutation was present in the PH-GFP construct, it prevented the membrane translocalization of BTK in response to stimulation. There are three different reasons why PH domains containing L111P do not bind to the plasma membrane: first, L111P produces a shortened beta strand in the PH domain; second, the hydrophobic interaction between the L111 and V104 is lost; and third the substitution of the leucine by proline affects the interactions between the Y112, W147, F146, and C154 residues, which leads to a disrupted metal center and prevents the folding of the motif. This loss of interactions may explain the absence of the protein in the patient

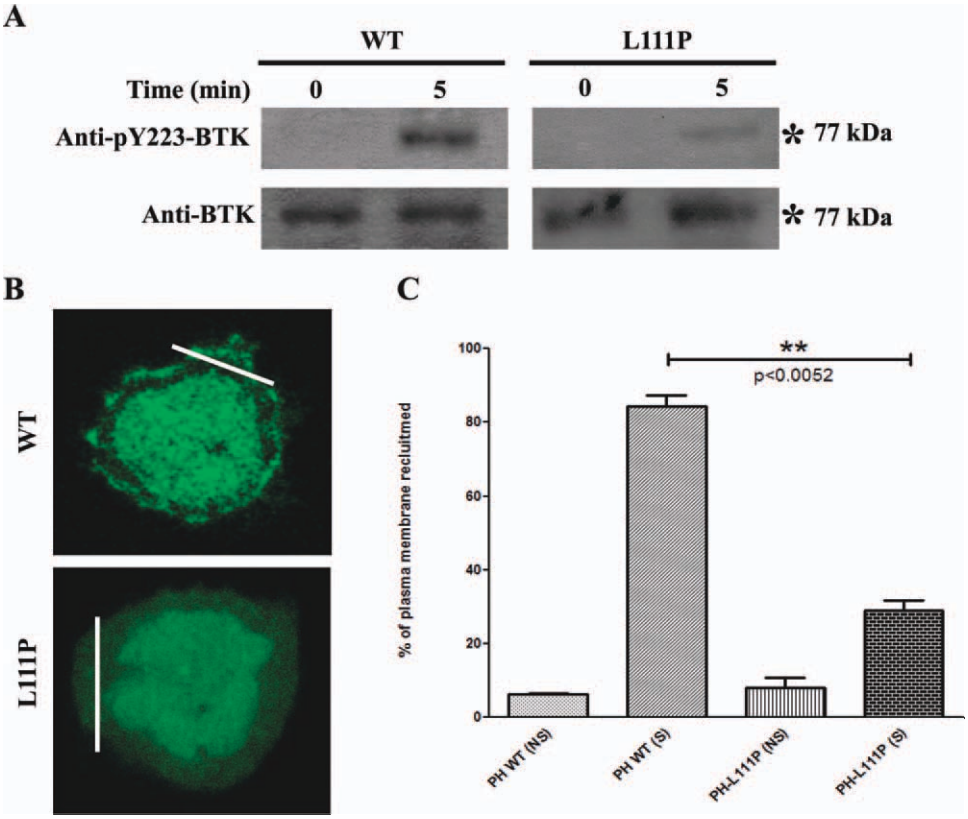


**Figure 4.** Localization of the PH domain of BTK in A20 lymphoma cells. (A) Kinase activity was analyzed by immunoblot with anti-phospho-Y223-BTK in $BTK^{-/-}$ DT40 cells reconstituted with WT and L111P mutant at the indicated time points after M4 stimulation (5 $\mu$g/mL). (B) The PH domain mutant or WT BTK (amino acids 1–177) was fused to the N terminus of GFP, and the respective constructs were transiently expressed in A20 lymphoma cells. Redistribution of fluorescence in A20 cells after a 10-min stimulation with 20 $\mu$g/mL of sheep anti-mouse IgG (left panels). Changes in fluorescence intensity were obtained by dividing the fluorescence intensity of the plasma membrane (Ipm) by that of the cytosol (Icyt), which gives a relationship that can be used as an index of membrane localization. (C) Redistribution to the plasma membrane of WT and mutant PH domains was analyzed in stimulated (S) and unstimulated (NS) cells. Five hundred cells were quantified, and an average was obtained. Statistical analysis was conducted using a Student's unpaired, two-tailed *t*-test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

who encoded the L111P mutation, because the mutation may affect the stability and half-life of BTK (*18*). This issue was not evaluated in this work, however; when we evaluated BTK kinase activity by measuring the amount of phospho-Y223 (pY223-BTK), the cells transfected with the L111P mutant lacked phosphorylation of Y223 in comparison to WT BTK.

The E605G mutation is located in the SH1 domain, which is the catalytic domain. Missense mutations located on alpha helices frequently perturb protein folding (*1, 6*). XLA-associated BTK mutations involving the N-terminal lobe of the kinase domain are less frequent that those involving the C-terminal lobe and many of the mutations identified in the C-terminal lobe are located in the alpha helix and loops that bind these structures. This finding suggests that these mutations may alter important interactions with neighboring residues (*17*). In accordance with modeling predictions, E605G mutant was unable to autophosphorylate and activates PLC$\gamma$2. The prediction that the abnormal molecular conformation of this mutant-affected BTK function was confirmed when we evaluated Y223 phosphorylation and calcium mobilization after BCR crosslinking.

This work has experimentally demonstrated the effect of L111P and E605G mutations on BTK functionality and described the structural basis of these results. Both positions are highly conserved in BTK family. Mutations to P and G are not conservative in the physical chemical nature of the mutant residue, and it would be interesting to explore if mutations of L111 to a more similar residue, such as V or I, and mutations of E 605 to D or Q, have the same effect, to know if the sequence conservation must be strict for the function or if the strong modifications into P or G are mandatory for the loss in BTK functionality. These results reveal the importance of both the L111 and E605 residues for BTK function and correlate with a typical XLA phenotype.

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