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ORIGINAL ARTICLE

Spectrum of *BTK* gene mutations in Vietnamese patients with X-linked agammaglobulinemia

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Abstract

Background X-linked agammaglobulinemia (XLA) is a rare primary immunodeficiency caused by mutations in the *Bruton's tyrosine kinase* (*BTK*) gene, resulting in severely impaired B-cell development and recurrent bacterial infections in children. Although over 2,000 *BTK* mutations have been reported worldwide, data from Southeast Asia remain limited.

Methods and results In this study, we conducted a comprehensive genetic analysis of the *BTK* gene in 82 unrelated Vietnamese male patients clinically diagnosed with XLA, using Sanger sequencing of both genomic DNA and cDNA, combined with in-silico pathogenicity prediction tools. Pathogenic *BTK* mutations were identified in 36 patients (43.9%), involving 35 distinct variants distributed across all functional domains of the BTK protein. Missense mutations were the most common (37.1%), followed by large deletions (25.7%), splice-site mutations (14.3%), nonsense (11.4%), frameshift (8.6%), and in-frame deletions (2.9%). Two novel variants were identified: a nonsense mutation (p.Q343X) in the SH2 domain and a splice-site mutation (IVS17 + 5G > C). All large deletions were detected only at the mRNA level, highlighting the importance of transcript-based analysis in cases where no mutation is found at the genomic level.

Conclusions This is the first study to characterize the *BTK* mutation spectrum in Vietnamese XLA patients, contributing novel variants to the global database and underscoring the value of integrative molecular strategies - particularly mRNA analysis - in enhancing early diagnosis and genetic counseling in this population.

Keywords X-linked · Agammaglobulinemia · Hemizygous · *BTK* gene · Vietnamese patients

Introduction

X-linked agammaglobulinemia (XLA) is a rare primary immunodeficiency disorder, affecting approximately 1 in 100,000 to 1 in 200,000 male births [1, 2]. It is caused by pathogenic variants in the *Bruton's tyrosine kinase* (*BTK*) gene located on the X chromosome. The BTK protein plays a critical role in early B cell development, and mutations in *BTK* gene result in a near-complete absence of peripheral B cells and markedly reduced levels of serum immunoglobulins

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[3]. Clinically, XLA is characterized by recurrent bacterial infections, severe hypogammaglobulinemia, and a lifelong susceptibility to infectious diseases [1].

The BTK protein consists of five functional domains: the pleckstrin homology (PH) and Tec homology (TH) domains form the N-terminal module, while the Src homology 3 (SH3), Src homology 2 (SH2), and kinase (SH1) domains constitute the Src module [2, 4]. These domains are connected by a proline-rich region [5]. The PH domain is responsible for membrane localization, the TH domain for structural stability, the SH2 and SH3 domains mediate critical protein–protein interactions, and the SH1 domain possesses essential kinase activity required for B cell development and antibody production [1, 5, 6]. Mutations in *BTK* can impair the structure or function of these domains, resulting in defective B-cell maturation and the clinical manifestation of XLA. Although immunoglobulin replacement therapy (IRT) remains the mainstay of treatment, XLA is still incurable and requires lifelong management [1]. Molecular diagnosis plays a crucial role in confirming the initial clinical assessement, enabling early intervention and genetic counseling. However, diagnostic challenges remain, particularly in regions with limited access to molecular testing, which can delay definitive diagnosis and appropriate care. Despite the identification of over 2,000 *BTK* mutations have been reported worldwide [7], data from Southeast Asian populations, including Vietnam, remain limited. Population-specific mutation profiling is essential as *BTK* variants exhibit high genetic heterogeneity, and the sensitivity of current diagnostic methods may vary across populations.

This study presents the first comprehensive analysis of *BTK* gene mutations in a cohort of Vietnamese patients with clinically diagnosed XLA. By identifying the mutation spectrum in this population, our findings aim to enhance the understanding of *BTK*-related genetic variation, support earlier and more accurate diagnosis, and contribute valuable data to global efforts in characterizing this rare disorder.

Table 1 Primer sequences for PCR amplification

Primer	Sequences (5'−3')	Length (bp)	Region
gDNA amplification primers			
BTK-g2F	GGATGAGGATTAATGTCCTG	912	Exons 2–3
BTK-g3R	TCACCAGTCTATTTACAGAG		
BTK-g4F	AAGGCTTCTAGTACCTAAGG	1860	Exons 4–5
BTK-g5R	TTTCCTTTTCCTCCCGTCTA		
BTK-g6F	AAATGGTGGCTTCTCCTCCA	638	Exons 6–7
BTK-g7R	GTGTTCTTAGGGCTTGACTA		
BTK-g8F	GTCTTGAGTAAGCCAGAGAG	340	Exon 8
BTK-g8R	GCAGCACTTTTTGCGTGTAG		
BTK-g9F	ATGAATCTGTCTCCTGGAGG	1978	Exons 9–12
BTK-g12R	CCTCTTATCACCTTGTCCTG		
BTK-g13F	TTAAGTGAGGATGTGTGAGG	1667	Exons 13–15
BTK-g15R	ATCTTCCACTGCTACTTCCA		
BTK-g16F	TGGTCAGCAGAAGCTTTGTG	993	Exons 16–17
BTK-g17R	GAACAATATCTCTGTGGAGG		
BTK-g18F	CTGGGGAAGATGCCATATGA	198	Exon 18
BTK-g18R	AATGGCCAGCTAAATGGGCA		
BTK-g19F	ATGCTACTGGGCATAGAGCA	258	Exon 19
BTK-g19R	GGGATTTCCTCTGAGAAAGT		
cDNA amplification primers			
BTK-F1	GTGAACTCCAGAAAGAAGA AGCT	2025	Full coding
BTK-R4	ACCAAGAAGCTTATTGGCG AGC		

Methods

Subjects

Patients with a clinical diagnosis of XLA were referred to the Center for Molecular Biomedicine, University of Medicine and Pharmacy at Ho Chi Minh City for genetic testing between 2018 and 2024. All patients met the diagnostic criteria for XLA, including recurrent bacterial infections, particularly of the respiratory tract and ears, as previously described [1, 8]. Because all specimens were from outside hospitals, we did not have access to clinical data and treatment information. The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (approval number 2658/HDDD-DHYD). Written informed consent was obtained from the parents or legal guardians of all minors.

Sample collection and extraction of DNA and RNA

Peripheral blood (4 mL) was collected from each patient.

Genomic DNA was extracted from whole blood using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) and stored at −30 °C.

Peripheral blood mononuclear cells (PBMCs) were isolated using 1X RBC lysis buffer and 1X phosphate-buffered saline (PBS). Total RNA was extracted from PBMCs using TRIzol® Reagent (Invitrogen, USA) and reverse-transcribed into complementary DNA(cDNA) using the PrimeScript™ RT Master Mix (Takara, USA).

Mutational analysis

PCR primers for exon and exon-intron boundary analysis of the *BTK* gene were designed based on the NCBI Consensus CDS reference sequences NG_009616.1 and NM_000061.3.

The entire coding regions of the *BTK* gene was amplified using primer pairs as listed in Table 1. Each 15 µL PCR reaction contained 25–50 ng of gDNA, 0.5 U Taq Hot

Start Polymerase (Takara Bio), 0.1 µM of each forward and reverse primer, 200 µM of each dNTP, and 1X PCR buffer. PCR was performed using a SimpliAmp Thermal Cycler (Thermo Scientific) with an annealing temperature of 60 °C.

PCR products were analyzed by 1.5% agarose gel electrophoresis and purified using ExoSAP-IT (Thermo Scientific). The purified amplicons were directly sequenced using the Sanger method with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in both forward and reverse directions. For large PCR products (those targeting exons 4–5, 9–12, and 13–15), we used additional primers for cycle sequencing to ensure that amplified products were sequenced in overlapping fragments. Sequencing reactions were analyzed on an ABI 3500 Genetic Analyzer (Applied Biosystems).

For samples in which no mutations were detected at the gDNA level, long-range RT-PCR was performed to amplify the full coding region of *BTK* using the primer pair BTK-F1 and BTK-R4 (see Table 1), to screen for potential large deletions detectable at transcript level. Each 15 µL PCR reaction contained 20–40 ng of cDNA, 0.5 U Taq Hot Start Polymerase (Takara Bio), 0.1 µM of each BTK-F1 and BTK-R4 primer, 200 µM of each dNTP, and 1X PCR buffer. Thermocycling was performed on a SimpliAmp Thermal Cycler (Thermo Scientific) using a three-step PCR program as follows: initial denaturation at 98 °C for 3 min, followed by 45 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 20 s, extension at 72 °C for 90 s, and final extension at 72 °C for 2 min.

In Silico predictions of variant pathogenicity

Sequencing data were analyzed using CLC Main Workbench v5.5 software based on the NM_000061.3 transcript reference. Nucleotide positions were numbered from the first ATG start codon, and variants were annotated according the HGVS nomenclature as recommended by T. den Dunnen [9]. Novel identified mutations were evaluated using multiple in-silico prediction tools and databases, including SpliceAI (https://spliceailookup.broadinstitute.org/), Genomnis (https://www.genomnis.com/), CADD (https://databases.lovd.nl/shared/variants/BTK), and Varsome (https://varsome.com), to provide a comprehensive evaluation of variant impact.

Results

In this study, mutation analysis of the coding regions (exons 1–19) of the *BTK* gene was performed on 82 unrelated Vietnamese male patients with clinical diagnosis of XLA. The mean age at diagnosis was 3 years (range: 1 to 18 years). All patients fulfilled the clinical diagnostic criteria for XLA.

Through comprehensive genetic analysis, we identified 35 distinct hemizygous pathogenic variants in 36 patients, corresponding to a mutation detection rate of 43.9% (Table 2). These variants were distributed across exons 2 to 18 of the *BTK* gene and affected all major functional domains of the BTK protein, including the PH, TH, SH3, SH2, and kinase domains (Fig. 1A).

Among the 35 identified mutations, missense variants were the most common, accounting for 37.1% (13 types), followed by large deletions (25.7%, 9 types). Other mutation types included splice-site mutations, nonsense mutations, frameshift mutations, and in-frame deletions. Almost all of the mutations identified were unique to individual patients, except for c.1898G>T (p.C633F), which was found in two unrelated individuals.

Within the exonic regions of genomic DNA, seven patients carried severe loss-of-function mutations, including nonsense and frameshift variants. Three previously reported nonsense mutations—p.R255X, p.Q497X, and p.W634X – were identified [10–13]. along with one novel nonsense mutation, p.Q343X. In addition, three well-known frameshift mutations (p.K71NfsX50, p.V537fsX3, and p.R562fsX6) were detected, all of which introduced premature stop codons, leading to truncated BTK proteins.

Five splice-site mutations were identified, four of which had been previously described in the literature: c.392–2 A>G [12], c.777-1G>A [10, 14], c.974+1G>T [15], and c.1567–2 A>G [14]. All four were evaluated at the mRNA level and were shown to cause exon skipping corresponding to the location of the mutation. While the IVS17+5G>A variant has been reported by Fiorini et al. [11], our study identified a novel splice-site mutation at the same position, IVS17+5G>C (c.1750+5G>C), which has not been previously reported. This variant was predicted to be pathogenic by several in-silico tools: SpliceAI (Pangolin score=0.73), Genomnis (high likelihood of splice disruption), CADD (score=22.2), and was classified as pathogenic by Varsome. mRNA analysis revealed the presence of both a normal transcript and an aberrant transcript with skipping of exons 16 and 17 (Fig. 2), further supporting its pathogenicity. These findings suggest that the guanine-to-cytosine substitution at the +5 position of intron 17 weakens but does not entirely abolish the function of this splice site.

Mutations detectable only at the mRNA level but not at the gDNA level included 9 large deletions and a 30 bp insertion between exons 5 and 6. The large deletions were distributed across various regions of the *BTK* gene, affecting multiple exons and disrupting critical functional domains of the protein (Fig. 1B). All 9 large deletions resulted in

Table 2 *BTK* gene mutations identified in 36 Vietnamese patients with XLA

No	Code	Localization	Nucleotide aberration		Amino acid aberration	Mutation type
			DNA level	RNA level		
1	XLA-AP21	Exons 2–8		c.92_768del	p.L31fsX10	Large deletion
2	XLA-AP27	Exons 2–7		c.110_563del	p.S38fsX10	Large deletion
3	XLA-AP19	Exons 2–10		c.125_841del	p.Y42fsX380	Large deletion
4	XLA-51	Exon 3	c.213_214delinsT		p.K71fsX50	Frameshift
5	XLA-AP15	Exons 5–6		c.391_392ins30	p.N130_V131insDVISLFFPAT	Inframe
6	XLA-36	Intron 5	c.392–2 A>G	c.392_520del	p.V131_S174>G	Splice site (Inframe)
7	XLA-AP18	Exons 6–13		c.394_1123del	p.R133fsX27	Large deletion
8	XLA-AP6	Exons 7–15		c.574_1445del	p.E194fsX23	Large deletion
9	XLA-14	Exon 8	c.763 C>T		p.R255X	Nonsense
10	XLA-38	Exon 9	c.777-1G>A	c.777_839del	p.Q260_E280del	Splice site (Inframe)
11	XLA-2	Exon 10	c.862 C>T		p.R288W	Missense
12	XLA-43	Exon 11	c.906_908delAGG		p.G303del	Inframe
13	XLA-47	Intron 11	c.974+1G>T	c.895_974del	p.K300fsX22	Splice site (Frameshift)
14	XLA-18	Exon 12	**c.1027 C>T**		**p.Q343X (NOVEL)**	Nonsense
15	XLA-45	Exon 12	c.1037T>A		p.L346Q	Missense
16	XLA-AP2	Exons 12–18		c.1059_1758del	p.S353fsX63	Large deletion
17	XLA-33	Exon 12	c.1061 C>T		p.T354I	Missense
18	XLA-AP30	Exons 12–17		c.1071_1708del	p.E357fsX3	Large deletion
19	XLA-AP13	Exons 12–15		c.1093_1531del	p.N365fsX19	Large deletion
20	XLA-AP16	Exon 14	c.1205T>C		p.L402P	Missense
21	XLA-13	Exons 15–16		c.1350_1631del	p.N451fsX116	Large deletion
22	XLA-50	Exon 15	c.1457T>G		p.L486R	Missense
23	XLA-6	Exon 15	c.1489 C>T		p.Q497X	Nonsense
24	XLA-44	Exon 15	c.1505G>A		p.C502Y	Missense
25	XLA-48	Exon 15	c.1522G>A		p.A508T	Missense
26	XLA-35	Intron 15	c.1567–2 A>G	c.1567_1631del	p.A523fsX5	Splice site (Frameshift)
27	XLA-29	Exon 16	c.1608_1609insA		p.V537fsX3	Frameshift
28	XLA-40	Exon 17	c.1684_1700delinsGGG		p.R562fsX6	Frameshift
29	XLA-42	Exon 17	c.1696 C>T		p.P566S	Missense
30	XLA-17	Exon 17	c.1745 C>A		p.A582D	Missense
31	XLA-41	Intron 17	**c.1750+5G>C**	**c.1567_1750del**	**p.A523fsX3 (NOVEL)**	Splice site (Frameshift)
32	XLA-AP1	Exon 18	c.1855 C>A		p.P619T	Missense
33	XLA-1	Exon 18	c.1898G>T		p.C633F	Missense
34	XLA-46	Exon 18	c.1898G>T		p.C633F	Missense
35	XLA-22	Exon 18	c.1902G>A		p.W634X	Nonsense
36	XLA-AP25	Exon 19	c.1921 C>T		p.R641C	Missense

frameshifts. Figure 3 illustrates one representative case with a large deletion spanning from exon 7 to exon 15.

Discussion

This study presents the first comprehensive analysis of *BTK* gene mutations in a Vietnamese cohort of patients with XLA. We analyzed 82 clinically diagnosed XLA patients and identified pathogenic *BTK* mutations in 36 individuals (43.9%). This detection rate is consistent with previous studies, which report rates ranging from 40 to 80%, depending on cohort size, inclusion criteria, and methods used [3, 11]16– [19]. Our findings expand the known *BTK* mutation spectrum by reporting 35 distinct variants, including two novel mutations: one nonsense mutation (p.Q343X) and one splice-site mutation (IVS17+5G>C). These findings contribute to the *BTK* mutation database and highlight the genetic diversity associated with XLA.

Consistent with prior research, missense mutations were the most common in our cohort, accounting for 37.1% of all detected mutations. This trend has also been observed in international studies from Europe, North America, and East Asia, indicating a shared mutation pattern across diverse populations [10, 11, 16, 18, 20].

Among the five splice-site mutations identified, the IVS17+5G>C variant was novel and caused skipping of exons 16 and 17 in the mRNA transcript. A mutation at the same position, IVS17+5G>A, was previously reported by Fiorini et al. [11], highlighting the critical role of this

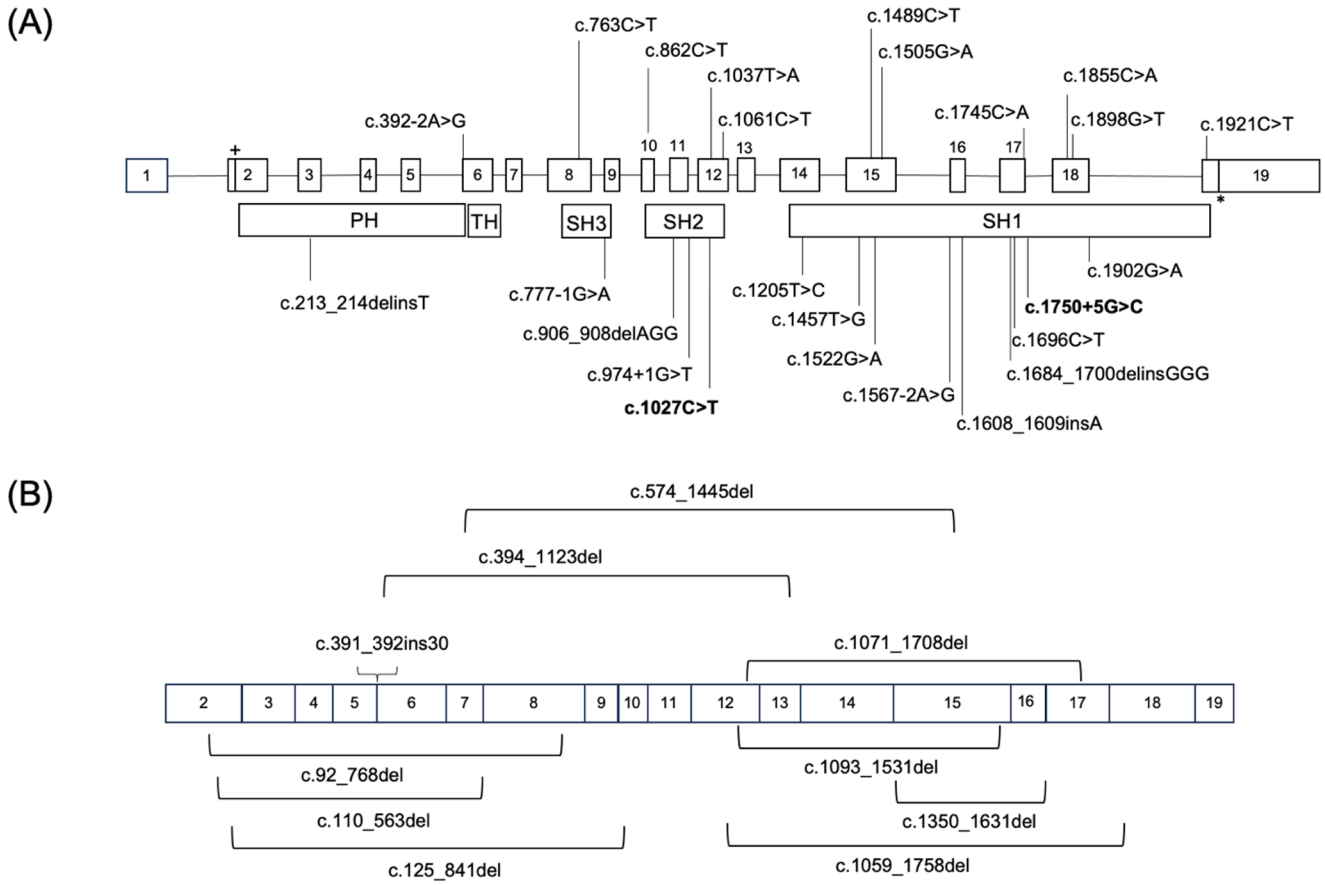

Fig. 1 Schematic representation of *BTK* gene mutations identified in Vietnamese patients with XLA (**A**) Distribution of small mutations identified with genomic DNA sequencing across exons and exon-intron boundaries of the *BTK* gene. Novel mutations are shown in bold. (**B**) Locations and extents of large deletions and in-frame insertion across *BTK* exons, identified with cDNA sequencing. Each bar indicates the coding region affected in the corresponding patients

nucleotide in normal exon recognition during RNA splicing. This finding emphasizes the importance of transcript-level analysis in evaluating the pathogenic effects of splice-site variants.

In addition to previously described nonsense mutations (p.R255X, p.Q497X, and p.W634X), we identified a novel nonsense mutation, p.Q343X, which introduces a premature stop codon within the SH2 domain. Given the domain's essential role in mediating protein–protein interactions during B-cell signaling, this mutation likely results in complete loss of BTK function and a classic XLA phenotype.

Large deletions were detected in 9 patients and involved multiple exons, disrupting various functional domains of the BTK protein. These mutations are known to lead to the production of truncated proteins with impaired or altered function and are often associated with severe disease phenotypes. Notably, all large deletions were detectable only via cDNA analysis and were absent in gDNA sequencing, highlighting the critical role of complementary diagnostic approaches to improve mutation detection.

Finally, two in-frame deletions were also identified, both affecting regions with important functional roles in the BTK protein. Overall, the mutation spectrum observed in our Vietnamese XLA cohort reflects significant genetic heterogeneity, and the novel variants described here contribute to the ongoing global effort to characterize the diverse genotypic landscape of XLA.

Conclusions

Our study provides the first comprehensive analysis of *BTK* gene mutations in Vietnamese patients with XLA. We identified a wide range of pathogenic variants, including two novel mutations, and emphasized the critical role of mRNA analysis in detecting large deletions and splice-site abnormalities. These findings not only expand the current understanding of *BTK*-related mutations in Southeast Asia but also underscore the importance of integrated molecular approaches in the diagnosis of primary immunodeficiency disorders.

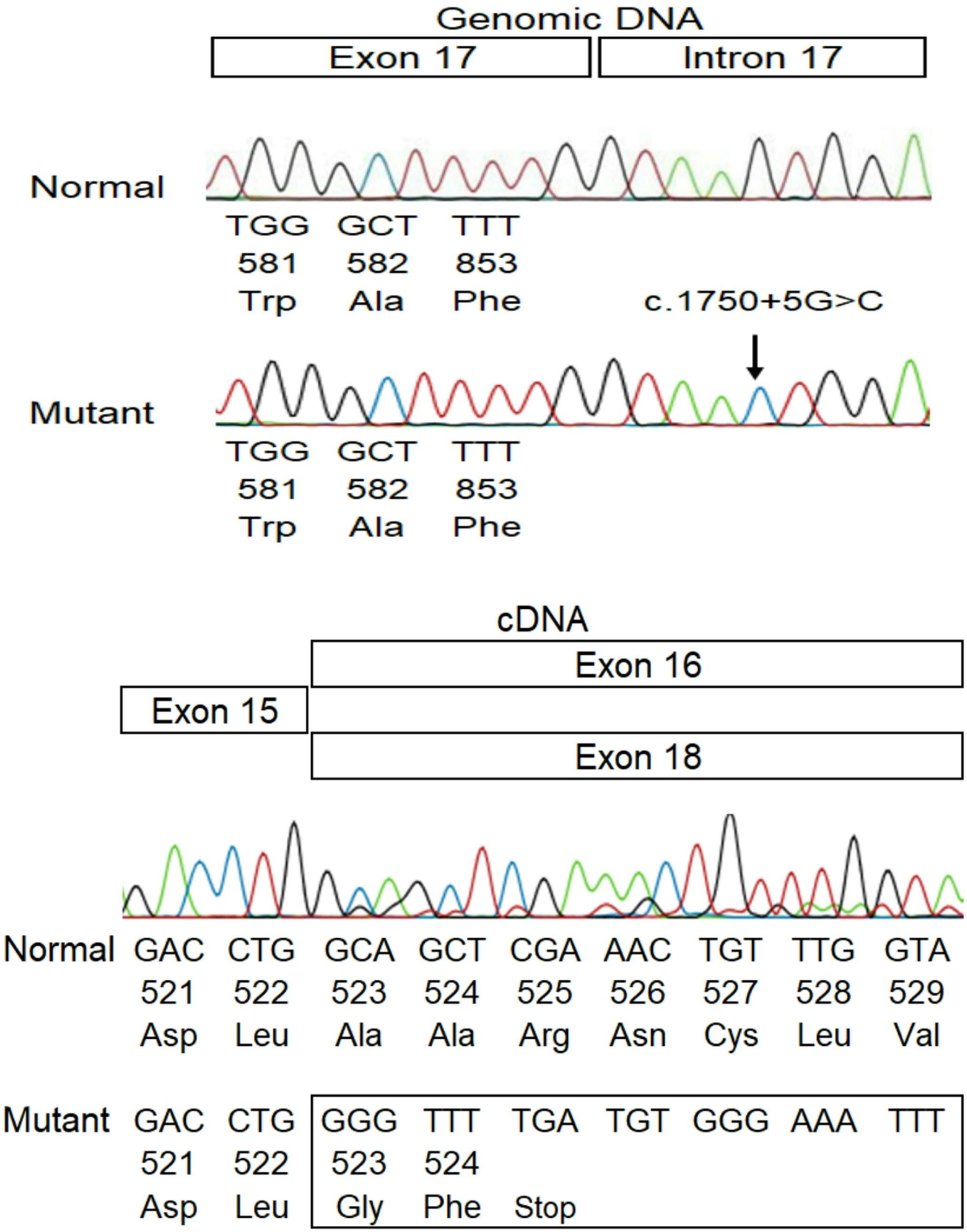

Fig. 2 The mutation c.1750+5G>C in genomic DNA of the *BTK* gene resulted in skipping of exons 16 and 17 at the mRNA level

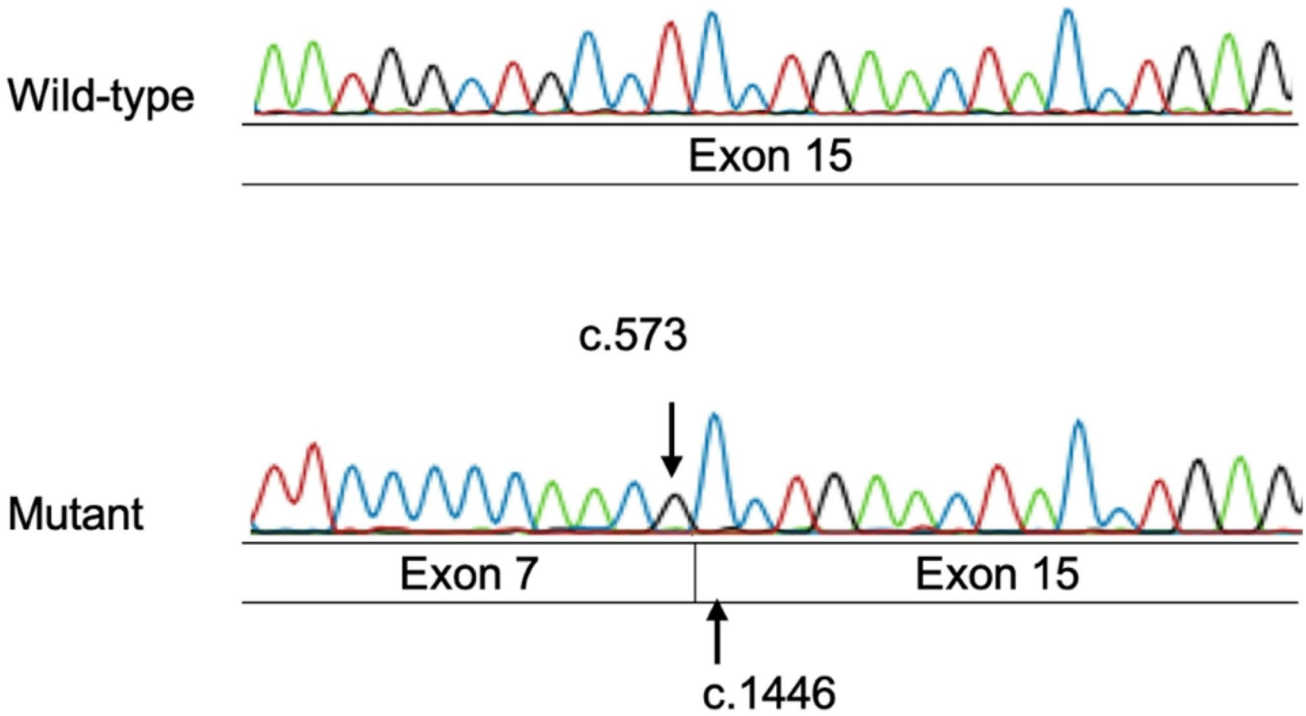

Fig. 3 The large deletion mutation c.574_1445del, spanning exons 7 to 15 was detected in patient XLA-AP6 through cDNA sequencing

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Author contributions PTX, HQC, HN, HLP, and HAV designed the study; HQC, HPTH, PNLA, NHMA, and NDV performed experiments; PTX, HQC, HPTH, NDV, NHMA, HN, and HAV collected and analyzed data; PTX, HQC, and HAV wrote the manuscript. All authors read and approved the final manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no conflict of interests.

Ethical approval The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (approval number 2658/HDDD-DHYD). Written informed consent was obtained from the parents or legal guardians of all minors.

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