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Beyond Recurrent Infections: Immune Dysregulation Manifestations, Genotype–phenotype Heterogeneity, and Three Novel *BTK* Variants in X-linked Agammaglobulinemia

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ABSTRACT

X-linked agammaglobulinemia (XLA) is an inborn error of immunity caused by *BTK* gene mutations, characterized by hypogammaglobulinemia and recurrent infections. This study evaluates a long-term pediatric cohort to assess clinical spectrum, therapeutic outcomes, and molecular findings.

We retrospectively reviewed clinical, immunological, treatment, and genetic data for 11 male XLA patients followed over 20 years at our center.

Median age at symptom onset was 12.7 months (range: 3–60), while median age at diagnosis was 4.5 years (range: 1–10), revealing marked diagnostic delays in patients without family history. Severe complications included bronchiectasis, septic arthritis, and immune dysregulation (e.g., juvenile idiopathic arthritis, autoimmune hepatitis, pancytopenia, celiac disease). Immunoglobulin replacement and antibiotic prophylaxis significantly reduced median annual infections from 16.9 to 2.09. Six distinct *BTK* variants were identified, including three novel mutations: c.90del, c.1922_1924delGTC, and c.1589dup. Inter-sibling phenotypic heterogeneity was notable. In the absence of functional expression studies, pathogenic contributions of novel variants remain exploratory.

XLA extends beyond recurrent infections to encompass significant immune dysregulation and phenotypic variability. Early diagnosis, family screening, genetic testing, and tailored immunoglobulin therapy are vital to improve long-term outcomes. Larger multicenter cohorts with functional testing are needed to validate genotype–phenotype associations.

Keywords: *BTK* gene mutations; Genotype–phenotype correlation; Immune dysregulation; Inborn error of immunity; X-linked agammaglobulinemia

INTRODUCTION

X-linked agammaglobulinemia (XLA) is a prototype of congenital humoral immunodeficiencies caused by mutations in the Bruton tyrosine kinase (*BTK*) gene.[1,2] Characterized by a profound block in B-cell differentiation, XLA traditionally presents with severe hypogammaglobulinemia and recurrent bacterial infections and is managed with lifelong immunoglobulin replacement therapy (IgRT).[3,4]

However, emerging clinical evidence over the last

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decade has challenged the classical paradigm of XLA as purely an infectious disease. It is increasingly recognized that immune dysregulation, encompassing autoimmune and autoinflammatory manifestations, represents an important component of the disease spectrum.[5,6] Despite the absence of mature B cells, inflammatory and autoimmune complications frequently complicate the clinical course, yet their precise pathophysiological mechanisms remain poorly understood. Furthermore, the relationship between specific *BTK* variants and clinical severity—the genotype–phenotype correlation—remains inconsistent across studies.[7,8] Identical mutations, even among siblings, frequently yield substantially different clinical phenotypes, complicating prognostic predictions.

Compounding these phenotypic uncertainties is the persistent challenge of diagnostic delay.[9] Despite increased global awareness of inborn errors of immunity (IEIs), many XLA patients undergo years of recurrent infections before diagnosis. Prolonged diagnostic delay is associated with irreversible tissue damage, such as bronchiectasis, which contributes to long-term morbidity. While adult registries or mixed-age reports exist, detailed long-term follow-up data specifically evaluating these multifaceted non-infectious complications and diagnostic delays within strictly pediatric cohorts remain notably limited.

Although previous XLA cohorts have substantially expanded our understanding of *BTK* mutations and infectious complications, several clinically relevant questions remain unresolved. Most published studies have primarily focused on infection susceptibility, immunoglobulin replacement outcomes, or molecular characterization, whereas long-term pediatric data integrating immune dysregulation-related complications, diagnostic delay, treatment response, and genotype–phenotype variability within the same cohort remain limited. Furthermore, the clinical impact of novel *BTK* variants is often reported without detailed longitudinal phenotypic characterization. Consequently, this study provides a comprehensive characterization of pediatric XLA by integrating long-term clinical follow-up, immune dysregulation outcomes, diagnostic delay trends, and molecular characterization at a single center. In particular, this study reports three previously unreported *BTK* variants, documents marked phenotypic heterogeneity among affected siblings carrying identical variants, and provides long-term clinical follow-up data emphasizing non-infectious complications, diagnostic delay, and treatment outcomes. Together, these findings expand the current clinical and molecular understanding of XLA and highlight the complexity of genotype–phenotype relationships.

MATERIALS AND METHODS

Patients and Study Design

This retrospective study analyzed 11 male patients diagnosed with XLA according to the European Society for Immunodeficiencies (ESID)[10] criteria, who were followed up at the Pediatric Allergy and Immunology Department of our hospital between 2006 and 2025. Demographic, clinical, and immunological data were collected from medical records.

Demographic Data

Sex, age of symptom onset, age at diagnosis, current age, history of immunodeficiency in the family, history of immunodeficiency in siblings, and consanguinity between parents.

Clinical Data

Presenting complaints, clinical presentations, history of previous severe infections, and annual infection counts. Annual infection frequency was defined as the total number of clinically documented infectious episodes occurring within a one-year period. For each patient, the pre-treatment annual infection frequency was calculated based on the last year before the diagnosis of XLA, whereas the post-treatment annual infection frequency was calculated based on the most recent one-year follow-up period after the initiation of immunoglobulin replacement therapy and prophylactic antibiotic treatment. Only infections documented in the medical records and requiring medical evaluation and/or antimicrobial treatment were included in the analysis. Autoimmune and autoinflammatory conditions were diagnosed according to established international classification and diagnostic criteria. Juvenile idiopathic arthritis was diagnosed according to the International League of Associations for Rheumatology (ILAR) classification criteria, autoimmune hepatitis according to the simplified International Autoimmune Hepatitis Group (IAIHG) criteria, celiac disease according to the ESPGHAN guidelines, and familial Mediterranean fever according to the Tel Hashomer criteria. All diagnoses were established by the corresponding pediatric subspecialists.

Immunological Data

Complete blood count, serum levels of IgA, IgM, IgE, total IgG, IgG subclasses (IgG1, IgG2, IgG3, IgG4), antibody responses to live (measles, rubella, mumps, varicella) and inactivated (Hepatitis B, pneumococcus) vaccines, isohemagglutinin antibody responses, and lymphocyte subset results (CD3, CD4, CD8, CD19, CD20, CD56/16, CD45RA, CD45RO) were retrospectively reviewed.

BTK gene mutations were identified in 9 patients, leading to a diagnosis of definite XLA. In the remaining 2 patients, no pathogenic *BTK* variant was detected despite molecular analysis. However, these patients fulfilled the ESID working definition for possible XLA, based on the presence of a compatible clinical phenotype, profound hypogammaglobulinemia, markedly reduced peripheral B cells, exclusion of secondary causes of antibody deficiency, and an X-linked family history. Due to the retrospective nature of the study, further molecular investigations were not feasible. These include whole-genome sequencing, extended gene panel analysis for alternative causes of congenital agammaglobulinemia, evaluation of deep intronic and non-coding *BTK* variants, and targeted sequencing of other associated genes. Therefore, these 2 patients were included in the overall clinical characterization of the cohort but were excluded from *BTK* variant-specific genotype–phenotype interpretation.

Laboratory Analyses

Serum Ig levels and IgG subclasses were measured by the nephelometric method (Dade Behring, Marburg, Germany). Peripheral blood lymphocyte subsets were measured by flow cytometry using the Beckman Coulter system (USA), following standardized staining and lysis procedures recommended by the manufacturer.

Peripheral blood samples were analyzed for *BTK* variants using next-generation sequencing (NGS) or conventional PCR-based Sanger sequencing, depending on the period during which genetic testing was performed. All variants initially identified by NGS were subsequently confirmed by Sanger sequencing before clinical interpretation. Written informed consent for genetic testing was obtained from the parents or the patients.

Variant pathogenicity was evaluated according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines. Interpretation incorporated the available clinical and molecular evidence and was supported using the VarSome and Franklin variant interpretation platforms. No functional assays were available to independently validate the biological effects of the identified variants. Variants were classified as pathogenic, likely pathogenic, variants of uncertain significance, likely benign, or benign according to the ACMG/AMP framework. For each variant, the specific ACMG/AMP evidence codes supporting pathogenicity classification (e.g., PVS1, PS3, PM1, PM2, PM4, PM5, PP2, and PP3) are provided in Table 4. Detailed sequencing quality metrics, including platform-specific sequencing coverage, were not consistently available because molecular analyses were performed over an extended study period in different accredited diagnostic laboratories. In addition, systematic copy number variation (CNV) analysis was not performed.

Statistical Analysis

Descriptive statistical methods were used to summarize the demographic, clinical, immunological, and genetic characteristics of the study population. Categorical variables are presented as frequencies (n) and percentages (%), whereas continuous variables are reported as medians and ranges because of the small sample size and the non-normal distribution of the data. Annual infection frequencies before diagnosis and after treatment were treated as paired observations for each patient and compared using the Wilcoxon signed-rank test. To improve the interpretation of the primary paired analysis beyond statistical significance alone, the Wilcoxon signed-rank effect size ($r = Z/\sqrt{N}$) was calculated. In addition, the Hodges–Lehmann estimate of the median paired difference together with its 95% confidence interval (CI) was reported. A 2-sided $p<0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 27.0.

Given the limited sample size and the exploratory nature of this retrospective study, statistical power was inherently limited, increasing the possibility of type II error. Therefore, non-significant findings should be interpreted cautiously and should not be considered evidence of the absence of clinically meaningful differences. Because this study included the entire available cohort of patients with X-linked agammaglobulinemia treated at our center, additional sensitivity analyses were considered unlikely to provide

meaningful additional information or substantially alter the interpretation of the findings. Accordingly, the statistical analyses were intended to support the descriptive and exploratory interpretation of the clinical observations rather than to establish definitive inferential conclusions.

RESULTS

Demographic and Immunological Characteristics

The study included 11 male patients evaluated according to the ESID diagnostic criteria for XLA.[10] Nine patients had a definite XLA diagnosis, and 2 had a possible XLA diagnosis. Parental consanguinity was present in 55% of the patients. Two families contributed multiple affected individuals: one family with 3 affected siblings (P1, P2, P3) and another with 2 affected siblings (P7, P8). The presence of these families resulted in a positive family history for XLA in 45% of the total cases. In the family with 3 affected brothers, there was also a history of early death in 2 male siblings. Overall, 55% of the patients had sporadic disease (no family history) (Table 1).

Ninety percent of the patients exhibited frequent infection symptoms during the first year of life. The median age of symptom onset was 12.7 months (range, 3–60 months). The median age at diagnosis was 4.5 years (range, 1–10 years), which was numerically earlier in cases with a positive family history (3.5 years; range, 1–8 years) compared to sporadic cases (5.3 years; range, 1.5–10 years). Diagnosis was established before the age of 5 in 45% of the patients and before the age of 1 in 18% of the patients. The median time elapsed between the onset of symptoms and the confirmed diagnosis was 39.9 months (range, 6–108 months). The median diagnostic delay in patients with a positive family history was 12.0 months (range, 4–30), whereas it was 54.0 months (range, 2–144) in those without a family history. Although the diagnostic delay was numerically shorter in the family history group, the difference did not reach statistical significance, likely due to the small sample size (p=0.082). However, these findings suggest a clinical trend toward earlier diagnosis in patients with a known family history of IEI.

As detailed in Table 2, all patients exhibited markedly reduced percentages of $CD19^+$ B cells and severely decreased serum immunoglobulin (IgG, IgA, and IgM) levels at the time of diagnosis. The $CD19^+$ B cell percentage ranged from 0% to 1% across all patients, with 5 of the 11 patients showing 0% B cells. Complete blood count and T-cell counts were within the normal range for all patients. All patients lacked both antibody responses to live (measles, rubella, mumps, varicella) and inactivated (Hepatitis B, pneumococcus) vaccines administered before diagnosis, as well as isohemagglutinin (anti-A and/or anti-B) antibody responses.

Table 1. Demographic and clinical characteristics of the study cohort.

Patient[a]	Consanguinity	Sibling death	Family history	Symptom onset, y	Diagnosis age, y	Diagnostic delay, mo	Annual infections before treatment	Annual infections after treatment	Current age/age at death, y
P1	(+)	(+)	(+)	0.5	1	6	8	2	21
P2	(+)	(+)	(+)	0.25	1	9	10	4	23[b]
P3	(+)	(+)	(+)	1	1.5	6	2	0	15
P4	(-)	(-)	(-)	0.5	10	108	32	2	12
P5	(-)	(-)	(-)	1	7	72	10	2	21
P6	(-)	(-)	(-)	0.5	2	18	20	1	17
P7	(+)	(-)	(+)	0.66	6	60	24	2	14
P8	(+)	(-)	(+)	1	8	84	20	2	16
P9	(-)	(-)	(-)	5	5.5	6	10	2	13
P10	(-)	(-)	(-)	0.58	1.5	10	32	3	12
P11	(+)	(-)	(+)	0.66	6	60	18	3	31

[a]P1, P2, and P3 are affected siblings from one family, while P7 and P8 are affected siblings from a different family. [b]Indicates the patient who died of liver failure during the follow-up period.

Table 2. Immunological characteristics and treatment of the study cohort.

Patient	IgG/IgA/IgM, mg/dL	IgE, kU/L	CD19 B, %	IGRT type	Trough IgG, mg/dL	Prophylactic antibiotic
P1	80 / <26.8 / <18.2	<2.43	0.0	IVIG	844	Azithromycin
P2	120 / <26 / <19.4	<2.11	0.0	IVIG	1140	TMP-SMX
P3	105 / <23.3 / <18.4	<2.43	0.3	IVIG	820	TMP-SMX
P4	24 / <23.3 / <16.8	0.1	0.0	SCIG	780	TMP-SMX
P5	150 / <26.1 / <18.4	<2.59	0.03	SCIG	975	TMP-SMX
P6	145 / <23.3 / <18.4	17.6	0.0	SCIG	790	TMP-SMX
P7	135 / 25 / 16	8.4	0.13	SCIG	910	TMP-SMX
P8	146 / 24 / 42	17	0.2	SCIG	827	TMP-SMX
P9	18 / 27 / 17	2.1	0.09	IVIG	870	TMP-SMX
P10	48 / 27 / 20	18	0.0	SCIG	810	Amoxicillin-clavulanate
P11	110 / 14 / 11	4	0.5	SCIG	794	Azithromycin

IGRT: immunoglobulin replacement therapy; IVIG: intravenous immunoglobulin; SCIG: subcutaneous immunoglobulin; TMP-SMX: trimethoprim-sulfamethoxazole.

Following diagnosis, all patients were initiated on IgRT administered regularly, along with prophylactic antibiotic treatments (trimethoprim-sulfamethoxazole, azithromycin, or amoxicillin-clavulanate). The median trough IgG level in our patients during the last follow-up year was 869.09 mg/dL (range, 790–1140 mg/dL). Four patients were treated via the intravenous route, and 7 via the subcutaneous route.

Following the initiation of IgRT and antibiotic prophylaxis, the median annual infection frequency decreased from 16.9 (range, 2–32) prior to diagnosis to 2.09 (range, 0–4) after treatment (p=0.004, Wilcoxon signed-rank test). The treatment effect was large (effect size, r=0.86). The Hodges–Lehmann estimate demonstrated a median paired reduction of 15.0 infections per year (95% CI, 8.0–21.0), indicating a clinically meaningful reduction in infection burden (Table 1). Due to the small sample size, a statistical subgroup analysis comparing the efficacy of subcutaneous immunoglobulin versus intravenous immunoglobulin could not be performed.

Clinical Features

No complications developed following routine live vaccinations (Bacillus Calmette–Guérin, measles, rubella, mumps, varicella) administered prior to diagnosis in our cohort. The most common infections at presentation were sinopulmonary infections, with recurrent pharyngitis observed in all patients (100%), followed by recurrent pneumonia, bronchopneumonia, and bronchitis (81%). Physical examination in all patients revealed hypoplastic or aplastic lymphoid tissue (tonsils, cervical lymph nodes, etc.). Two patients developed a combination of autoimmune hepatitis, pancytopenia, and juvenile idiopathic arthritis (JIA) during follow-up. One of these cases was also complicated by familial Mediterranean fever (FMF) and celiac disease. Regarding survival, only 1 patient died at the age of 23 due to liver failure (Figure 1, Table 3).

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Table 3. Presenting complaints, follow-up complications, and mortality.

Presenting Complaints	No. (%)	Follow-up Complications / Mortality	No. (%)
Recurrent pharyngitis	11 (100)	Juvenile idiopathic arthritis	2 (18)
Recurrent pneumonia / bronchitis	9 (81)	Autoimmune hepatitis	2 (18)
Recurrent sinusitis	8 (72)	Pancytopenia	2 (18)
Recurrent otitis media	5 (45)	Atopic dermatitis	2 (18)
Recurrent skin infections	4 (36)	Septic arthritis	2 (18)
Chronic diarrhea with malabsorption	3 (27)	Familial Mediterranean fever	1 (9)
Failure to thrive	3 (27)	Celiac disease	1 (9)
Bronchiectasis	1 (9)	Bronchiectasis	1 (9)
Anal abscess	1 (9)	Acute liver failure	1 (9)
Bacterial meningitis	1 (9)	Mortality	1 (9)

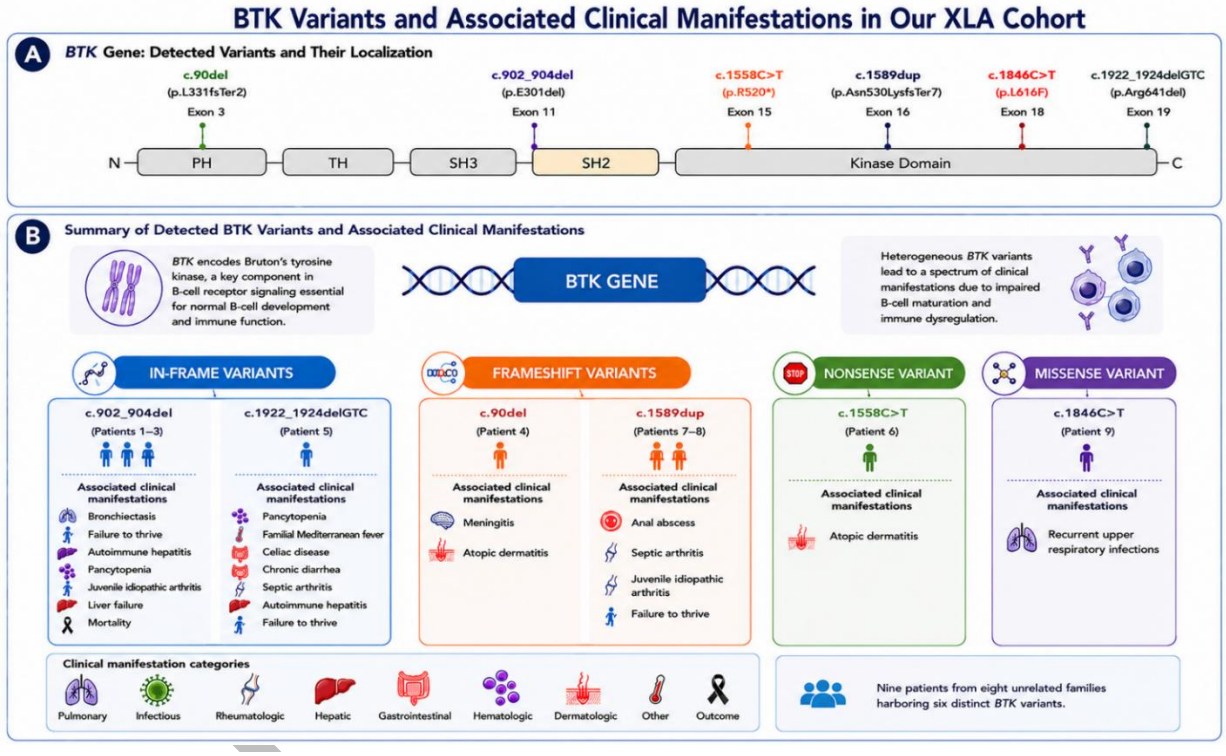

Figure 1. A. Schematic representation of the *BTK* protein showing the location of the identified variants across functional domains. B. Summary of the detected *BTK* variants, their classification, and the associated clinical manifestations. Patients without molecular confirmation (P10 and P11) were excluded. *BTK*, Bruton's tyrosine kinase.

Genetic Characteristics

The diagnosis of XLA was genetically confirmed in 9 of our patients. Only the 9 genetically confirmed patients were considered for *BTK* variant-specific genotype–phenotype comparisons. Six distinct genetic variants were identified, 3 of which were novel. The pathogenicity scores for each variant were determined based on the standard ACMG classification criteria. These variants were distributed throughout the *BTK* gene, predominantly localized to the Kinase domain (Figure 1, Table 4).

Despite sharing the same c.902_904del variant, the 3 affected siblings (P1, P2, P3) exhibited different clinical presentations. Among the 3 siblings with the same mutation, long-term follow-up revealed: bronchiectasis in the middle brother, autoimmune hepatitis, pancytopenia, and death in the eldest brother, and only growth retardation in the youngest brother. These clinical observations are consistent with the clinical heterogeneity previously described in XLA; however, they should not be interpreted as functional evidence of variant pathogenicity. Of the 2 siblings (P7, P8) sharing the same mutation, one developed an anal abscess and septic arthritis, while the other developed JIA and growth retardation. No consistent relationship between *BTK* variant type and clinical severity could be established in this cohort. Although some patients carrying frameshift or in-frame variants experienced severe complications and some patients with missense or nonsense variants had relatively milder clinical findings, these observations should be interpreted as descriptive and exploratory rather than evidence of a definitive genotype–phenotype correlation. However, because of the limited sample size and familial clustering, these observations should be regarded as descriptive findings rather than evidence of a definitive genotype–phenotype correlation (Figure 1, Table 5).

Table 4. Genetic variations detected in X-linked agammaglobulinemia patients.

Variant	Variant type	Domain	Novel	Exon	Transcript	Pathogenicity score
c.902_904del (p.E301del)	Inframe	SH2	No	11	NM_000061/NM_00006 1.3	LP (PM2+PM4+PM1+PS3)
c.90del (p.L31SfsTer2)	Frameshift	PH	Yes	3	NM_000061	LP (PVS1+PM2)
c.1922_1924delGTC (p.Arg641del)	Inframe	Kinase	Yes	19	NM_000061.2	LP (PM4+PM5+PM2+PM1)
c.1558C>T (p.R520*)	Nonsense	Kinase	No	15	NM_000061	P (PVS1+PM3+PM2)
c.1589dup (p.Asn530LysfsTer7)	Frameshift	Kinase	Yes	16	NM_000061.3	LP (PVS1+PM2)
c.1846C>T (p.L616F)	Missense	Kinase	No	18	NM_000061.2	LP (PM1+PP2+PM2+PP3)

Only genetically confirmed patients (n=9) are included in this table. LP: likely pathogenic; P: pathogenic.

Table 5. Clinical features of genetic variations.

Patient	Variant	Variant type	Clinical features
P1	c.902_904del	Inframe	Bronchiectasis
P2	c.902_904del	Inframe	Autoimmune hepatitis, pancytopenia, JIA, FTT, liver failure, mortality
P3	c.902_904del	Inframe	FTT
P4	c.90del	Frameshift	Meningitis, atopic dermatitis
P5	c.1922_1924delGTC	Inframe	Pancytopenia, FMF, celiac disease, diarrhea, septic arthritis, autoimmune hepatitis, FTT
P6	c.1558C>T	Nonsense	Atopic dermatitis
P7	c.1589dup	Frameshift	Anal abscess, septic arthritis
P8	c.1589dup	Frameshift	JIA, failure to thrive
P9	c.1846C>T	Missense	Upper respiratory infections
P10	Not detected (Possible XLA)	NA	Diarrhea, skin infections
P11	Not detected (Possible XLA)	NA	Bronchiectasis

FMF: familial Mediterranean fever; FTT: failure to thrive; JIA: juvenile idiopathic arthritis. Patients P10 and P11 fulfilled the ESID working definition for possible XLA but lacked molecular confirmation and were therefore not included in *BTK* variant-specific genotype–phenotype interpretation.

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DISCUSSION

Over a 20-year period, we comprehensively evaluated a pediatric XLA cohort with long-term follow-up. Beyond confirming the well-recognized infectious phenotype of XLA, our study provides 3 key findings: (1) identification of 3 previously unreported *BTK* variants, (2) marked phenotypic heterogeneity among affected siblings carrying identical variants,[7] and (3) comprehensive characterization of immune dysregulation in a single long-term pediatric cohort.[11]

Although autoimmune manifestations and clinical heterogeneity have been reported previously, our principal contribution is the integration of these clinical observations with detailed molecular characterization, long-term therapeutic outcomes, and diagnostic delay analysis within a single pediatric cohort.[12]

Although our findings are broadly consistent with larger national and international registries, the relatively small size of our cohort limits direct comparisons and precludes definitive conclusions regarding genotype–phenotype relationships. Accordingly, our observations should be interpreted as descriptive and hypothesis-generating.[9,13,14]

In our cohort, immunoglobulin replacement therapy and prophylactic antibiotic use were associated with a marked reduction in infection frequency during follow-up. Although these observations are consistent with the established clinical benefits of IgRT in XLA, the retrospective uncontrolled design of our study does not permit causal inference. Therefore, the observed reduction in infections should be interpreted as an association rather than definitive evidence of treatment efficacy within this cohort. These findings emphasize the need for increased clinical awareness, earlier diagnosis, and a multidisciplinary long-term management approach for XLA. Nevertheless, because our cohort was derived from a tertiary referral center, the observed clinical spectrum may be enriched for patients with more severe or diagnostically challenging disease.

Despite well-established diagnostic criteria, delayed diagnosis remains a significant challenge in XLA.[9] Insufficient awareness of IEIs, together with limited access to immunologic and genetic testing, may contribute to delayed recognition and increased morbidity. Enhancing clinician awareness is therefore critical for early diagnosis and improved patient outcomes.[15] In our cohort, the median age at diagnosis was 4.5 years, with a median diagnostic delay of 39.9 months. Patients without a family history were often diagnosed only after severe complications, highlighting the importance of early clinical suspicion for XLA.[13] Our findings also have important practical implications for reducing diagnostic delay in XLA. Earlier recognition through increased clinician awareness, family screening, genetic counseling, and broader access to immunological and molecular testing may facilitate timely diagnosis and earlier initiation of IgRT, thereby reducing long-term morbidity.

Lower respiratory tract infections were the most common clinical presentation, in line with previous studies.[14,16,17] Recurrent sinopulmonary infections accompanied by absent tonsils or hypoplastic lymph nodes should raise suspicion for XLA, especially in the presence of reduced immunoglobulin levels across multiple classes.[13,16] Routine live vaccines administered before diagnosis were not associated with adverse outcomes in our patients, likely due to preserved T-cell function despite profound humoral immunodeficiency. Consistent with previous reports, these findings suggest that intact cellular immunity in XLA may provide partial protection against vaccine-related complications, in contrast to patients with combined immunodeficiencies in whom live vaccines may lead to severe disease.[18]

Immune dysregulation is increasingly recognized within the spectrum of IEIs and may present with inflammatory, autoimmune, or lymphoproliferative manifestations.[5] In our cohort, juvenile idiopathic arthritis, autoimmune hepatitis, pancytopenia, celiac disease, and familial Mediterranean fever represented the major immune dysregulation manifestations. These findings further support the concept that XLA extends beyond recurrent infections despite profound B-cell deficiency.[6,19]

Several biological mechanisms may contribute to immune dysregulation in XLA despite the profound deficiency of mature B cells. *BTK* is expressed not only in B lymphocytes but also in monocytes, macrophages, neutrophils, dendritic cells, and mast cells, where it participates in Toll-like receptor (TLR), Fc receptor, and inflammasome signaling pathways.[6,19,20] Impaired *BTK* signaling may therefore disrupt innate immune homeostasis, resulting in dysregulated cytokine production, defective clearance of pathogens and apoptotic cells, persistent inflammatory stimulation, and aberrant activation of autoreactive immune

pathways.[6,19-21] In addition, chronic or recurrent infections secondary to antibody deficiency may further amplify inflammatory responses and promote bystander immune activation.[5,6] These mechanisms have been proposed to contribute to autoimmune and autoinflammatory manifestations despite profound B-cell deficiency.[6,19,20] Nevertheless, the relative contribution of these pathways remains incompletely understood, and additional genetic modifiers and environmental factors are also likely to influence disease expression.[6,19]

However, whether these findings reflect atypical manifestations of XLA or independent coexisting conditions remains unclear, and further functional studies are required to clarify the underlying pathophysiological mechanisms. Although immune dysregulation is increasingly recognized as part of the clinical spectrum of XLA, not all autoimmune or autoinflammatory conditions observed in individual patients can be assumed to represent direct consequences of BTK deficiency. In particular, disorders such as familial Mediterranean fever and celiac disease may represent coincidental coexisting diseases, especially in populations with a relatively high background prevalence. Nevertheless, the occurrence of these conditions in the context of a broader immune dysregulation phenotype, together with previous reports describing autoimmune and inflammatory complications in XLA, suggests that impaired immune homeostasis may contribute to disease expression in at least a subset of patients. Because of the observational design and limited cohort size, causality cannot be established, and these findings should be interpreted as descriptive rather than evidence of a direct pathogenic relationship.

Gastrointestinal manifestations are less common in XLA compared with other antibody deficiency syndromes, likely due to the generally preserved T-cell function in these patients.[22] Dermatologic manifestations may represent early indicators of IEIs and can reflect infectious susceptibility, atopy, or immune dysregulation.[23] With regard to arthritis, septic arthritis is the most common form observed in XLA, whereas noninfectious etiologies such as JIA are considered rare.[24,25]

Previous studies investigating genotype–phenotype correlations in X-linked agammaglobulinemia have reported conflicting results. While some studies found no significant association between *BTK* variants and clinical phenotype,[8] others reported correlations with age at disease onset and severity of infections.[7] Genetic analysis plays a critical role in distinguishing XLA from other antibody deficiency disorders and in establishing an early diagnosis before the development of severe infections.[26] Previous reports have identified *BTK* mutations in approximately 90% to 95% of male patients with suspected XLA.[27] Consistent with the literature, pathogenic or likely pathogenic *BTK* variants were identified in 90% of our patients, including 3 novel variants. Patients carrying identical *BTK* variants, including affected siblings, demonstrated marked clinical heterogeneity ranging from relatively mild phenotypes to severe complications such as bronchiectasis, autoimmune hepatitis, pancytopenia, liver failure, and mortality. Conversely, some patients harboring different *BTK* variants exhibited similar clinical phenotypes. These findings suggest that *BTK* genotype alone may not fully predict disease severity in XLA. However, given the retrospective design, small cohort size, and familial clustering of some cases, these observations should be interpreted cautiously and should not be considered definitive genotype–phenotype associations. Rather, they represent hypothesis-generating findings that highlight the complexity of clinical variability in XLA. Differences in genetic background, potential modifier genes, environmental exposures, and other non-*BTK*-related factors may contribute to the observed phenotypic diversity. Larger multicenter studies incorporating functional *BTK* expression analyses and broader genomic approaches are required to better define the contribution of *BTK* variants to clinical outcomes.[28] The marked clinical variability observed among affected siblings carrying identical *BTK* variants represents one of the most distinctive findings of our cohort and further illustrates the complexity of genotype–phenotype relationships in XLA. In the present cohort, the distribution of clinical severity across *BTK* variant classes was heterogeneous. Although severe complications were observed in some patients with frameshift or in-frame variants, and relatively milder findings were observed in some patients with missense or nonsense variants, the small number of genetically confirmed patients precludes any definitive conclusion regarding variant class–specific disease severity. Therefore, these findings should be regarded as exploratory and hypothesis-generating observations rather than evidence of a true genotype–phenotype correlation. Consistent with this interpretation, previous findings by López-Granados et al demonstrated reduced clinical severity among patients

harboring less deleterious mutations, suggesting that residual *BTK* function may influence disease severity.[29] Therefore, variant type alone should not be used to predict individual disease severity in this cohort, and the observed differences should be interpreted as exploratory clinical observations requiring validation in larger and genetically independent cohorts.

The identification of 3 novel *BTK* variants in our cohort contributes to the expanding molecular spectrum of *BTK* alterations associated with XLA. These variants were classified as pathogenic or likely pathogenic according to the ACMG/AMP guidelines based on the available genetic, clinical, and immunological evidence. Therefore, the inferred pathogenicity of these novel variants is primarily supported by ACMG/AMP classification rather than direct functional validation. Specifically, *BTK* protein expression analyses, flow cytometric assessment of intracellular *BTK* expression, and *BTK* phosphorylation or kinase activity assays were not available. Thus, the biological effects of these novel variants remain to be confirmed through future functional studies. Furthermore, the absence of maternal carrier testing and familial segregation analyses limited our ability to obtain additional genetic evidence supporting variant pathogenicity, highlighting the need for future family-based studies.

In our cohort, the annual median number of infections before diagnosis was 16.9, which significantly decreased to 2.09 following initiation of IgRT and antibiotic prophylaxis (p=0.004). Although the reduction in infection frequency was statistically significant, this finding should be interpreted within the context of the small cohort size and retrospective study design. Therefore, the observed treatment effect should be considered supportive rather than definitive and warrants confirmation in larger prospective studies.

Beyond reducing infection frequency, our findings have important implications for the long-term management of patients with XLA. Immunoglobulin replacement therapy should be individualized according to each patient's clinical phenotype, infection burden, organ involvement, and treatment response rather than relying solely on standard weight-based dosing. Optimization of IgG trough levels through regular monitoring may further reduce breakthrough infections and help prevent irreversible complications such as bronchiectasis.[30] Moreover, long-term follow-up should include not only surveillance for infectious episodes but also periodic assessment of chronic lung disease, immune dysregulation, gastrointestinal complications, and treatment-related adverse events. A multidisciplinary approach involving pediatric immunologists and other relevant specialists may therefore improve long-term clinical outcomes and quality of life in patients with XLA.[17,31] Nevertheless, the development of sequelae such as bronchiectasis and septic arthritis in some patients despite appropriate treatment further underscores that early diagnosis, before irreversible tissue damage occurs, remains the most effective strategy for improving long-term prognosis.[15,32] These observations should be interpreted within the context of the observational study design and are intended to support clinical practice rather than establish causal treatment effects.

Although our study provides a comprehensive long-term clinical characterization of a pediatric XLA cohort, its findings should be interpreted within the context of a retrospective observational design. Therefore, the observed genotype–phenotype variability and the clinical relevance of the novel *BTK* variants should be considered hypothesis-generating rather than definitive. Future multicenter studies with larger cohorts, standardized longitudinal follow-up, and functional characterization of BTK protein expression and kinase activity will be essential to validate these observations and further clarify the biological and clinical implications of individual *BTK* variants. Although these 2 patients fulfilled the ESID working definition for possible XLA and had a clinical phenotype highly compatible with XLA, the absence of molecular confirmation means that alternative genetic forms of congenital agammaglobulinemia cannot be completely excluded.[10] Accordingly, these patients were retained only for the descriptive clinical characterization of the cohort and were not included in the interpretation of *BTK* variant-specific genotype–phenotype findings.

In conclusion, this retrospective single-center cohort demonstrates that X-linked agammaglobulinemia is associated with a broad spectrum of infectious and non-infectious manifestations, including immune dysregulation, substantial diagnostic delay, and marked clinical heterogeneity. The principal contribution of this study is the comprehensive long-term characterization of pediatric X-linked agammaglobulinemia by integrating clinical, immunological, therapeutic, and molecular findings, including the identification of 3 previously unreported *BTK* variants, the documentation of marked phenotypic heterogeneity within affected

families, and clinically relevant associations between delayed diagnosis, treatment, and long-term outcomes. However, because the pathogenic significance of the novel variants is inferred primarily from ACMG/AMP classification together with the compatible clinical phenotype, these findings should be interpreted cautiously in the absence of functional validation studies while emphasizing the importance of early recognition, comprehensive immunological and genetic evaluation, and individualized long-term management. Given the retrospective design, limited sample size, familial clustering, and potential referral and selection bias, these observations should be considered hypothesis-generating and require confirmation in larger multicenter cohorts incorporating functional analyses to better define genotype–phenotype relationships and the biological significance of novel *BTK* variants.

Future Research Directions

Our findings highlight several important priorities for future research. First, larger multicenter prospective registries are needed to validate the clinical associations observed in our cohort, improve the understanding of genotype–phenotype variability, and increase the generalizability of findings across diverse populations. Second, functional characterization of the novel *BTK* variants through *BTK* protein expression, kinase activity assays, and complementary cellular studies will be essential to establish their biological significance.[33] Future studies should also incorporate maternal carrier testing and familial segregation analyses to strengthen the molecular interpretation of newly identified *BTK* variants.

Emerging multi-omics approaches, including transcriptomic and proteomic profiling, may further clarify the downstream molecular pathways affected by *BTK* deficiency and help explain the marked clinical heterogeneity observed even among patients carrying identical variants.[21] Integration of genomic, transcriptomic, proteomic, and functional immunophenotyping approaches may improve our understanding of disease heterogeneity and facilitate the identification of biomarkers associated with disease severity and clinical outcomes.[21] Finally, mechanistic studies investigating the immunological basis of immune dysregulation—including autoimmunity, autoinflammation, and chronic inflammatory complications—may facilitate more individualized patient management and improve risk stratification in X-linked agammaglobulinemia.[20,33]

Strengths and Limitations

This study has several strengths, including a 20-year follow-up period, the identification of 3 novel *BTK* variants, and the quantitative demonstration of the clinical impact of diagnostic delay and treatment efficacy. In addition, the detailed characterization of immune dysregulation and atypical phenotypes enhances the clinical relevance of the study.

However, several limitations should be acknowledged. First, this was a retrospective single-center study with a relatively small sample size, which limits the generalizability of the findings, reduces statistical power, increases the possibility of type II error, and precludes definitive genotype–phenotype correlations. Therefore, non-significant statistical findings and genotype–phenotype associations should be interpreted with caution.

Second, although the novel *BTK* variants were classified as pathogenic or likely pathogenic according to ACMG/AMP criteria and were identified in patients with compatible clinical and immunological features, functional validation studies-including *BTK* protein expression, intracellular flow cytometric *BTK* analysis, and *BTK* phosphorylation or kinase activity assays-were not available. Consequently, the pathogenicity of these variants is inferred primarily from ACMG/AMP classification and should be interpreted cautiously until confirmed by future functional studies.[34,35] Furthermore, maternal carrier testing and familial segregation analyses could not be performed because parental DNA samples were unavailable. Consequently, segregation evidence supporting the pathogenicity of the novel *BTK* variants could not be obtained, limiting the strength of the molecular interpretation despite ACMG/AMP-based classification. Future family-based segregation studies will therefore be important to further validate these variants.

Third, potential missing data inherent to retrospective medical records may have influenced some clinical observations. In addition, detailed NGS quality metrics, including sequencing coverage, systematic copy number variation analyses, and detailed immunophenotyping data—including intracellular *BTK* protein expression, B-cell maturation subsets, switched memory B cells, and other advanced flow cytometric analyses—were not routinely available because of the retrospective design and the extended study period. The absence of these molecular and immunological data limited further biological characterization of the cohort and precluded

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exploration of potential associations between immunophenotypic profiles and clinical outcomes.

Fourth, 2 patients without molecular confirmation were included because they fulfilled the ESID working definitions for possible XLA based on severe hypogammaglobulinemia, markedly reduced B-cell counts, and a compatible X-linked family history. Because additional molecular analyses were not available, alternative genetic causes of congenital agammaglobulinemia, including undetected deep intronic or non-coding *BTK* variants or defects affecting other components of the pre-B-cell receptor signaling pathway, could not be completely excluded. To minimize potential bias, these patients were excluded from *BTK* variant-specific genotype–phenotype analyses and were included only in the overall clinical characterization of the cohort.

Finally, referral and selection bias should be considered when interpreting our findings. Because this study was conducted at a tertiary pediatric allergy and immunology referral center, patients with more severe, atypical, or clinically complex phenotypes may have been overrepresented, whereas individuals with milder disease may have remained undiagnosed or been managed at other healthcare facilities. Consequently, the observed frequencies of immune dysregulation, severe complications, and genotype–phenotype variability may not fully reflect the broader XLA population. In addition, the 3 novel *BTK* variants have not yet been submitted to a publicly accessible database such as ClinVar, which may limit external validation and comparison with future reported cases.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of our university (Session number 2024/12-60, dated 11.09.2024).

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CONFLICT OF INTEREST

The authors declare that they have no known financial or non-financial conflicts of interest relevant to this study or the publication of this manuscript.

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DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are not publicly available because they contain potentially identifiable patient information but are available from the corresponding author upon reasonable request and with approval from the institutional ethics committee, where applicable.

AI ASSISTANCE DISCLOSURE

No artificial intelligence (AI) tools were used in the preparation of this manuscript.

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