Case report of a novel mutation in Bruton's tyrosine kinase gene with confirmed agammaglobulinemia and absent B lymphocytes

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ABSTRACT

Background: X-linked agammaglobulinemia type 1 (XLA) is one of the most common pediatric inborn errors of immunity affecting the humoral immune system. The condition is caused by a mutation in the Bruton's tyrosine kinase gene (*BTK*), located in the long arm of the X-chromosome. BTK is crucial for B lymphocyte differentiation and activation. Therefore, a defect in BTK results in B lymphocyte maturation arrest, absence of plasma cells, and failure of immunoglobulin production. XLA affected individuals present with a history of frequent severe pyogenic infections such as pneumonia, conjunctivitis, otitis media, and bacteremia. Laboratory evaluation classically reveals undetectable immunoglobulins and the absence of B cells. The mainstay treatment is immunoglobulin replacement which can be administered intravenously (IVIG) or subcutaneously (SCIG). Aggressive antimicrobial treatment is also administered to reduce complications such as bronchiectasis or invasive bacterial infections during active infections.

Aim: To report the clinical presentation, immune features, and genetic mutation in a case of a four-year-old boy with a novel mutation in the *BTK* gene leading to XLA.

Results: The patient's chart was reviewed. We describe the phenotypical and diagnostic characteristics of an established case in a four-year-old boy who suffered from recurrent infections. Genetic analysis revealed a pathogenic novel mutation in the *BTK* gene (c.1953C>A; p.Tyr651*), while flow cytometry found 0% CD19+ (B cells), and low serum Ig levels.

Discussion: We report the clinical presentation, immune features, and genetic mutation in a patient with a novel mutation in the *BTK* gene causing XLA. Genetic analysis along with patient history, physical examination, and laboratory results are necessary to identify and diagnose XLA associated with pathogenic mutations in the *BTK* gene.

Statement of novelty: We present an established case of a novel mutation in the *BTK* gene (c.1953C>A; p.Tyr651*), based on genetic analysis, absent CD19+cells (B cells), and low Ig serum levels.

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Introduction

X-linked agammaglobulinemia type 1 (XLA) is one of the most common pediatric inborn errors of immunity affecting the humoral immune system. It is inherited in an X-linked recessive manner, thus, only males are affected and females are asymptomatic carriers. The estimated prevalence of agammaglobulinemia in Saudi Arabia is 250 cases per 100 000 males. In the United States, the prevalence is 1 case per 190 000 males (Al-Attas et al.1998; Taneja et al. 2020).

The condition is caused by a mutation in the Bruton's tyrosine kinase gene (*BTK*), located in the long arm of the X-chromosome (Xq21.3 – Xq22). Bruton's tyrosine kinase (BTK) is crucial for B lymphocyte differentiation and activation. Therefore, a defect in BTK results in B lymphocyte maturation arrest, absence of plasma cells, and failure of immunoglobulin (Ig) production (Lackey and Ahmad 2021).

XLA affected individuals are at risk of serious infections. The most common responsible pathogens are encapsulated bacteria, and patients may present with frequent severe pyogenic infections such as pneumonia, otitis media, and bacteremia. In the first year of life, enteroviruses cause serious and sometimes life-threatening infections such as meningoencephalitis, hepatitis, or dermatomyositis (Person and Chin 2019). Diagnosis can be established by quantitative Ig assessment and B cell enumeration. Genetic testing may reveal mutations in the *BTK* gene. The mainstay treatment is Ig replacement which can be administered intravenously (IVIG) or subcutaneously (SCIG). Aggressive antimicrobial treatment is needed to reduce complications such as bronchiectasis or invasive bacterial infections during active infections.

There are more than 600 different pathogenic variants reported in the *BTK* gene, and no single pathogenic variant accounts for more than 3% of individuals (Holinski-Feder et al. 1998; Conley et al. 2005; Lindvall et al. 2005; Väliaho et al. 2006). A study conducted in Turkey found 544 mutations were linked with the disorder. The variants were missense, nonsense, splice-site mutations, deletions, and insertions. Among these mutations, a missense mutation was the single most frequent genetic occurrence. Spontaneous mutations occurred in 60% of people while only 40% had a positive family history (Doğruel et al. 2019; Taneja et al. 2020; Justiz Vaillant and Ramphul 2021).

In this case report, we present an established case of a novel mutation in the *BTK* gene (c.1953C>A; p.Tyr651*). The patient had 0% CD19+ cells (B cells) and low Ig serum levels.

Functional and clinical presentation

We present a case of a four-year-old boy with a family history of a brother who died of sepsis at infancy. Initially, he presented at the age of 18 months with knee arthritis that progressed over the next year to involve all large joints including, knees, ankles, elbows, and wrists. He was diagnosed at the age of 3 years with undifferentiated juvenile idiopathic arthritis (JIA). Investigations revealed negative anti-nuclear antibody (ANA), Cyclic Citrullinated Peptide (anti-CCP), and significant elevation of Erythrocyte sedimentation rate (ESR) and C-Reactive protein (CRP). Since his arthritis failed to be controlled completely by JIA standard therapy, including weekly methotrexate and adalimumab, the possibility of *LACC1* gene-associated JIA was considered. However, direct Sanger sequencing of the *LACC1* gene was negative. Interestingly, a 2-year trial of arthritis treatment saw an increase in frequency of chest infections requiring hospital admission (four to five times per year), raising the possibility of primary immunodeficiency. He was then referred to the immunology clinic. Initial blood workup revealed a low white blood cell count of 3.31×10^9/L and a normal lymphocyte count of 2.09×10^9/L. Flow cytometry showed a total lymphocyte count of 2350 cells/μL, CD3 (T cells) count of 2126.8 cells/μL, CD4 (T-helper cells) count of 1222 cells/μL, CD8 (T-cytotoxic cells) count of 904.8 cells/μL, Natural Killer T (NK cells) count of 176.3 cells/μL, CD3+ CD45RA (naïve T cells) count of 1339.5 cells/μL, CD3+ CD45RO (memory T cells) count of 881.3 cells/μL, Human Leukocyte Antigen–DR isotype (HLA-DR) count of 611 cells/μL that represents Major Histocompatibility Complex Class II (MHC-II), and CD19+ (B cells) count of 0 cells/μL. Ig levels testing showed reduced IgG < 0.33 g/L, IgA < 0.07 g/L, IgM < 0.05 g/L. Based on these results, molecular genetic analysis was performed and a heterozygous pathogenic variant was identified in the *BTK* gene (c.1953C>A; p.Tyr651*). According to these findings and the immunological workup, he was

diagnosed with XLA in Dec 2020. Currently, the patient is on Amoxicillin prophylaxis and IVIG replacement every 28 days. Adalimumab and MTX were discontinued. Since then, he has been clinically well with no episodes of infections.

Discussion

XLA is the culprit defect in 85% of patients with agammaglobulinemia (El-Sayed et al. 2019). This disorder results from mutations in a crucial B cell development protein, BTK, encoded by the *BTK* gene. BTK is a cytoplasmic tyrosine kinase expressed mainly in hematopoietic cells. As a member of the Tec kinase family, BTK is activated by an Src kinase. Activated BTK then activates PLCγ2 via phosphorylation resulting in calcium influx and downstream signaling. A defect in BTK leads to absent or very low numbers of peripheral B cells and agammaglobulinemia, a crucial arm of the adaptive immune system in fighting infections. Indeed, patients with XLA suffer from serious recurrent infections. A large cohort of 226 patients with XLA showed that more than 70% had upper and lowered respiratory tract infections, one-third suffered from skin infections, and 12% had CNS infections (Groth et al. 2020).

Other clinical manifestations of XLA include arthritis, neutropenia, thrombocytopenia, and inflammatory bowel disease. Hernandez-Trujillo et al. (2014) reported 128 patients with XLA collected from the United States Immune Deficiency Network (USIDNET) registry; 69% reported having at least one inflammatory symptom, 20% had joint pain, and 11% noted joint swelling. Although several subjects reported symptoms compatible with joint disease, only 7% had been formally diagnosed with arthritis. Specifically, 2% reported being diagnosed with rheumatoid arthritis, and 5% having "other" arthritis (Hernandez-Trujillo et al. 2014. El-Sayed et al. (2019) described 783 patients from 40 centers around the world with XLA; 62 patients (7.9%) were reported to have arthritis (El-Sayed et al. 2019).

Our patient shared the previously reported clinical manifestations of XLA, such as recurrent respiratory infections and arthritis and the classical immunological findings of absent B cells and agammaglobulinemia. Our case revealed a novel pathogenic mutation in the *BTK* gene (c.1953C>A; p.Tyr651*). This nonsense mutation introduces a stop codon to the gene sequence by replacing the nucleotide cytosine with adenosine. Therefore, the amino acid tyrosine changes to a stop codon at the 651 position, resulting in premature chain cessation. Over 900 variants have already been described in this gene; however, new pathogenic variants remain to be identified (Kraft et al. 2021). In this article, we report a new novel variant of the *BTK* gene.

Conflict of interest

The authors declare that they have no conflict of interest.

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