# Atypical Manifestation of X-linked Agammaglobulinemia – the Importance of Genetic Testing

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ABSTRACT

X-linked agammaglobulinemia (XLA) was one of the first inborn errors of immunity to be described. It is caused by pathogenic variants in the gene for Bruton tyrosine kinase (BTK), which has important functions in B cell development and maturation. Recurrent bacterial infections in the first two years of life and hypogammaglobulinemia with absent B cells in male patients are the most common symptoms.
A four-month-old male patient underwent surgical removal of *urachus persistens* complicated with recurrent scar abscesses. Hypogammaglobulinemia (IgG, IgA, and IgM), low phagocytic activity, mild neutropenia, and a normal percentage of B cells were observed in the patient's immune laboratory profile. Over time, he suffered recurrent respiratory infections (otitis media and rhinosinusitis) and developed B cell depletion, but interestingly, this was with a normalisation of IgG and IgA levels along with undetectable IgM. Molecular-genetic testing confirmed the presence of the pathogenic variant c.1843C>T in the BTK gene, which is associated with a milder phenotype of XLA.
Molecular-genetic testing uncovers the variability of clinical and laboratory features of apparently well-known inherited disorders. Patients with mild "leaky" XLA may have normal levels of non-functional or oligoclonal immunoglobulins.

KEYWORDS

atypical leaky phenotype; Inborn error of immunity; X-linked agammaglobulinemia; molecular-genetic testing; BTK gene

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Received: 9 June 2024
Accepted: 7 August 2024
Published online: 22 October 2024

Acta Medica (Hradec Králové) 2024; 67(2): 60–63
https://doi.org/10.14712/18059694.2024.21

## INTRODUCTION

X-linked agammaglobulinemia (XLA) is a long-known inborn error of immunity (IEI) that was first described by paediatrician Ogden Bruton in 1952 (1). Affected male patients typically present with recurrent bacterial infections; hypogammaglobulinemia; rudimentary adenoidal, tonsillar tissue; peripheral lymphoid hypoplasia; and an absence of mature B cells in peripheral blood. Typical identified pathogens are the encapsulated bacteria *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*, but patients also suffer from giardiasis (lambliasis) and meningoencephalitis caused by echoviruses (2). In addition, autoimmune and inflammatory complications have been reported, but only rarely (3). Clinical diagnostic criteria established by the European Society for Immunodeficiencies (ESID) are shown in Table 1 (4). XLA is caused by pathogenic loss of function variants in the gene for BTK, which has an essential function in B cell development and maturation in the bone marrow. The most profound defect is in development from the pre-B cell stage to the immature B cell stage; maturation of pro-B cells and immature B cells is also defective in this condition (5). BTK expression is primarily restricted to hematopoietic cells – B cells, monocytes, myeloid cells, erythrocyte precursors, and megakaryocytes. In XLA patients, only a decrease in B cells has been observed, suggesting that BTK may be redundant for the maturation of other cell types (5). Immunoglobulin replacement and antibiotic prophylaxis is used in XLA therapy. In selected, very severe cases, hematopoietic stem cell transplantation is another treatment option (6). In rare cases, females can also develop symptoms due to skewed (non-random) inactivation of the non-mutated X-chromosome in hematopoietic cells (7, 8).

More pathogenic variants associated with a milder course of disease have been reported in the literature (9–11); they lead to decreased BTK expression but not to a total absence of BTK expression or the production of a partially functional mutated protein. This condition is referred to as leaky or mild XLA – the patients become symptomatic with typical associated-infection complications but at an older age. Patients with leaky XLA can have higher proportion of B cells as in typical XLA (more than 2%) and only slightly decreased to normal immunoglobulin values. Patients with mild XLA can be misdiagnosed with common variable immunodeficiency (11). There is no definite correlation between the type of variant (missense, nonsense, etc.) in the BTK gene and the severity of the disease (12).

**Tab. 1** Clinical diagnostic criteria for X-linked agammaglobulinemia.

| |
|---|
| **Definitive diagnosis** |
| **Male patient with < 2% CD19⁺ B cells and at least one of the following:** |
| 1. Mutation in BTK gene<br>2. Absent BTK mRNA on northern blot analysis of neutrophils or monocytes<br>3. Absent BTK protein in monocytes or platelets<br>4. Maternal cousins, uncles or nephews with less than 2% CD19⁺ B cells |
| **Probable diagnosis** |
| **Male patient with < 2% CD19⁺ B cells in whom:** |
| Other causes of hypogammaglobulinemia have been excluded and all of the following:<br>1. Onset of recurrent bacterial infections in the first 5 years of life<br>2. Serum IgG, IgM and IgA more than 2SD below normal for age<br>3. Absent isohemagglutinins and /or poor response to vaccines |
| **Possible diagnosis** |
| **Male patient with < 2% CD19⁺ B cells in whom:** |
| Other causes of hypogammaglobulinemia have been excluded and at least one of the following:<br>1. Onset of recurrent bacterial infections in the first 5 years of life<br>2. Serum IgG, IgM and IgA more than 2SD below normal for age<br>3. Absent isohemagglutinins and /or poor response to vaccines |

Legend: SD – standard deviation.

## CASE REPORT

At the age of four months, a male patient born after an uneventful first pregnancy and with normal postnatal adaptation underwent a surgical removal of *urachus persistens* that was complicated by recurrent scar abscesses (three times), a C-reactive protein level of 208 mg/L (reference range: 0–5 mg/L), and poor healing. Cultures of from the abscess fluid were repeatedly positive for *Staphylococcus aureus*. Hypogammaglobulinemia, low phagocytic activity (50%, reference range: 80–100%), low phagocytic index (4, reference range: >30), and mild neutropenia were observed in the patient's laboratory parameters. Flow cytometry showed no significant deviations; the patient's percentage of B cells was normal but with a significant decrease in the proportion of switched memory B cells. Selected laboratory parameters are shown in Table 2.

Over time, the patient suffered recurrent respiratory infections, recurrent otitis media with positive cultivation of *Streptococcus pneumoniae*, and mouth ulcers. He underwent *Salmonella* gastroenteritis and SARS-CoV2 infection with mild respiratory symptoms, and he was diagnosed with allergic rhinitis caused by grass allergy. After the solving of abscess complications, the patient's phagocytic activity, phagocytic index, and neutropenia normalised. He developed B cell depletion in blood, but, interestingly, this was accompanied by the normalisation of IgG and IgA levels and a persisting depletion of IgM. Flow cytometry showed an elevation of transitional B cells, $CD21^{low}$ B cells, and a normal proportion of marginal zone cells such as B cells, switched memory B cells, naïve B cells, and double-negative T cells. Mild $IgG_1$ subclass deficiency (3,27 g/L, reference range: 3.80–9.30 g/L) was identified at 8 years of age, but other IgG subclasses had normal values. Diagnostic vaccination was not performed because of parents' refusal. The patient had a negative family history for IEI-associated complications or monogenic disorders. Molecular-genetic testing was performed (massive parallel sequencing focusing on a panel for primary immunodeficiencies) because of recurrent infections and significant B cell depletion, which confirmed the presence of the

**Tab. 2** Changes of laboratory parameters over time.

| Age | 4 months | 7 months | 16 months | 4 years | 6 years | 8 years | 10 years |
|---|---|---|---|---|---|---|---|
| IgG [g/l] | **1.59** | **1.12** | **3.27** | 6.11 | 6.57 | 6.17 | 6.58 |
| IgA [g/l] | **0.05** | **0.05** | **0.07** | 0.84 | 0.86 | 1.17 | 1.43 |
| IgM [g/l] | **0.05** | **0.24** | **0.14** | **0.29** | **0.06** | **0.05** | **0.12** |
| IgE [IU/l] | 25.00 | 46.00 | 27.70 | 51.70 | 56.90 | 24.00 | 48.90 |
| leukocytes [x $10^9$/l] | 6.60 | 11.30 | 7.50 | 7.90 | 6.70 | 5.50 | 4.60 |
| neutrophils [x $10^9$/l] | **1.20** | 3.90 | 2.20 | 4.20 | 1.30 | 2.80 | 2.90 |
| lymphocytes [x $10^9$/l] | 4.70 | 5.80 | 4.20 | 2.90 | 2.26 | 2.12 | 2.70 |
| $CD3^+$ T cells [%] | **84.00** | 80.00 | **82.00** | **90.00** | **81.00** | **90.00** | 79.00 |
| $CD3^+$ T cells [x $10^9$/l] | **4.03** | **4.66** | **3.38** | **2.64** | 1.82 | 1.91 | 1.64 |
| $CD19^+$ B cells [%] | 10.00 | 12.00 | **3.00** | **1.00** | **3.00** | **1.00** | **2.00** |
| $CD19^+$ B cells [x $10^9$/l] | 0.25 | 0.72 | **0.11** | **0.022** | **0.064** | **0.021** | **0.041** |
| transitional B cells [%] | | | | | **27.80** | **32.00** | **32.50** |
| switched memory B cells [%] | | | | | **2.10** | 11.00 | 13.60 |
| $CD21^{low}$ B cells [%] | | | | | **9.10** | **22.70** | 5.00 |

Legend: Values below reference range specific for age are marked with bold writing in blue color, values above reference range specific for age are marked with bold writing in red color. Mild neutropenia: 1.0–1.5 × $10^9$/l of neutrophils in blood. All laboratory parameters were collected during the stable course of disease without ongoing infection complications. We did not perform B cell phenotypic profiling until 6 years of age because of technical limitations of flow cytometry analysis in our laboratory.

pathogenic c.1843C>T, p.Arg615Cys missense variant in the BTK gene (NM_000061.3).

The identified missense variant is located in the kinase domain of the gene that is responsible for the catalytic activity of BTK; it is considered pathogenic according to ACMG criteria (PS3, PM1, PM2, PM5, PP3). The variant was evaluated twice as a variant of uncertain significance in the ClinVar database. In silico prediction tools classify this variant either pathogenic, pathogenic supporting, or uncertain. The CADD score for this variant is high with a value of 32, its population frequency is very low (0.000000913 in gnomAD), and its conservation score is high (10.003 in PhyloP100way). The variant has previously been described by Kraft et al. in a 34-year-old male patient with recurrent infections, hypogammaglobulinemia, a decreased percentage of B cells (2.9%), and decreased BTK protein expression as determined by flow cytometry in $CD20^+$ B cells and $CD14^+$ monocytes (13). Alternative variants at the same amino acid position (p.Arg615Ser, p.Arg615Pro) were described in XLA patients, both in a male patient with a mild course of disease and IgG hypogammaglobulinemia (1,8 g/L), and in another male patient with decreased *BTK* expression (12, 14).

## DISCUSSION

A male patient with recurrent respiratory infections, otitis media, scar abscesses, and the significant depletion of B cells was genetically diagnosed with XLA. Interestingly, the patient did not have this B cell depletion at the age of 4 months, and IgG and IgA levels normalised over time. A missense variant c.1843C>T in the BTK gene met the genotype–phenotype correlation in our patient, and we consider it causal for mild "leaky" XLA.

As we did not perform B cell phenotypic profiling at the beginning of follow up, we cannot say which B cell developmental stage was responsible for the normal B cell proportion, but we later saw the elevation of transitional B cells that represent a developmental stage between immature B cells in the bone marrow and mature peripheral B cells. The BTK gene is also important in the development of immature B cells, which could be the explanation for this observation. The identified pathogenic variant was not associated with a total absence of BTK expression, but only with its decrease, so we expect that a small proportion of pre-B cells has been able develop to stages capable of immunoglobulin production. The observed proportion of switched memory B cells is probably responsible for the increased production of endogenous immunoglobulins between 2 and 4 years of age. A small number of leaked mature B cells had possibly been distributed in local lymphoid tissues, such as bone marrow or lymph node; the produced immunoglobulins were non-functional or oligoclonal and therefore less effective than immunoglobulins produced by B cells of the healthy individual. There is an indication for immunoglobulin replacement therapy in case of recurrent infections.

## CONCLUSIONS

Patients with leaky XLA may have normal levels of non-functional or oligoclonal immunoglobulins, and there is the indication for immunoglobulin replacement therapy in case of recurrent infections. Molecular-genetic testing uncovers the variability of clinical and laboratory features of apparently well-known inherited disorders. We should therefore consider molecular-genetic testing in IEI patients with atypical presentation or mild symptoms.

## ACKNOWLEDGMENTS

This publication has been produced with the support of the Integrated Infrastructure Operational Program for the following project: Systemic Public Research Infrastructure – Biobank for Cancer and Rare Diseases, ITMS: 313011AFG5, which was co-financed by the European Regional Development Fund.

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