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Short communication

# Genetic and demographic features of X-linked agammaglobulinemia in Eastern and Central Europe: A cohort study

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ABSTRACT

Primary immunodeficiency disorders are a recognized public health problem worldwide. The prototype of these conditions is X-linked agammaglobulinemia (XLA) or Bruton's disease. XLA is caused by mutations in Bruton's tyrosine kinase gene (*BTK*), preventing B cell development and resulting in the almost total absence of serum immunoglobulins. The genetic profile and prevalence of XLA have not previously been studied in Eastern and Central European (ECE) countries. We studied the genetic and demographic features of XLA in Belarus, Croatia Hungary, Poland, Republic of Macedonia, Romania, Russia, Serbia, Slovenia, and Ukraine. We collected clinical, immunological, and genetic information for 122 patients from 109 families. The *BTK* gene was sequenced from the genomic DNA of patients with a high susceptibility to infection, almost no $CD19^+$ peripheral blood B cells, and low or undetectable levels of serum immunoglobulins M, G, and A, compatible with a clinical and immunological diagnosis of XLA. *BTK* sequence analysis revealed 98 different mutations, 46 of which are reported for the first time here. The mutations included

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single nucleotide changes in the coding exons (35 missense and 17 nonsense), 23 splicing defects, 13 small deletions, 7 large deletions, and 3 insertions. The mutations were scattered throughout the *BTK* gene and most frequently concerned the SH1 domain; no missense mutation was detected in the SH3 domain. The prevalence of XLA in ECE countries (total population 145,530,870) was found to be 1 per 1,399,000 individuals. This report provides the first comprehensive overview of the molecular genetic and demographic features of XLA in Eastern and Central Europe.



## 1. Introduction

X-linked agammaglobulinemia (XLA; MIM#300300) is a primary immunodeficiency disorder characterized by an early defect in B-lymphocyte differentiation. It is caused by defects in Bruton's tyrosine kinase gene (*BTK*), which encodes a cytoplasmic tyrosine kinase expressed throughout myeloid and B cell differentiation (Tsukada et al., 1993; Vetrie et al., 1993). Affected individuals have almost no peripheral blood B cells and only very small amounts of immunoglobulins of all isotypes. XLA patients are therefore highly susceptible to infections with various types of pathogens, including encapsulated pyogenic bacteria, enteroviruses, and *Giardia lamblia*, against which host defenses are largely based on antibodies (Ochs and Smith, 1996; Plebani et al., 2002). Clinical manifestations of XLA include recurrent infections of the upper and lower respiratory tract and the skin, meningoencephalitis, gastroenteritis, and conjunctivitis (Winkelstein et al., 2006). Infections usually start at four to six months of age, coinciding with the catabolism of IgG of maternal origin. XLA patients may also develop purulent and non-purulent arthritis, hepatitis, osteomyelitis, and protracted enterovirus infection (Winkelstein et al., 2006). Intravenous or subcutaneous immunoglobulin replacement therapy can attenuate, but not completely prevent infectious complications.

The genetic and epidemiological features of primary immunodeficiency disorders (PIDs) have remained largely unexplored in Eastern and Central European (ECE) countries. We provide here the first report of the demographics and mutational spectrum of *BTK* in 122 XLA patients from 10 ECE countries. We report 46 previously unknown mutations in *BTK*. This study was carried out in the framework of the *J Project*, an ECE initiative for increasing awareness and improving the diagnosis of PIDS, including genetic testing for these conditions.

## 2. Methods

### 2.1. Study population

We analyzed 122 XLA patients from 109 unrelated families from 10 ECE countries. XLA diagnosis in all these patients was based on family history, typical clinical and immunological findings, including recurrent otitis media, sinusitis, bronchitis and pneumonia, an almost total lack of peripheral blood B cells (<2%), and very low levels of serum immunoglobulin isotypes. XLA diagnosis was confirmed genetically by screening for mutations of the *BTK* gene. Blood samples were collected into EDTA, from patients giving informed consent. Fifty-two of the 122 patients studied underwent genetic diagnosis at the Jeffrey Modell Diagnostic Laboratory at the University of Debrecen (Hungary), 29 were underwent genetic diagnosis at the Erasmus University in Rotterdam (The Netherlands), 16 at the Research Center for Medical Genetics in Moscow (Russia), 7 at the Department of Pediatrics in Brescia (Italy), 6 at the Belarusian Research Center for Pediatric Oncology and Hematology in Minsk (Belarus), 6 at St. Jude Children's Research Hospital in Memphis (USA), and 4 at Karolinska University Hospital at Huddinge (Sweden). Samples were transported overnight or on the same day, at room temperature, to one of the seven molecular genetics centers. Two additional patients were analyzed genetically and have been described elsewhere (Jyonouchi et al., 2008). In total, 12 of the patients had been studied before (Jyonouchi et al., 2008; Rohrer et al., 1999; Richter et al., 2001; Noordzij et al., 2002; Fiorini et al., 2004; van Zelm et al., 2008). All investigations were carried out after informed consent had been obtained from the patients or their parents, and were approved by the appropriate institutional review board.

### 2.2. Immunological assays

B cell counts and serum immunoglobulin determinations were carried out at the various participating ECE immunology centers. Peripheral blood mononuclear cells were isolated from heparin-treated venous blood by density gradient centrifugation. The percentage of $CD19^+$ B-lymphocytes was determined by flow cytometry after incubating the cells with labeled anti-CD19 antibodies or an isotype control. Concentrations of serum IgM, IgG, and IgA isotypes were determined by standard immunochemical assays.

### 2.3. Mutational analysis of BTK

Genomic DNA was extracted from blood leukocytes according to standard protocols. Mutations were analyzed by amplifying exons 1–19 and the flanking intronic regions of *BTK* by PCR. The primer sequences used at the Debrecen Center were kindly provided by Dr. T. Freiberger. Amplicons were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), and analyzed with an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, CA). Sequence variations were described with respect to a reference sequence, GenBank accession no. NM_000061 for *BTK* cDNA, in which the c.1 position corresponds to the A of the ATG translation initiation codon. Mutations were designated as recommended by den Dunnen and Antonarakis (2001).

## 3. Results

### 3.1. Types and location of BTK mutations

In our cohort of 122 XLA patients from 15 referral centers, we identified 98 different *BTK* mutations, 46 of which were previously unknown (Table 1 and Fig. 1). Diverse mutations, including missense or nonsense base substitutions, splicing mutations, large and small deletions and insertions, were detected (Fig. 1). Missense mutations were the most frequently identified (35; 36%), followed by splice-site mutations (23; 23.2%), nonsense mutations (17; 17.2%), frameshift due to insertions or deletions (16; 16.2%), and large deletions (7; 7.2%). The mutations were scattered throughout the *BTK* gene, but most frequently affected the SH1 domain of the protein (45; 45.3%), followed by the PH domain (22, 22.3%), the SH2 domain (13; 13.3%), the SH3 domain (7; 7.3%) and the TH domain (6; 6.3%) (Fig. 1). We also identified four large deletions affecting at least two domains of BTK and one large deletion affecting exon 1 (Table 1 and Fig. 1). None of the mutations affected the tyrosine residues in positions 223 and 551, which are phosphorylated during the regulation of the protein. No missense mutation was detected in the SH3 domain.