# BRUTON'S TYROSINE KINASE (BTK): A PROTEIN IMPLICATED IN HUMAN X-LINKED AGAMMAGLOBULINEMIA (XLA).

Michael P. Okoh.[1,2], Ilkka Lappalainen[1,2], Juha Ollila[2], Kaj Stenberg[2], and Mauno Vihinen[1,3].

[1] Institute of Medical Technology, FIN-33014, University of Tampere, Finland;
[2] Department of Biosciences, Division of Biochemistry, P. O. Box 56, FIN-00014 University of Helsinki,Finland;
[3] Tampere University Hospital, FIN-33520 Tampere, Finland.

## ABSTRACT

Bruton's tyrosine kinase (Btk) is a cytoplasmic protein tyrosine kinase belonging to the Tec family of protein tyrosine kinases (PTK). It consists of 659 amino acids with five different domains. The Tec family contain the Src homology 2 (SH2) and SH3 and the kinase domain. In addition, Tec family cytoplasmic tyrosine kinases with the exception for Txk also contain a pleckstrin homology (PH) domain in their N-terminal, followed by a Tec homology (TH) domain with its characteristic Btk motif and proline rich sequences. Deficient function of Btk is responsible for the human X-linked agammaglobulinemia (XLA) and murine X-linked B cell immunodeficiency (XID). Over the years, our group has focused on the studies of Btk and its involvement in signalling and in XLA. The aim of our work is to study the function of the Tec family members and effects of disease-causing mutations in structural details. Here, we describe the role of Btk in XLA and its relations with other Tec family members in cell signalling.

## INTRODUCTION

X-linked agammaglobulinemia (XLA) was first described in 1952 by O.C Bruton. It was the first primary immunodeficiency diseases (PID) in which an underlying defect causing the absence of gammaglobulins was clearly identified (1). The disease is manifested as a B cell differentiation defect. Mutations in the gene coding for Btk block B cell maturation, thus, it leads to a decrease in the numbers of B-lymphocytes and an almost (complete) lack of plasma cells. The immunoglobulin levels in affected individuals are very low. The disease afflicts about 1/200,000 males (2).

The *Btk* gene has been mapped to the Xq21.3-22 region in the mid-portion of the long arm of the X-chromosome (3). Btk together with Tec, Itk, Txk (4) and Bmx forms a distinct family. This family is called the Tec family. The members of the Tec family share the same organization consisting of PH, TH, SH3, SH2, and kinase domains. Txk, however, contains at the N-terminal a unique cysteine string. They have in their N-terminus a pleckstrin homology (PH) domain, which has membrane-localizing function. The TH region is unique to the Tec family. The SH2 and SH3 domains have binding functions, whereas the kinase domain is catalytic and phosphorylates tyrosine residues of the substrate proteins. A mutation in all of the domains of Btk causes XLA (5). The majority of all mutations lead to truncation of the enzyme. The XLA mutations data has been collected to a database called BTKbase available at

http://www.uta.fi/imt/bioinfo/btkbase (5). In addition to mutations, it also contains clinical information regarding the disease.

B cell differentiation via pro-B, pre-B, and B-lymphocytes to plasma cells from the hematopoietic stem cell occurs in several stages (6). Signals from each stage of B cell development are thought to be crucial for B cell survival. Upon maturation, the stimulation of the B cell receptors (BCR) by the surface immunoglobulin leads to activation of several events, including a multitude of cellular responses such as proliferation, activation and differentiation (6). Currently, several cytoplasmic protein tyrosine kinases including Btk are known to regulate this process.

## BTK SIGNALLING AND REGULATION

The Tec family proteins are involved in a vast array of signal transduction pathways. The signalling impairment in Xid mice due to mutations in Btk suggests a pivotal role for Btk in lympho-hematopoietic growth and differentiation (7). The domains of Btk play a critical role in Btk associated signalling, as demonstrated by mutations found in XLA patients (5,8-9). Several mutations in Btk PH domain are known, including missense and nonsense mutations, insertions and deletions leading to classical XLA (10). Thus far, Btk is the only protein were mutations in the PH domain cause a disease. The importance of Btk PH domain in Btk signalling had earlier been brought into focus using molecular modelling. With the model, most of XLA causing mutations were shown to occur in residues that form functionally important charged patches on the surface of the molecule (10,11). Furthermore, alterations of these charge patches due to mutations, either reduce or reverse the electrostatics affecting Btk binding to targets (10,11). Tec family PH domains and Btk motifs present in the TH domain share similar three-dimensional structure (10). Overall, PH domains of these kinases are highly polar (10). Thus, the electrostatic polarization of Tec family PH domains, suggest domain that have related, but not identical properties and functions (10).

The TH domain consists of two motifs, the Btk motif and the proline rich region (12). To interpret the effects of mutations molecular modelling has been combined with experimental methods such as binding and activity assays and spectroscopic methods. The Btk motif contains highly conserved histidine and cysteine residues involved in $Zn^{2+}$ binding (12,13). The $Zn^{2+}$ binding region of the Btk motifs are in identical positions in Tec family (10). The proline rich motifs on the other hand binds to the adjacent SH3 domain in an intramolecular fashion with possible regulatory ability (14). Missense mutations in the TH domain leading to classical XLA alter the conserved cysteines (12). By using circular dichroism and phosphotyrosine-binding assays, mutations that affect Btk SH2 domain leading to XLA could be classified to three categories, i.e. functional mutations, structural mutations, and structural-functional mutations, all with negative consequences on Btk SH2 interaction (15). Further, analysing missense mutation causing classical XLA, it is obvious that intact SH2 domain is important for both the biological function and regulation of Btk, and other Tec family members (15).

The crucial role of Btk, in B cell differentiation, has been studied by searching molecules regulating the activity of Btk and its linkage to various signal transduction pathways. To date, the main pathways Btk have been shown to participate in are the B-cell antigen receptor (BCR), the high affinity IgE receptor ($Fc_{\varepsilon}RI$) in mast cells (16), IL-3 (17), IL-5 (18) and IL-6 receptors (19), G-protein coupled receptors via association with $G\alpha 12$, $Gq\alpha$ or $\beta\gamma$ subunits (20) and the CD32, collagen or thrombin receptors in platelets (21).

Recently, the Btk PH domain has been reported to bind F-actin (22) and cytoskeletal regulation, mediated by small GTPases, has been demonstrated for Btk (23). The PH-TH and kinase domains of Btk have also been shown to be responsible for the regulation of nuclear localization and transcriptional activity of TFII-I (BAP-135), a multifunctional transcription factor, suggesting a novel pathway for Btk (24). The activation of another nuclear factor, NF-κB, is found to be a downstream target of Btk in response to BCR engagement (24). NF-κB has also been implicated in the up-regulation of Bcl-x (25) and this, together with the observation that Btk acts as an anti-apoptotic protein upstream Bcl-x (25), might contribute to the B-cell deficiences in XLA and xid. In fact, Btk has been identified to act as a dual-function regulator of apoptosis promoting radiation-induced apoptosis, but inhibiting Fas-activated apoptosis in chicken DT-40 lymphoma B-cells, (25).

## CONCLUSIONS.

Work carried out in our group, and other independent research groups, have yielded much information on mutations causing XLA. BTKbase is available at World Wide Web at http://www.uta.fi/imt/bioinfo/btkbase (5). This site currently contains data for over 700 patients, with clinical information about there mutation.

The diversity of the group of molecules interacting with Btk and other family members suggest functions that are significant and strictly controlled. Several signal mediators are now known as effectors of Tec family signalling. The understanding of the molecular interactions of Tec family is essential for addressing issues, relating to the bases of signal transduction and diseases arising from mutations in the involved genes and proteins.

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