

A novel Bruton's tyrosine kinase gene (*BTK*) invariant splice site mutation in a Malaysian family with X-linked agammaglobulinemia

Chai Teng Chear,^{1,2} Harvindar Kaur Gill,² Nazatul Haslina Ramly,³ Jasbir Singh Dhaliwal,² Noraini Bujang,⁴ Adiratna Mat Ripen² and Saharuddin Bin Mohamad¹

Summary

X-linked agammaglobulinemia (XLA) is a rare genetic disorder caused by mutations in the Bruton's tyrosine kinase (*BTK*) gene. These mutations cause defects in early B cell development. A patient with no circulating B cells and low serum immunoglobulin isotypes was studied as were his mother and sister. Monocyte *BTK* protein expression was evaluated by flow cytometry. The mutation was determined using PCR and followed by sequencing. Flow cytometry showed the patient lacked *BTK* protein expression in his monocytes while the mother and sister had 62% and 40% of the monocytes showing *BTK* protein expressions respectively. The patient had a novel base substitution in the first nucleotide of intron 9 in the *BTK* gene, and the mutation was IVS9+1G>C. This mutation resulted in exon 9 skipping. This defect rendered the patient susceptible to asthma, failure to thrive, recurrent pyogenic infections, otitis media and bronchopneumonia. His mother and sister

were heterozygous for this mutation. The combination of flow cytometry and genetic study is necessary in the diagnosis of X-linked agammaglobulinemia and may be used for subsequent genetic counseling, carrier detection and prenatal diagnosis. (*Asian Pac J Allergy Immunol* 2013;31:320-4)

Key words: *BTK* gene, splice site mutation, X-linked agammaglobulinemia

Introduction

X-linked agammaglobulinemia (XLA) is a rare disease with an estimated prevalence of 1 in 200,000 live births.¹ It is caused by mutations in the Bruton's tyrosine kinase (*BTK*) gene² which block the differentiation of pre-B cells into circulating, mature B cells and plasma cells. Affected males have normal number of pre-B cells in the bone marrow.³ However, they have low or absent circulating B cells and markedly reduced immunoglobulin isotypes in the serum,⁴ which renders them susceptible to recurrent pyogenic bacterial infections.³ Hence, immunoglobulin replacement therapy via intravenous or subcutaneous route must be given to patients with XLA to protect them from severe recurrent infections.⁵

The definitive diagnostic criteria for XLA are male patient with less than 2% circulating CD19+ B cells along with either a mutation in *BTK* gene; absent *BTK* mRNA in neutrophils or monocytes; absent *BTK* protein in monocytes or platelets; or a positive family history.⁶ Probable diagnostic criteria of XLA are early onset of recurrent bacterial infections, hypogammaglobulinemia, absent isohemagglutinins and/or poor response to vaccines.⁶

The *BTK* gene is located at Xq21.3-Xq22 and codes for a 659 amino-acid protein.² This gene comprises 19 exons which span over 37.5kb of genomic DNA.⁷ The *BTK* protein is cytoplasmic tyrosine kinase which is involved in signal transduction for B cell proliferation and development.⁷

From 1. Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

2. Allergy and Immunology Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia.

3. Pediatric Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia.

4. Centre of Research for Computational Sciences and Informatics for Biology, Bioindustry, Environment, Agriculture and Healthcare (CRYSTAL), Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Corresponding author: Saharuddin Bin Mohamad

E-mail: saharuddin@um.edu.my

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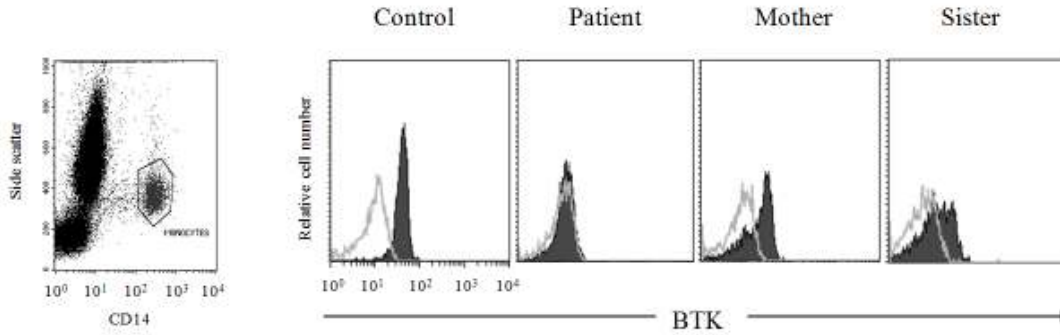


Figure 1. Flow cytometric analysis of monocyte BTK expression in a normal control (Control), the patient (Patient), his mother (Mother) and his sister (Sister). Two thousand events of monocytes were gated from side scatter versus CD14 dot plot. BTK expression was further analyzed in the gated monocytes, as shown in the histograms. The line indicates the isotype control antibody expression and shaded area indicates BTK expression.

Up to 2007, 620 unique mutations from 974 unrelated families have been recorded in a BTK database.¹ These mutations are varied in type and scattered throughout all domains of the BTK protein. They can be categorized as missense mutations, 32%, nonsense mutations, 12%, deletions, 27%, insertions, 8%, splice site mutations, 19%, multiple mutations, 1% and upstream mutation, 1%.¹ However, a genotype-phenotype correlation has not been shown to date for these mutations. Furthermore, there are very few reports describing the clinical features and laboratory findings of Malaysian XLA patients. In this report, we describe a Malaysian XLA patient with a novel *BTK* gene splice site mutation, and the carriers from his family.

Case report

A seven year-old Malay boy with no circulating B cells (CD19+ cells) and low serum immunoglobulin isotypes was studied. He had a history of recurrent pyogenic infections since he was one year old. He had recurrent otitis media and was admitted almost every year for recurrent bronchopneumonia that usually had slow response to antibiotics treatment. At seven years of age, he was admitted to the hospital with another episode of bronchopneumonia, the serial chest X-ray showed recurrent middle lobe consolidation. He also had episodes of wheezing, suggestive of asthma, therefore was started on metered dose inhaler prophylaxis. Physical examination showed a failure to thrive without clubbing but pectus carinatum was observed. Respiratory examination revealed crepitations with minimal rhonchi.

No hepatosplenomegaly or enlarged lymph nodes were observed.

Laboratory finding revealed that he had no circulating B cells and his serum immunoglobulin levels (compared to that of an age matched child) were as follows: IgG = 41mg/dL (550-1200mg/dL), IgA = 48mg/dL (60-170mg/dL), IgM = <12mg/dL (40-95mg/dL). Furthermore, high resolution computed tomography of the thorax showed features of early bronchiectasis. Hence, a clinical diagnosis of XLA with bronchiectasis was made. He was then started on three-weekly IVIg therapy. Since then, he remained well and suffered no further recurrent episodes of pneumonia.

The patient’s mother and sister appeared healthy. However, two maternal uncles died at below two years of age, due to high fever.

Flow cytometric analysis of monocyte BTK expression in Figure 1 revealed 94% of patient’s monocytes did not express BTK protein with a mean fluorescence intensity (MFI) of 11. In contrast, 98% of the normal control’s monocytes expressed the BTK protein, MFI=39. Sixty-two percent of the mother’s monocytes expressed the BTK protein, MFI= 29, whereas 38% monocytes did not express BTK protein, MFI=10. The sister had 40% of her monocytes expressed BTK protein, MFI = 22, and 60% monocytes did not express BTK protein, MFI = 7.

The *BTK* gene was amplified from the study subjects’ cDNA and Figure 2A depicts the RT-PCR products generated with an exon 6 forward primer, Btk 6F, and an exon 10 reverse primer, Btk 10R.⁸

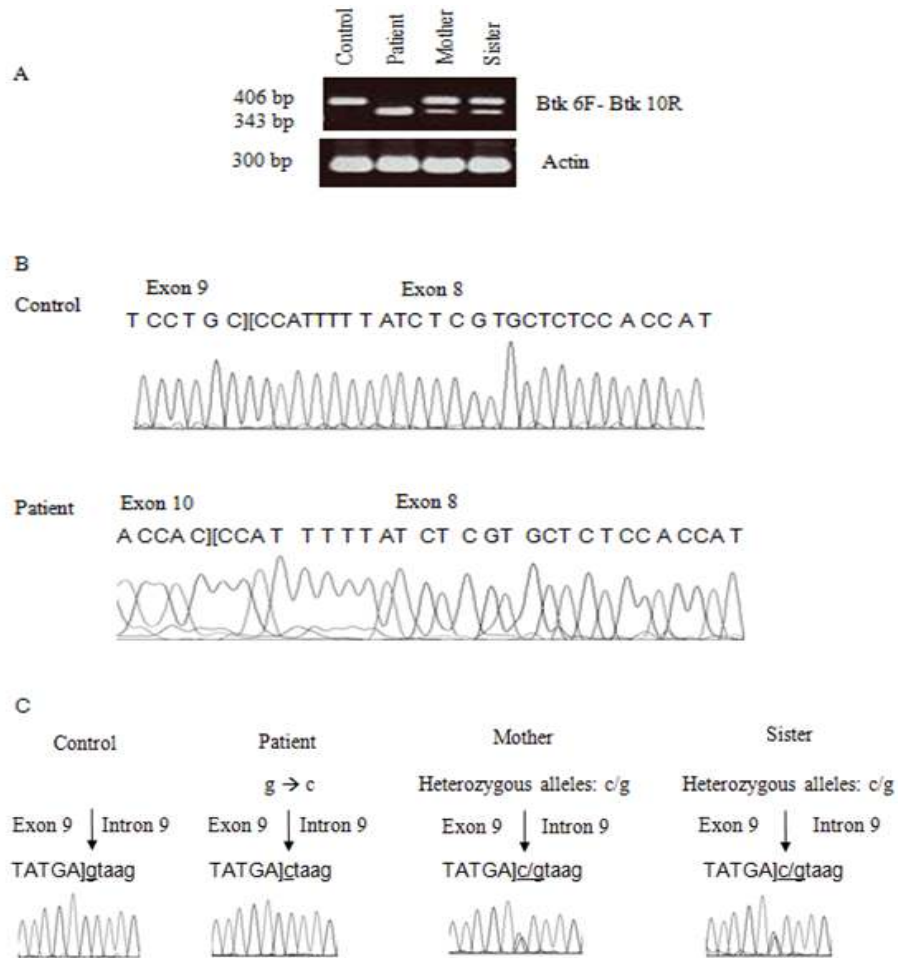


Figure 2. RT-PCR analysis of *BTK* cDNA followed by sequence analysis.

A: RT-PCR products from a normal control, patient, mother, and sister were amplified using a primer set, Btk 6F-Btk 10R, and were visualized on 2% agarose gel. Actin was used as a housekeeping gene.

B: Direct sequence analysis of RT-PCR products from a normal control (Control) and patient (Patient). Reverse sequence is shown.

C: Direct sequence analysis of genomic DNA from a normal control (Control), the patient (Patient), his mother (Mother) and sister (Sister). Forward sequence is shown. Capital letters represent the sequence of exon 9 and small letters represent the intron 9 sequence. The underlined nucleotides represent the mutation point.

The patient's cDNA yielded a single shorter product of 343 base pairs (bp), while the normal control's product was 406 bp long. Amplification of the mother and sister's cDNA yielded both products. Sequencing showed the patient's cDNA PCR product was shorter due to exon 9 skipping (Figure 2B). Sequencing of the cDNA PCR products from

the mother and sister confirmed that the 343 bp product was the same as the patient's, while the 406 bp product was the same as the normal control's product. The PCR reactions using the other six primer pairs⁸ generated fragments that were normal in size and sequence in all the study subjects.

The mechanism of exon skipping was studied by amplification of genomic DNA from the study subjects using primers located in intron 8 and intron 9 respectively, which covered exon 9 and both invariant splice sites, as described by Vorechovsky et al.⁹ Forward sequencing results showed that the first nucleotide of intron 9 had changed from G>C, in the patient as compared to the normal control. However, his mother and sister showed both G and C nucleotides at that position (Figure 2C), indicating they were carriers.

Discussion

BTK is a cytoplasmic tyrosine kinase that plays crucial role in B cell proliferation and differentiation. Other than B cells, *BTK* is also expressed in monocytes¹⁰ and platelets,¹¹ but it is down-regulated in T cells, natural killer cells and neutrophils.¹⁰ In this study, our patient had no circulating B cells. To study the *BTK* protein expression in this patient, monocyte *BTK* protein expression was evaluated using flow cytometric analysis. Flow cytometric analysis is a rapid and sensitive method to analyze monocyte *BTK* protein expression and has been widely used to diagnose XLA patients and to identify female carriers.^{4,10,12}

Sequencing result of the genomic DNA revealed that our patient had a base change from nucleotide G to C, in the splice donor site, the first nucleotide at the 5' end of intron 9 in the *BTK* gene (Figure 2C). The dinucleotide, GT, at the 5' end of the intron and dinucleotide, AG, at the 3' end of the intron are invariant splice sites and critical for a proper splicing of exon and intron. Mutations in any nucleotide in these splice sites may lead to splicing errors, such as exon skipping, intron inclusion or activation of a proximal or distal cryptic splice site.¹³ Point mutations affecting nucleotide 'G' of invariant splice donor site of intron 9, IVS9+1G>A, which resulted in exon 9 skipping and severe phenotype, have been described previously.^{11,14} Compared to these reported mutations, our patient had a different base substitution in the first nucleotide of intron 9, and the mutation was IVS9+1G>C (Figure 2C), which also resulted in a skipping of exon 9 (Figure 2B). Apart from the mutation described here, a base change or deletion of nucleotides 3' downstream of the invariant donor splice site of intron 9, c.839+4A>G or c.839+(4_7)delAGTA, has also been described elsewhere, resulting in either a frameshift mutation (E280fsx 281) or skipping of exon 9.^{15,16,17} The mutation

c.838delGAGT involving exon 9 and intron 9 has been described and also resulted in either a frameshift mutation (E280fs)¹⁸ or a skipping of exon 9.¹⁹ In other words, invariant splice donor site of intron 9 may be a hotspot for *BTK* gene mutation.

The common clinical features of XLA patients with invariant splice donor site of intron 9 are recurrent pyogenic infections since young age, recurrent otitis media, bronchiectasis, low level of serum immunoglobulin and low B cells. They might have different clinical features. The patient with mutation IVS9+1G>A, as described by Zhu et al.,¹⁴ had chronic sinusitis and severe arthritis involving multiple joints; whereas our patient had bronchopneumonia, asthma, failure to thrive and lower serum IgG level. Asthma is uncommon in XLA patients. So far, there is only one report on asthma and allergic rhinitis in XLA patient.²⁰ It is speculated that patients with IVS9+1G>C might have unusual XLA phenotypes.

In conclusion, our patient had a novel base substitution in the first nucleotide of intron 9, and the mutation was IVS9+1G>C. This mutation resulted in the absence of *BTK* expression, circulating B cells and antibodies, which rendered the patient susceptible to recurrent infections. The combination of flow cytometry and genetic study is necessary in the diagnosis of XLA and may be used for subsequent genetic counseling, carrier detection and prenatal diagnosis.

Conflict of interest

We declare that we have no conflict of interest.

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