A novel mutation leading to a deletion in the SH3 domain of Bruton's tyrosine kinase

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X-linked agammaglobulinemia (XLA) is a primary B cell immunodeficiency disorder, caused by a defect in the Bruton tyrosine kinase (BTK) gene. Here, we describe a novel four base pair mutation (838delGAGT) in intron 9 of the BTK gene leading to the skipping of exon 9 in a 2.5-year-old boy with this disorder.

Key words: X-linked agammaglobulinemia, Bruton's tyrosine kinase, mutation.

X-linked agammaglobulinemia (XLA), identified by Bruton in 1952, is a human immunodeficiency disorder caused by B lymphocyte differentiation arrest affecting the transition of B cell progenitors into mature B lymphocytes[1]. It is also known as Bruton's agammaglobulinemia occurring with an average frequency of 1/50,000 male births. XLA is characterized by an increased susceptibility to infections mainly caused by extracellular bacteria. Definitive diagnosis is based on the presence of at least one of the following: mutation in BTK; absent Btk mRNA on Northern blot analysis of neutrophils or monocytes; absent Btk protein in monocytes or platelets; or maternal cousins, uncles or nephews with less than 2% $CD19^+$ B cells, in a male patient with less than 2% circulating B cells[2]. Obligate carriers do not exhibit immunological defects. The responsible gene for this disorder, Bruton's tyrosine kinase (BTK), was found to encode a novel cytoplasmic (non-receptor) tyrosine kinase[2-4].

Bruton's tyrosine kinase is a cytoplasmic protein kinase containing a pleckstrin homology (PH), Tec homology (TH), Src homology (SH3, SH2) and kinase (SH1) domains. Mutations found in XLA patients revealed that these domains are associated with signal transduction[2]. To date, 512 unique mutations in all domains were identified in 855 patients from 746 unrelated families (http://www.uta.fi/imt/bioinfo/BTKbase/). Here we describe a novel mutation leading to skipping of exon 9 of the BTK gene in a patient with XLA.

Case Report

The patient was a 2.5-year-old boy who presented with chronic diarrhea and malnutrition during early infancy. He was the first child of consanguineous parents and had a healthy five-year-old brother. He was shown to have hypogammaglobulinemia (IgA 6.5 mg/dl, IgM 33 mg/dl and IgG 35 mg/dl) with absence of B cells (CD19 0%). He had been doing well since being placed on replacement therapy with intravenous immunoglobulin (IVIG).

Polymerase chain reaction (PCR) was carried out using primers encompassing all exon/intron boundaries of the BTK gene as described[5]. The amplified DNA was mixed with the denaturing solution in a 1:1 ratio, denatured for 5 minutes at 95°C and chilled on ice immediately and loaded onto Genegel SSCP starter kit (Amersham Pharmacia Biotech, Buckinghamshire, England).

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RNA was isolated from peripheral MNCs using Nucleospin RNAII (Macherey-Nagel, Duren, Germany) kit. cDNA was synthesized with Reverted Aid First Strand cDNA synthase (Fermentas, Vilnius, Lithuania) kit. Reverse transcriptase (RT)-PCR was performed with the sense primer (5'-ATGCTATGGGCTGCCAAATT-3') for exon 8 and antisense primer (5'-TTTAGCAGTTGCTCAGCCTG-3') for exon 10.

The PCR product migrated abnormally on SSCP gel, was re-amplified, purified through Qiaquick PCR columns (Qiagen) and sequenced with BigDye Terminator v.3 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Purified sequencing products were run on ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The effect of genomic mutation on RNA splicing was determined by sequencing of the RT-PCR product. All samples were re-amplified and sequenced.

All 19 exons of the BTK gene were screened for mutations by SSCP. PCR-SSCP analysis revealed an abnormal band on exon 9 of the patient (Fig. 1a). The PCR product of corresponding genomic DNA showed four nucleotide deletions in exon 9 and intron 9 boundary (Fig. 1b).

This novel mutation, 838delGAGT, was used for segregation analysis. None of the eight family members, including the mother, was found to carry the mutation. To identify the possible consequence of this genomic deletion on RNA splicing, RT-PCR encompassing exon 8 to 10 was performed. Agarose gel electrophoresis showed that the PCR product was shorter than those obtained from normal controls (Fig. 2). Sequence analysis of RT-PCR product revealed 63 nucleotide deletions, which resulted in the skipping of the whole exon 9 (Fig. 3).

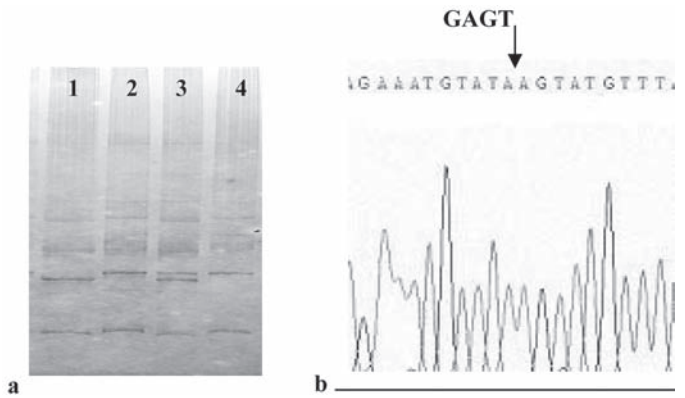

Fig. 1. a. SSCP profile of exon 9. Lane 1, patient; Lane 2, mother of the patient; Lane 3, mix of patient and normal control; Lane 4, normal control. b. DNA sequence electropherogram of exon 9 and intron 9 boundary showing GAGT deletion.

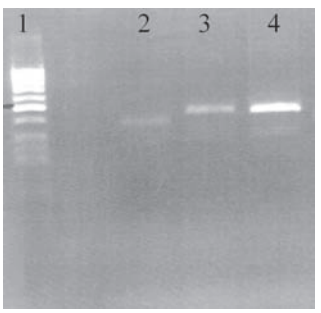

Fig. 2. Agarose gel electrophoresis profile of exon 8 to exon 10 RT-PCR. Lane 1, 50 bp Ladder; Lane 2, patient; Lane 3, mother of the patient; Lane 4, normal control.

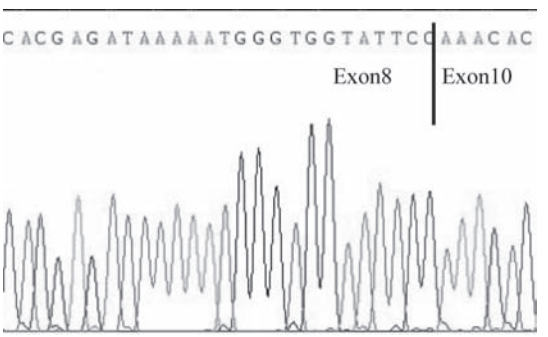

Fig. 3. RT-PCR sequence electropherogram of exon 8 to exon 10.

None of the 41 healthy individuals screened for this novel mutation showed altered SSCP profile.

Discussion

About half of the BTK mutations reported are located in the kinase domain (http://www.uta.fi/imt/bioinfo/BTKbase/). One-third of the mutations in the database are missense types. However, the site and type of mutation within the BTK cannot predict the phenotype of XLA[1]. Here, we describe a patient who had a novel mutation leading to skipping of exon 9 with a clinical phenotype of XLA without any family history.

To date, skipping of exon 9 has been reported several times[5-11]. The first reported exon 9 skipping mutation by Zhu et al.[7] was a G to A transition in the donor splice site of intron 9. Two other groups reported exon 9 skipping in their set of XLA cases at the cDNA level[8,10,11]. Whether the genomic mutation(s) leading to the loss of exon 9 in these cases are the same as ours or Zhu et al.'s[7] is not clear. In our patient, the mutation led to the production of a protein lacking 21 amino acids between residues 260 and 280. This mutation is located in the boundary of SH3 and SH2 domains with Src homology (HUGO database).

Bruton's tyrosine kinase plays a key role in B cell development and activation. The protein consists of five structural domains. Among these domains, phosphotyrosine-binding SH2 and polyproline-binding SH3 domains constitute the middle portion of BTK, and exons 8-13 reside in these domains. These domains are required for the regulation of enzyme activity and signaling cascades by binding to their partners[2]. If SH2 and SH3 domains are prevented from binding, e.g. due to a mutation, the enzyme is depressed[7]. In previous studies in which different aberrant splicing and skipping of exon 9 were identified, it has been demonstrated that, despite the skipping of exon 9, the protein was expressed in a stable form and had full kinase activity in vitro[7,8]. However, the deletion of 21 amino acids within the BTK SH3 domain may alter the protein structure so that it disturbs interactions with one or more crucial SH3 binding proteins and interrupt the signal transduction process required for B cell differentiation[7]. Although functional assays were not performed, as in the previously reported exon 9 skipping mutations, the novel 4 bp deletion identified in this study was found to be associated with XLA phenotype.

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