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.

This study was funded by Hacettepe University Research Foundation (Project number: 00.02.101.013).
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).
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 XLA1. 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 times5-11. 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 level8,10,11. Whether the genomic mutation(s) leading to the loss of exon 9 in these cases are the same as ours or Zhu et al.’s7 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 partners2. If SH2 and SH3 domains are prevented from binding, e.g. due to a mutation, the enzyme is depressed7. 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 vitro7,8. 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 differentiation7. 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.

Acknowledgement

The authors are grateful to Dr. Hirokazu Kanegane (Department of Pediatrics, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan) for kindly providing primers for BTK mutation analysis and for his critical discussion.

REFERENCES