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Short Communication

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Analysis of Bruton's Tyrosine Kinase Deficiency in Patients with Presumed X-Linked Agammaglobulinemia

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Introduction

X-linked agammaglobulinemia (XLA) is a fully penetrant X-linked recessive disorder characterized by the early onset of recurrent bacterial infections, profound hypogammaglobulinemia and a marked decrease in the number of B-lymphocytes [1]. The gene defective in XLA has been identified as a non-receptor proteine tyrosine kinase, *BTK* (Bruton´s tyrosine kinase) [2,3]. Over 600 mutations have been described in the *BTK* gene and they are spreaded throughout the gene [4]. Mutational analysis has revealed that there is considerable heterogeneity in the clinical spectrum of XLA, with atypical individuals having small numbers of mature B lymphocytes and some immnoglobulin production [5]. Analysis of Btk protein expression in primary cells of XLA patients has shown that the large majority of individuals do not express protein regardless of their mutation [6]. As it is yet known Btk is expressed not only in B cells but also in granulocytes and monocytes. In the present work we applied a flow cytometric test employing whole blood to detect Btk expression in monocytes to identify XLA patients. We also applied this method to detect Btk in monocytes from females relatives of XLA patients through demonstration of cellular mosaicism of Btk expression reflecting the random inactivation of X chromosomes in monocytes [6]. In this study, 13 male patients from 7 different families were enrolled. Informed consent was obtained from the patients and their family according to the guidelines of the Hospital Virgen del Rocio Bioethic Committee. After genomic DNA isolation, all the exons and exon/intron boundaries of the *BTK* gene were amplified by PCR with appropriate primers (available by the authors upon request). Sequencing was performed in a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Computer analysis of DNA sequences was performed using the CEQ 8000 Sequence Analysis Software Package. Complementary DNA (cDNA) analysis was performed in patients with non-coding mutations.

The protein expression and mutational analysis results are shown in the table. Sequence analysis of exons 1-19 and intron boundaries of the *BTK* gene revealed three novel different mutations in families A, B and C. Direct sequencing of the *BTK* gene in patient A.1 revealed a novel mutation in exon

Table. Characteristics of the patients with XLA

Family	Patients	%B-cells (CD19+)	Btk expression (FCM)	Nucleotide change	Protein alteration	Exon
A	A.1	0.0	negative	c.1791_1792delAT	p.Y548fsX0	18
	A.2	0.1	negative			
B	B.1	0.0	negative	c.-30G>A	non-coding region	1
	B.2	0.0	negative			
	B.3	2.0	negative			
C	C.1	0.9	weak	c.470_474delins GGGCAGTATCTCTGCTGCTCTCAGACT	p.Q157fsX43	6
D	D.1	0.7	negative	NMF* NMF		
E	E.1	0.1	negative			
F	F.1#	2.0	weak	c.-30G>A	non-coding region	1
	F.2	4.0	weak			
	F.3	0.9	weak			
G	G.1	0.0	positive	c.1722G>A	p.R520Q	15
	G.2	0.2	positive			

Underlined: index case
*NMF: no mutation found
#exitus

18: a 2-base pair deletion of nucleotides leading to the generation of a downstream stop codon causing the premature truncation of the protein product of the *BTK* gene. If translated, the mutant protein would lack the carboxy terminal 61 amino acid residues; this truncated part contains part of kinase domain (TK) that is key for Btk functionality. This mutation was confirmed in patient A.2, nephew of the index case. The mother and one sister of patient A.1 (mother of patient A.2) were heterozygous for this mutation.

Patients B.1, and B.2 were uncle and nephew respectively; patient B.3, brother of patient B.2, was born during the course of this work. Direct sequencing of the *BTK* gene in the index case revealed a novel point mutation in the last base of exon 1; there was an A instead of a G. This transversion may affect to the donor splicing site and could affects to the mRNA stability, since no cDNA could be found. We confirmed this mutation in patients B.2 and B.3, being their mother heterozygous for this mutation [7,8]. A previous study by flow cytometry confirmed that mother of patient B.1 showed cellular mosaicism of Btk expression.

Patient C.1 was of Moroccan origin. A novel mutation in exon 6 was disclosed by direct sequencing of the *BTK* gene, a 26bp insertion and a 2 bp deletion. This mutation did not alter the reading frame leading to a protein with a bigger size. The mutation affects the PH/TH domain. The monocytes of this patient exhibited a reduced expression of Btk by flow cytometry and his mother, heterozygous for this mutation, showed a two-peak expression profile of Btk in monocytes.

No mutations could be identified in the *BTK* gene of patients D.1 (of Moroccan origin) and E.1; the absence of expression of Btk in monocytes by flow cytometry previously found confirmed the diagnosis of XLA. No familiar history was reported for these two cases. Mother of patient D.1 showed cellular mosaicism of Btk expression in monocytes, whereas mother of patient E.1 exhibited positive expression of Btk in monocytes by flow cytometry. It has been proposed that in patients with null Btk expression and no coding or regulatory mutations, some deep intronic mutations are possible [9].

The index case in family F, patient F.1, bore the same mutation in the BTK gene described in family B, although both families were not related. Patients F2 and F3, were bichorionic maternal twins, being his mother cousin of patient F1 and heterozygous for this mutation, as well as the mother of patient 1 and her sister (grandmother of patients F2 and F3). No cDNA could be isolated from the patients. Interestingly, the expression of Btk in the patients of this family was markedly diminished in contrast with the absence of expression in family B. This could be explained by the use of a different monoclonal anti-Btk antibody; family B was analyzed by indirect immunofluorescence using monoclonal antibody 48-2H [6], whereas a commercially available monoclonal reagent (BDPhosflow clone 53/BTK) and direct immunofluorescence was used for the study of family F.

Patient G.1 exhibited positive expression of Btk in monocytes. Sequence analysis of the BTK gene identifying an already published mutation in the patient, as well as in his nephew (G.2) also with positive expression of Btk. So far, at least 17 cases of this mutation have been reported (BTK-base-a database of XLA-related mutations). Mother of patient G.1 and his two sisters were found to be carriers. The mother of patient G.2 was pregnant (10 weeks of gestation) and a sample of chorionic villus was obtained to perform mutational analysis with the result that the male fetus was affected, subsequently, genetic counseling was offered.

In conclusion, direct mutation analysis of the BTK gene and analysis of Btk expression in monocytes by flow cytometry may be considered complementary methods in the diagnosis of Bruton's syndrome.

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