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Case report

A mutation in the promoter region of *BTK* causes atypical XLA

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ABSTRACT

X-linked Agammaglobulinemia is a primary immunodeficiency caused by mutations in BTK, a tyrosine kinase essential for B lymphocytes differentiation. Patients usually have very low or absent B lymphocytes and are not able to develop humoral specific responses. Here we present a boy, diagnosed with XLA due to a mutation on the promoter region of the gene, whose phenotype is characterised by low percentage of B cells, hypogammaglobulinemia, oscillating neutropenia, antibodies responses to some antigens after vaccination and IgE-mediated allergy. Additional technology as flow cytometry was needed to demonstrate the pathological status of the variant. We focus on the idea that XLA should be suspected in males with B lymphopenia and hypogammaglobulinemia, even if they make humoral specific responses. We also highlight the importance of sequencing BTK's promoter region, as mutations on it can be disease-causing.

X-linked Agammaglobulinemia (XLA) is a primary immunodeficiency characterized by profoundly decreased serum levels of immunoglobulins, markedly reduced or even absent circulating B cells, incapacity to produce antibodiy-specific responses and susceptibility to severe bacterial infections [1]. It has been estimated to affect one in every 150.000 human males. Patients should be diagnosed in early childhood as it can be life-threatening if treatment with intravenous gammaglobulin is not immediately established.

XLA is caused by loss of function mutations in *BTK* (Bruton's tyrosine kinase, MIM 300300), located on chromosome Xq22.1, which encodes a Tec tyrosine kinase family member. Several pathogenic variations have been reported in this gene, including missense mutations (40%), deletions (20%), nonsense mutations (17%), splice-site mutations (16%) and insertions (7%) [2]. Causal mutations in the promoter region have also been found, although in a significantly lower rate, with only two cases reported to date [3, 4]. One of them showed absent B cells with hypogammaglobulinemia and, as reported by the authors, a less severe phenotype with minimum expression of BTK and onset of first symptoms at age 5. Both mutations are located in the transcription factor PU.1 binding site sequence, whose conservation seems to be crucial for gene transcription.

Over the last years, XLA patients with atypical immunological features and mild phenotypes have also been described [3, 5, 6, 7, 8, 9], highlighting a clinical heterogeneity that can complicate the suspicion of the disease.

Here we describe an XLA patient with atypical clinical and immunological findings caused by a non-coding variation on the promoter sequence of *BTK*.

Informed consent was obtained from the patient and his family. Our patient is a 40-month-old male born full-term to healthy unrelated parents. From the second month of life he has presented recurrent episodes consisting of fever and neutropenia. At the age of nine months he had one episode of bronchiolitis which resolved without antibiotherapy. During the first year of life he suffered IgE-mediated allergy to cow's milk protein. At the age of 32 months he had an otitis which required antibiotherapy and one month after he developed an episode of diarrhoea

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Table 1. Immunological findings of the patient.

Humoral Immunophenotype	Age: 32 months	Age: 40 months
IgG levels	318 mg/dl	450 mg/dl
IgA levels	<6 mg/dl	<6 mg/dl
IgM levels	9 mg/dl	37 mg/dl
IgE levels	119 UI/ml	84 UI/ml
Vaccination response to tetanus	Positive	Positive
Vaccination response to diphteria	Positive	Positive
Vaccination response to measles	Negative	ND
Vaccination response to neumococo	Negative	Negative
Vaccination response to mumps	Negative	ND
Vaccination response to BHV	Negative	ND
Cellular Immunophenotype		
Total neutrophils counts	ND	50/μl
Total lymphocytes counts	4000/μl	3850/μl
CD3+	91%	96%
CD4+	50%	40%
CD4+CD45RA+	71.1%	45.9%
CD4+CD45RA+CD31+	68.6%	ND
CD4+CD45RO+	14.7%	17.2%
CD8+	40%	54%
CD8+CD45RA+	62.7%	39.6%
CD8+CD45RO+	11.5%	10.6%
CD19+	1%	1%
CD16+CD56+	5%	5%
Proliferation assays (PHA, ConA, PWM, αCD3)	Normal	Normal

ND: Not done. PHA (phytohemagglutinin), ConA (concanavalin A), PWM (pokeweed), αCD3 (anti CD3 antibody).

without abdominal pain, which lasted one month. At the age of 34 months he developed two episodes of urticaria, starting in the face and spreading to the whole body, with predominance of arms and legs and without burning. At the age of 40 months, he had a viral infection with a rash in the trunk and lower limbs and a profound neutropenia which required hospital admission. His immunological findings are summarised on Table 1.

Despite the heterogeneous clinical findings and the presence of humoral specific responses, *BTK* sequencing was performed (supplementary material), attending to the persistently low percentage of B cells and hypogammaglobulinemia as well as the oscillating neutropenia.

The nucleotide substitution c.-193A>G (NM_000061) in the PU.1 binding site sequence of the promoter region was found. His mother was a carrier of the mutation (Figure 1A). This change had been previously described in 1998 in one affected patient with clinical and immunological findings compatible with XLA and was considered by the authors as disease-causing [3].

To test whether this variation might be pathogenic or not, BTK expression by flow cytometry was measured (supplementary material). As it can be observed, neither B lymphocytes nor monocytes of the patient expressed BTK (Figure 1B), demonstrating that the substitution c.-193A>G, is indeed disease causing. Furthermore, his mother showed the classical image of an XLA carrier, with 100% of B lymphocytes expressing BTK, but only part of the monocytes.

In addition, qPCR (supplementary material) showed total absence of BTK messenger RNA in patient's monocytes (Figure 1C).

The low degree of correlation between phenotype and genotype among XLA patients has been discussed for years. Furthermore, new forms of presentation of the disease are being described nowadays, demonstrating that, in this entity, phenotypes are more heterogeneous than it was though when the first patients started to be published.

It has been pointed out that mutations in the transcription factor PU.1 binding site, located in the promoter region of the BTK gene, are disease causing, but the repercussion these mutations have at messenger RNA and protein level has not been so far evaluated.

Here we demonstrate that the nucleotide change c.-193A>G in the consensus DNA binding site sequence of the transcription factor PU.1 avoids the synthesis of mRNA and, consequently, its translation into a functional protein. We also describe the atypical phenotype of a patient affected with XLA due to this promoter mutation, whose main manifestations are low percentage of B cells, neutropenia in the context of infections and presence of humoral specific responses, as allergic pathology or vaccination responses. This case report definitely highlights that XLA might be an unrecognised immunodeficiency in some patients with atypical courses and should be suspected in patients with B lymphopenia and hypogammaglobulinemia, regardless of the presence of specific antibodies.

Considering the possibility of affected XLA patients due to mutations in the promoter region of *BTK*, we recommend sequencing this part of the gene. We also recommend flow cytometry as a simple and quick assay to distinguish XLA patients from others with similar phenotypes, before

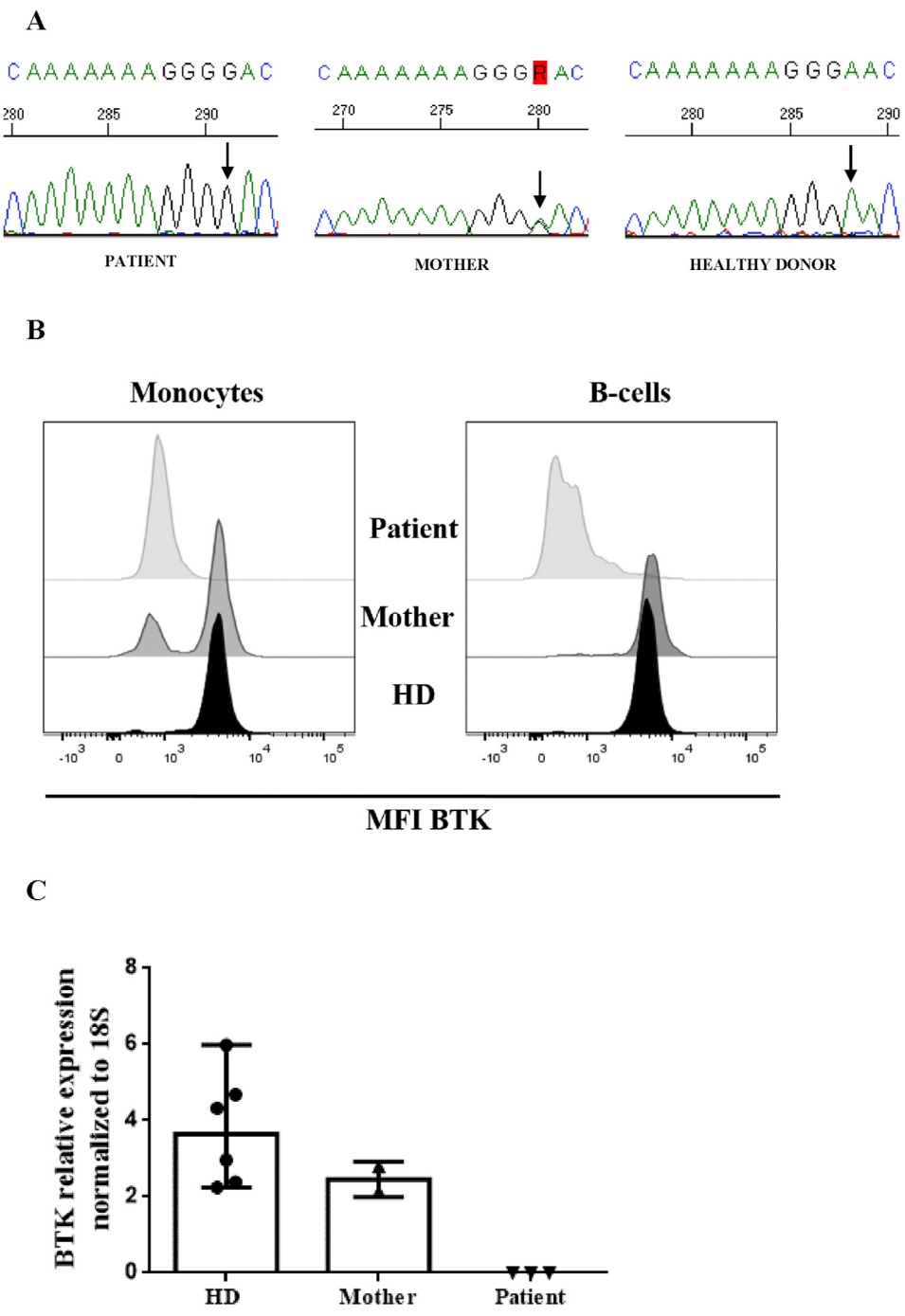

Figure 1. A) The nucleotide substitution c.-193A>G (NM_000061) in the PU.1 binding site sequence of the promoter region was found. His mother was a carrier of the mutation. B) BTK expression by flow cytometry represented as Mean fluorescence intensity (MFI) in B lymphocytes and monocytes of the patient, his mother and a healthy donor. C) Relative BTK expression by q-PCR in monocytes from healthy donors, the patient and his mother. Relative mRNA expression levels was normalized with 18S ribosomal RNA. Box and whiskers represent median with range.

starting molecular biology studies, which are more costly in terms of work and money.

Declarations

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Additional information

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