Case report

A mutation in the promoter region of BTK causes atypical XLA

María Bravo García-Morató a,b,*, Lucía del Pino Molina a,b, Juan Manuel Torres Canizales a,b, Teresa del Rosal Rábades b,c, Ana Méndez Echevarría b,c, Berta González Martínez d, Eduardo López-Granados a,b, Rebeca Rodríguez Pena a,b

a Clinical Immunology Department, La Paz University Hospital and Lymphocyte Pathophysiology in Immunodeficiencies Group, La Paz Institute for Health Research (IdiPAZ), Madrid, Spain
b Center for Biomedical Network Research on Rare Diseases (CIBERER U767), Madrid, Spain
c Department of Pediatrics, La Paz University Hospital, Madrid, Spain
d Department of Pediatric Hematology, Oncology, La Paz University Hospital, Madrid, Spain

ARTICLE INFO

Keywords:
Genetics
Proteomics
Immunology
Imune disorder
Diagnostics
Primary immunodeficiencies
XLA
Promoter region
Flow cytometry
qPCR

ABSTRACT

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 antibody-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 antibiotic. At the age of 32 months he had an otitis which required antibiotherapy and one month after he developed an episode of diarrhoea.

* Corresponding author.
E-mail address: maria.bravo@salud.madrid.org (M. Bravo García-Morató).

https://doi.org/10.1016/j.heliyon.2020.e04914

Received 25 June 2020; Received in revised form 28 July 2020; Accepted 8 September 2020

2405-8440/© 2020 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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

| 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 diphtheria | 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-CD3+ | 66.6% | 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).
starting molecular biology studies, which are more costly in terms of work and money.

Declarations

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04914.

References

[1] C.I. Smith, K.B. Islam, I. Vorochovský, O. Olerup, E. Wallin, H. Rabbani, et al., X linked agammaglobulinemia and other immunoglobulin deficiencies, Immunol. Rev. 138 (1994 Apr) 159–183.
[2] J. Valáško, C.I. Smith, M. Vihinen, BTKbase: the mutation database for X-linked agammaglobulinemia, Hum. Mutat. 27 (12) (2006 Dec) 1209–1217.
[3] E. Lopez-Granados, R. Pérez de Diego, A. Ferreira Germain, G. Fontán Casariego, M.C. García Rodríguez, A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia, J. Allergy Clin. Immunol. 116 (3) (2005 Sep) 690–697.

[4] E. Holinski-Feder, M. Weiss, O. Brandau, K.B. Jedele, B. Nore, C.M. Backesjo, et al., Mutation screening of the BTK gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations without correlation to clinical course, Pediatrics 101 (2) (1998 Feb) 276–284.

[5] T. Fujioka, H. Kawashima, S. Nishimata, H. Ioi, K. Takekuma, A. Hoshika, et al., Atypical case of x linked agammaglobulinemia diagnosed at 45 years of age, Pediatr. Int. 53 (4) (2011 Aug) 611–612.

[6] S. Alyasin, F. Abolnezhadian, A. Rezaei, A case of brutons disease with normal immunoglobulin G level, Iran J Immunol 11 (1) (2014 Mar) 59–63.

[7] H. Kaneko, N. Kawamoto, T. Asano, Y. Mabuchi, H. Horikoshi, T. Teramoto, et al., Leaky phenotype of X-linked agammaglobulinemia in a Japanese family, Clin. Exp. Immunol. 140 (3) (2005 Jun) 520–523.

[8] J.R. Sigmon, E. Kasasbeh, G. Krishnaswamy, X-linked agammaglobulinaemia diagnosed late in life: case report and review of the literature, Clin. Mol. Allergy 6 (2008 Jun 2) 5.

[9] K. Maekawa, M. Yamada, Y. Okura, Y. Sato, Y. Yamada, N. Kawamura, et al., X linked agammaglobulinaemia in a ten year old boy with a novel non invariant splice site mutation in Btk gene, Blood Cells Mol. Dis. 44 (4) (2010 Apr 15) 300–304.