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RESEARCH LETTER

X-linked agammaglobulinaemia. Mutation A1246G (R372G)

To the Editor:

X-linked agammaglobulinaemia (XLA) is a primary immune deficiency characterised by a reduction in circulating B lymphocytes, hypogammaglobulinaemia and recurrent infections. The disease is caused by a mutation of Bruton's tyrosine kinase (Btk). Few studies in large groups of patients have been published; a large cohort of XLA patients in Eastern and Central European countries was recently published, in which the genetic and demographic features of XLA were studied; and clinical, immunological and genetic information was collected for 122 patients from 109 families.¹¹

We present a case of XLA which we believe to be of interest in view of its classical clinical presentation, diagnostic confirmation based on molecular biological techniques, and positive familial history.

The patient was a three-year-old Caucasian male from Ecuador, who had arrived in Spain in June 2003. On occasion

of the first routine primary care visit, the paediatrician noted retarded progression of body weight and height – 10.4 kg (percentile 3) and 89 cm (percentile 25) – without significant findings in the physical examination. History of disease: Starting at one year of age the patient developed two episodes of pneumonia (one requiring hospital admission), recurrent bronchitis, multiple upper airway conditions and one episode of gastroenteritis. Family history: three siblings had died as a result of respiratory infections – the first two at four and two years of age, respectively (corresponding to pneumonia and sepsis), and the third at four months of age, due to severe bronchitis. The mother explained that the first two deceased children were from her first marriage, while the third deceased child and our patient (Figure 1) were the result of her second marriage. She reported no familial or disease antecedents of interest in either herself or her two partners. The paediatrician completed the vaccination protocol. The tuberculin (PPD) test proved negative, while the study of parasites in stools revealed the presence of *Giardia*, which was treated with metronidazole. Blood testing in turn revealed an important decrease in immunoglobulin titres: IgA 66.7 mg/l (reference range 400–2000), IgG 441 mg/l (6400–14,400), IgM 158 mg/l (700–2300). Based on these findings and the mentioned antecedents, an immune deficiency of probable hereditary nature was suspected. The patient was therefore referred to our centre for further evaluation. We performed a complete immune study, revealing intense immunoglobulin and circulating B lymphocyte depletion: IgA <63 mg/l (244–1510), IgG 399 mg/l (4540–12,000), IgM 182 mg/l (436–1890), B lymphocytes 1% (21–28%). The T lymphocyte population proved normal in number and function, as did the natural killer (NK) cells. This was a three-year-old male with a history of recurrent respiratory infections from one year of age, with three male siblings who had died at an early age as a result of infectious processes, and a reduction of both immunoglobulins and circulating B lymphocytes. The diagnostic impression was X-linked agammaglobulinaemia (XLA). At this point treatment was started with intravenous gammaglobulin (400 mg/kg/month), with a good response, no serious infections, and the recovery of a normal growth pattern. Only one admission proved necessary, in the year 2005, due to knee arthritis in response to a viral process, with negative culture findings, which was favourably resolved without antibiotic therapy. In this patient the diagnosis of XLA was confirmed by molecular biological techniques used to study the Btk gene. An A1246G (R372G)

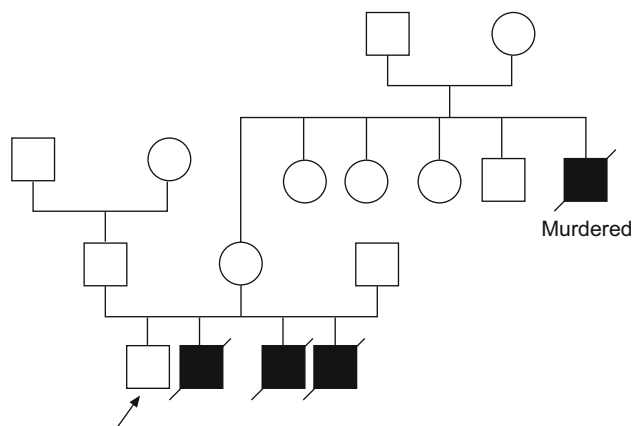


Figure 1 Three siblings died: the first two at four and two years of age, respectively, corresponding to pneumonia and sepsis, and the third at four months of age, due to bronchitis. The first two deceased children were from her first marriage. The mother reported no familial or disease antecedents of interest in either herself or her two copules.

mutation was located in exon 14, causing the agammaglobulinaemia. The study of the mother in turn revealed heterozygous status for the R372G mutation identified in her offspring, i.e., she is a carrier of the disease. Mutation detection was performed in Hospital La Paz, Madrid, Spain, by single strand conformation polymorphism (SSCP) screening followed by direct sequencing of relevant polymerase chain reaction (PCR) products.

XLA is a recessive X-linked immune deficiency characterised by the blocking of B lymphocyte differentiation. The condition manifests with serious and recurrent bacterial infections from the first year of life and enteroviral infections, intense B cell depletion (absent or under 2%), and agammaglobulinaemia.¹ Since 1993 it is known that the disease is caused by mutations of Bruton's tyrosine kinase (Btk) – a circumstance that is of great use in relation to genetic counselling and confirmation of the diagnosis.^{1,2}

Btk is a cytoplasmic tyrosine kinase belonging to the Tec kinases family; it is essential for B lymphocyte development, differentiation and signaling.³ Btk is expressed by all haematopoietic cell lines except the T cell lineage and plasma cells.¹⁻⁴ It intervenes in the transmission of signals from the pre-B cell receptors needed for continuation of the maturation of these cells. The absence of Btk does not allow B lymphocyte maturation in the bone marrow compartment, and the cells do not advance beyond the pre-B stage. The activation of Btk triggers a cascade of signals which result in the displacement of intracellular calcium, reordering of the cytoskeleton, and activation of nuclear factor NF- κ B and other signals necessary for cell survival.³

The Btk protein consists of 659 amino acids distributed into 5 domains: PH (pleckstrin homology), TH (Tec homology), SH2 and 3 (src homology), and kinase.¹⁻⁴ Approximately 800 different Btk mutations have been described,³ of which over 95% are substitutions of a single base pair or only a few base pairs; the rest are large deletions or duplications, insertions or inversions.⁴ These mutations can yield a classical phenotype of the disease or sometimes atypical presentations, characterised by late onset and milder clinical manifestations. Different phenotypes have even been observed within one same family.^{4,5} It has been seen that the substitution of amino acids, particularly those associated to a stable Btk protein, tend to correlate to a more benign disease phenotype, an older patient age at diagnosis, higher IgM titres, and a mild decrease in B lymphocyte count in peripheral blood.⁴ The different phenotypes in turn may be related to the type of mutation involved or its location, although it is possible that the within-family differences and the less severe presentations of the disease are influenced by other genetic and environmental factors.^{4,5} Determination of the underlying mutation is of great diagnostic use, but lacks prognostic application.⁵

The classical clinical picture appears in the second semester of life when the maternal IgG levels transmitted through the placenta fade. Affected patients are boys with recurrent bacterial infections such as otitis, sinusitis, conjunctivitis, pneumonia, pyodermitis, meningitis and sepsis. The implicated microorganisms are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas*. The patients are susceptible to enteroviral infections that cause

chronic meningoencephalitis. Oral polio virus vaccination with attenuated viruses may induce poliomyelitis. Patients with XLA in turn show a higher incidence of juvenile rheumatoid arthritis, aseptic polyarthritis, dermatomyositis and colorectal neoplasms.⁶ The condition sometimes appears associated to other genetic disorders such as neurosensory deafness and growth hormone deficiency.^{7,8} The patients lack tonsils and palpable lymphoid tissue, and the immunoglobulin levels and B lymphocyte counts are either reduced or absent.⁶ Cellular immunity is normal. There may be neutropenia in 15–25% of the cases.⁶

The diagnosis of XLA is easy to establish in the presence of a male with intense hypogammaglobulinaemia, a reduced or absent B cell count (<2% of CD19+ or CD20+ cells) and normal T lymphocytes, in the context of other similarly affected males in the family – although in one third of all cases there is no positive family history.^{1,2} The study of the Btk gene based on molecular biological techniques allows confirmation of the diagnosis if the causal mutation is found. Such techniques also allow us to establish a differential diagnosis, study carriers, provide genetic counselling, and establish a prenatal diagnosis of the disease.^{1,2}

Treatment consists of the administration of gammaglobulin via the intravenous route at high doses which must be individualised (≥ 400 mg/kg every 3–4 weeks). In this context, higher doses are needed in the presence of bronchiectasis and enteroviral infections.⁶ The early introduction of gammaglobulin via the intravenous route allows patients to lead a normal life, and generally without organ disease. The prognosis is favoured when an early diagnosis is established, with the prompt introduction of therapy.^{2,5} At present, thanks to advances in the diagnostic techniques and treatments, the disease is detected early (mean age 3 years), and the affected children survive and reach adulthood. Although XLA can affect adult daily life (with more frequent hospital admissions and sick leave), quality of life is similar to that of the rest of the population, and these patients can become productive individuals for society (with increased educational and income levels).⁹ The development of gene therapy involving Btk gene transfer in haematopoietic progenitor cells could represent a definitive solution for management of the disease.¹⁰

Our patient involved a typical presentation of XLA. In effect, the disease was diagnosed in a three-year-old boy with recurrent infections from the first year of life, intense hypogammaglobulinaemia and the absence of B lymphocytes. Of note is the important positive family history in this case, with three male siblings who had died of infectious diseases at an early age, which proved to be of great diagnostic utility. Indeed, based on the above antecedents, the diagnosis could have been established in our setting much sooner, and even in the prenatal period – with subsequent early treatment and counselling of the mother after the death of her first child.

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- I. Barón Ruiz^{*}, M.A. Martín Mateos, A.M. Plaza Martín, M.T. Giner Muñoz, M. Piquer, O. Domínguez
- Section of Pediatric Allergy and Immunology, Sant Joan de Déu Hospital – Clinic Hospital, Esplugues de Llobregat, Barcelona, Spain*
- ^{*}Corresponding author.
E-mail address: 36823ibr@comb.cat (I. Barón Ruiz).