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Prevalence of BTK mutations in male Algerian patterns with agammaglobulinemia and severe B cell lymphopenia☆

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ABSTRACT

X linked agammaglobulinemia (XLA) is the first described primary immunodeficiency and the most common form of agammaglobulinemia. It is characterized by susceptibility to recurrent infections, profound decrease of all immunoglobulin isotypes and very low level of B lymphocytes in peripheral blood. The disorder is caused by mutations in the Bruton's Tyrosine Kinase (*BTK*). Nine male patients suspected to have XLA from nine unrelated families were enrolled in this study. We performed sequencing of the *BTK* gene in all nine patients, and in the patients' relatives when possible. The XLA diagnosis was confirmed for six patients with six different mutations; we identified a novel mutation (c.1522G>A) and five known mutations. One third of nine unrelated patients do not have mutations in BTK and thus likely suffer from autosomal recessive agammaglobulinemia in the setting of consanguinity. Our results support that the autosomal recessive agammaglobulinemia can be more common in Algeria.

1. Introduction

The prototypical humoral immunodeficiency, the X-linked agammaglobulinemia (XLA; MIM#300300), was first described by Ogden C. Bruton in 1952 [1]. It is a fully penetrant X-linked recessive disorder and occurs in approximately one in 200,000 individuals [1,2]. It is characterized by an increased susceptibility to bacterial infections, severe reduction in all serum immunoglobulin isotypes and a profound decrease or absence of peripheral blood B lymphocytes [3]. It is caused by mutations of the Bruton's tyrosine kinase (*BTK*) gene resulting in profound block of B cell differentiation at early stages in the bone marrow. The *BTK* gene containing 19 exons is localized at the Xq21.3-Xq22 locus, encompasses 37.5 kb and codes for a 659 amino-acid protein which plays a crucial role in B cell development and function. BTK consists of five structural domains: a Pleckstrin Homology (PH) domain, a Tec Homology (TH) domain, an Src Homology 3 (SH3) domain, an SH2 domain and an SH1 domain (catalytic kinase domain) [2,4]. Mutations in patients with XLA are spread throughout the *BTK* gene in coding and non-coding regions. Many of the reported mutations are gathered in the international BTKbase [http://bioinf.uta.fi/BTKbase/]. Most of them are missense mutations in all domains except SH3, possibly because the stability of this domain is refractory to missense mutations, but also deletions, splice-site mutations and insertions. The frequency of mutations depends on the length of the domain [5].

In Algeria, the prevalence of XLA is still unknown. XLA diagnosis is based only on clinical manifestations such as recurrent infections, hypogammaglobulinemia and decreased circulating B cells which are

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insufficient to establish a correct diagnosis. In this study, we describe nine unrelated Algerian male patients clinically diagnosed with XLA. Using genetic analysis we confirm the presence of mutation in the *BTK* gene and XLA diagnosis in six of them.

2. Materials and methods

2.1. Patients

We report nine unrelated male patients with agammaglobulinemia native from different areas of Algeria. Patients were referred by physicians to our laboratory during the 2010–2014 period. Patients' epidemiological and clinical information were collected. Agammaglobulinemia suspicion was based on increased susceptibility to bacterial infections, severe reduction in all serum immunoglobulin isotypes and significant decreased or absence of peripheral blood B lymphocytes (according to the diagnostic criteria for agammaglobulinemia from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency) [3]. The rate of consanguinity reaches 66%. The patients AGM1, 3, 6, 7, 8 and 9 are from related parents. AGM3, 7 and 8 parents' are first cousin, AGM3 and AGM8 had a familial history of recurrent infections. Clinical features are presented in Table 1. Consent of participation in this study was obtained from the patients or their parents. Blood samples from the patient's, and when possible their family members, were collected on EDTA and processed in our laboratory.

2.2. Immunological analysis

Serum immunoglobulin concentration was determined by nephelometry, using the BN ProSpec™ System (Siemens). Peripheral blood leucocytes immunophenotyping was carried out by flow cytometry with a BD FACS Canto™, using a panel of monoclonal antibodies directed against CD45, CD3, CD4, CD8, CD19 and CD20, labeled with either fluorescein (FITC), phycoerythrin (PE), PerCP or PerCP-Cy 5.5 or PE-Cy™7 or APC (Becton Dickinson).

2.3. Mutation detection in the BTK gene

Genomic DNA was isolated from patients, family members and control healthy individuals using a conventional Salting Out method [6].All *BTK* gene exons and the intron-exon junctions were amplified by PCR using a set of primers described by Yu et al. [20]. In brief, 50 ng of gDNA was amplified in 50 μl containing: 4 μM of each primer, 10 μM of dNTP mix, 5 μl of buffer, and 0.4 μl of Taq DNA Polymerase. The following program was used for all exons amplifications: pre-heating at 94 °C during 5 min and then 35 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min 30 s, with 5 min final extension at 72 °C. The PCR fragments were sent for sequencing (Eurofins). Results were analyzed and aligned using the UGEN and BioEdit softwares to detect mutations in the coding sequences and exon/intron junctions. The annotations and numbering of amino acids and nucleotides were done referring to the *BTK* gene sequenceENST00000308731 (NM_000061 and NP_000052).

3. Results

3.1. Clinical features

Nine male patients with agammaglobulinemia or severe hypogammaglobulinemia from nine unrelated families were enrolled in this study. The median age of onset of agammaglobulinemia symptoms was 15 months (range: 3 months–4 years) and the median age of diagnosis was 6.7 years (range: 9 months–26 years). All the nine patients presented with respiratory infections, and the majority of them have bronchiectasis (77%). Otitis media (2/9), arthritis (1/9) and skin infection (1/9) are less present. Patients AGM1, AGM3, AGM6, AGM7, AGM8 and AGM9 exhibit inbreeding while patients AGM2, AGM4 and AGM5 are not from wedding consanguine (Table 1).

AGM3 had family history of early death because of recurrent infections in maternally related males (four uncles and a brother) (Fig. 1). AGM8 also had a family history of early death. His first sister presented with recurrent urinary infections and his second sister died of septicemia at the age of 2.

3.2. Immunoglobulins and circulating B cells percentages

Patients did not receive intravenous immunoglobulin (IVIG) substitution therapy before the XLA diagnosis. The nine patients had very low levels of serum immunoglobulins (Table 2) which is characteristic of XLA. The concentrations were: less than 300 mg/dl for IgG, less than 25 mg/dl for IgA and less than 40 mg/dl for IgM immunoglobulins. Peripheral blood leucocytes immunophenotyping showed 0% circulating B Lymphocytes (Table 2).

3.3. A novel mutation and five reported mutations

Nine male patients recruited in this study were analyzed at the genetic level by *BTK* gene sequencing.AGM1 carries a de novo mutation (c.1522G>A) in exon 15 corresponding to the SH1 domain of the BTK protein (Table 3) which has never been reported in the past. AGM2, AGM3, AGM5and AGM6 carry mutations previously reported, c.1952T>G, c.1922G>A, c.552-557insA and c.1574G>A respectively

Table 1
Clinical features of XLA suspected patients.

Patient	Age at onset	Age at diagnostic	Inbreeding	Family history	Pneumonia	Bronchiectasis	Otitis media	Arthritis	Skin infection
AGM1	3 months	9 months	+	−	+	+	−	−	−
AGM2		26 years	−	−	+	+	−	−	−
AGM3	12 months	3 years	+ (2°)	+ (a brother and 4 maternal uncles died after recurrent infections)	+	+	−	−	+
AGM4		8 years	−	−	+	+	−	−	−
AGM5	48 months	7 years	−	−	+	+	+	−	−
AGM6	24 months	5 years	+	−	+	+	−	−	−
AGM7	3 months	7 years	+ (2°)	−	+	+	−	+	−
AGM8	12 months	3 years	+ (2°)	+ (sister 1 died at 2 years after septicemia, sister 2 presents recurrent urinary infection)	+	−	−	−	−
AGM9	6 months	9 months	+	−	+	−	+	−	−