Clinical, Immunological and Genetic Characterization of Patients With X-linked Agammaglobulinemia in Costa Rica

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Abstract

X-linked agammaglobulinemia is caused by mutations in the gene encoding Bruton tyrosine kinase. It produces an arrest in the maturation and differentiation of B cells with very low levels of all immunoglobulins isotypes. The aim of the study was to characterize the clinical, immunological and genetic defect in patients with XLA in Costa Rica. Sixteen cases were identified over a period of 30 years, a case every 2 years, approximately. Three patients were asymptomatic and diagnosis was made by family history. the average age of onset of symptoms was 1.46 years-old (0.08-6.1). Six patients (44%) had onset of symptoms before 1 year of age and 12 (81%) patients before 5 years of age. The average age of diagnosis was 3.63 years-old (0.17-13, SD 3.51 years-old the average time between the onset of symptoms and the diagnosis was 2.5 years (2.5 months to 12 years, SD 3 years). Initial reason to study the patients was recurrent infection, family history of XLA, arthritis and neutropenia. Four patients had pneumonia and two had suppurative lung disease. Nine patients had recurrent infection: acute otitis media, sinusitis, mastoiditis and recurrent diarrhea. Three patients presented with arthritis. Neutropenia as an isolated event was not identified in any case. All patients receive monthly IVIG and no deaths were reported. Three new likely pathogenic/pathogenic variants in BTK gene have been described in our population. This is the first report of XLA Costa Rican patients and their BTK mutations.

Introduction

X-linked agammaglobulinemia (XLA, OMIM # 300300) is a primary immunodeficiency (PID) caused by mutations in the gene encoding Bruton tyrosine kinase (BTK). It produces arrest in the maturation and differentiation of B cells, that results in absence of B cells in peripheral blood and very low levels or absence of all immunoglobulins isotypes with inability to produce specific antibodies (1–4). Patients have susceptibility to bacterial and enteroviral infections. They present with recurrent sinusitis, bronchitis, otitis media, pneumonia and chronic diarrhea (2–5). Recently, it is not clear if the specific infections vary from region to region and *Pseudomona* infection at the time of diagnosis has been reported in XLA (6–7). Chronic lung disease is a common sequel. Since the description of the disease, the use of parenteral gammaglobulin replacement reduces morbidity, mortality and significantly improves quality of life (2, 6).

BTK gene codes for a tyrosine kinase protein expressed in most hematopoietic cells. It is in Xq21.3-22 and contains 19 exons. Mutations have been described over the entire gene but most of them are in the tyrosine kinase domain (8–10). A clear genotype-phenotype relationship has not been established (11). The aim of this study was to characterize clinically, immunologically and genetically the patients with XLA from Costa Rica.

Methods

The study was approved by the HNN Bioethics and Research Unit (code CLOBI-HNN-003-2012 and CEC-HNN-024-2017) and the permission to publish was obtained by the corresponding Research Subarea. The diagnostic criteria of PAGID and ESID (4) were applied to clinically define XLA cases. Patients with other

causes of hipogammaglobulinemia were identified and excluded. A review of each patient's clinical record was done and information on socio-demographic, clinical and immunological characteristics was obtained. Patients that met the criteria for XLA signed the informed consent or their parents in case of patients younger than 18 years of age. Genomic DNA was extracted from blood leukocytes according to standard protocols. They were sampled for automated DNA extraction using platform MagNA Pure 2.0 (Roche Diagnostics, Switzerland).

BTK gene mutational analysis was carried out using standard PCR protocols, Sanger sequencing (10) and methods established by Danielian et al (12). PCR products were evaluated with a Qiaxell system (Qiagen Company, Hilden, Germany) and the mutations were evaluated by Sanger sequencing on an ABI 3130 Genetic Analyzer (at the University of Costa Rica) and ABI 3500 (at the National Children´s Hospital) (Life Technologies, California, United States) by using BigDye 3.1 chemistry. They were analyzed amplifying all exons and the flanking intronic regions of BTK by either PCR and subsequent Sanger sequencing or Next Generation Sequencing by using a commercial kit (Inmunodeficiencias-GeneSGKit® and GeneSystems® software from Sistemas Genómicos, Valencia Spain) and MiSeq platform (Illumina Inc, San Diego, CA, USA). Amplified sequences were compared to the *BTK* canonic transcript (NM_000061.3) by using the Basic Local Alignment Search Tool for nucleotides (BLASTn) software. Clinically relevant variants detected by NGS were confirmed by either Sanger sequencing or MLPA techniques. Sanger sequencing were based on Big Dye Terminator cycle chemistry (Applied Biosystems, Foster City, CA), and analyzed with an ABI 3500 capillary sequencer (Applied Biosystems, Foster City, CA). Sequence variants were described with respect to a canonical *BTK* transcript NM_000061.3.

Results

Seventeen cases met the clinical case definition of XLA, nevertheless, after genetic testing only 16 patients had identified mutations on *BTK* gene. In a period of 31 years: one case every 2 years approximately. Fourteen patients came from the central, urban area of Costa Rica. One case was born in Cuba but diagnosed in Costa Rica. The mean age of the patients was 17.41 years-old (5.13-32.25, SD 9.45).

Considering the family history of PIDs, there were two patients that had the family history of one brother who died due to pulmonary infection. Two pairs of siblings were found with XLA. In addition, an extended family had 3 affected members in 2 different generations. Therefore, three patients were diagnosed based on their family history while being asymptomatic; one at one month of age and two at 2 months of age. For the remaining 13 patients, the average age of onset of symptoms was 1.46 years-old (0.08-6.1). Six patients (44%) had onset of symptoms before 1 year of age and 12 (81%) patients before 5 years of age and only one after 5 years of age. This patient with later manifestations coincided with the onset of respiratory symptoms in his brother who died 15 days before his diagnosis.

The average age of diagnosis was 3.63 years-old (0.17-13, SD 3.51 years-old). Five patients (31.25%) were diagnosed before one year of age and only 6 patients (37,5% after 5 years of age. Of the 13 patients

that had symptoms before the diagnosis, the average time between the onset of symptoms and the diagnosis was 2.5 years (2.5 months to 12 years, SD 3 years). Table 1 summarizes the data on age, onset of symptoms and diagnosis.

Table 1
Distribution according to family history, age of onset of symptoms and diagnosis of patients with XLA in Costa Rica.

Pt	History of XLA, PIDs or deaths due to infection	Year of birth	Age[1] (years)	Age of onset of symptoms (years)	Age of diagnosis (years)	Interval between the onset of Symptoms and diagnosis (years)
1	No	1993	26.53	1.26	1.56	0.30
2	Yes, brother of 3	2008	11.95	asymptomatic	0.13	asymptomatic
3	Yes, brother of 2	1999	20.13	0.08	2.78	2.70
4	Yes, brother of 5	1995	24.61	0.17	0.53	0.36
5	Yes, brother of 4	1989	30.25	1.00	2.55	1.55
6	Yes, nephew of 7 and maternal cousin of 8	1997	22.82	asymptomatic	0.35	asymptomatic
7	Yes, maternal uncle of 6 and 8	1987	32.25	1.04	5.01	3.98
8	Yes, nephew of 6 and 7	2014	5.13	asymptomatic	0.22	asymptomatic
9	No	2001	18.43	3.47	3.77	0.29
10	Yes, maternal cousin with ADA-SCID	1992	27.20	1.00	12.98	11.98
11	No	2010	9.33	0.25	5.01	2.63
12	No	2011	9.00	0.50	3.87	3.37
13	Yes, 2 brothers died of pulmonary infection	2006	13.82	6.01	8.87	2.86
14	No	2001	18.66	1.88	6.24	4.36
15	No	2013	6.49	1.71	3.64	1.93
16	Yes, brother died due septic shock	2018	1.99	0.56	0.58	0.02

1 Age at 2 Feb, 2020.

The most important symptoms and laboratory data, that led to the diagnosis of XLA are presented in Table 2. Patients were suspicious of XLA because they presented with: recurrent infection, family history of XLA or family death due to infection, arthritis and neutropenia. Infection was the most common finding in 12 patients (75%) where pneumonia and recurrent otitis media are the most relevant. Invasive pneumococcal infection presented in three patients and *H. influenzae* in one patient. Atypical presentation of ectima gangrenosum and septicemia due to *Psedomona aeruginosa* was the initial presentation of one patient. In four patients (25%), arthritis was the most important manifestation; in one as a single finding and in three accompanied by a history of recurrent infection. The family history of death due to infection was relevant in two patients for the clinical suspicion of PID.

Table 2
Clinical, laboratory and genetic data at diagnosis of patients with XLA*.

	Initial symptoms	Lymphocytes (%)			Immunoglobulins (mg/dl)			Neutropenia (<1500 cells/mm^3)
		CD3	CD19	NK	IgA	IgG	IgM	
1	Arthritis	93	0	0	<8	<6.7	<8	no
2	Family history of XLA	86	2	5	25.1	97.2	17.6	yes, 1290
3	Arthritis, recurrent infection	87	0	13	<7.8	15	18	no
4	Recurrent infection	90	0	9	<7.8	50	33	no
5	Arthritis, recurrent infection	88	0	0	<7.8	50	33	no
6	Family history of XLA	83	0	5	<7.8	146	9	no
7	Recurrent infection, neutropenia	93	0	3	<7.8	0	11	yes, 1290
8	Family history of XLA	87	0.4	3	<7.8	319a	<8	yes, 952
9	Recurrent infection	87	0	5	<18	533	<22.7	no
10	Recurrent infection	91	0	12	<22.75	261.9	20.48	no
11	Arthritis, recurrent infection	75	0	12	<26.1	<34.1	38.5	no
12	Recurrent infection	67	0	22	<7.8	<6.7	<8	no
13	Recurrent infection	83	0	5	<7.8	58.1	5.78	no
14	Recurrent infection	90	0	6	10	1280b	18	no
15	Recurrent infection, family death due to infection	80	0	6	<7.8	139	10.4	yes, 294
16	Family death due to infection and acute infection	85	0	6.2	<25.8	<13.6	<18.1	yes, 1232

* Genetic data is not available in all patients. [a] First measurement performed in the patient at 1 month of age. [b] Normal IgG levels in two separate samples. NA not available

Neutropenia was present in 6 cases (37.5%) at the diagnosis, but not as an isolated event. Physicians were aware of the neutropenia only in one patient, it was not noted in the 5 others left. All patients had normal CD3, CD4, CD8 and CD16/56 lymphocytes; while all fulfilled the criteria of less than 2% of B cells. Immunoglobulins levels varied as seen in Table 2. One patient had normal IgG levels in two different measurements at the diagnosis. Specific antibody responses were performed only in 7 patients because initially this assay was not available in Costa Rica.

All patients receive monthly intravenous gammaglobulin replacement. Patients are receiving an average dose of 606 mg/kg per dose of IVIG (DS 206mg/kg/dose) with average trough IgG levels 903mg/dl (SD 191 mg/dl). Half of the patients had infections even after gammaglobulin replacement, specially rhino-sinusitis. Serious adverse events were not reported with the use of IVIG.

The most frequent complications observed were: recurrent or chronic upper respiratory tract infections (otitis media and sinusitis) and chronic diarrhea. There were two patients with suppurative acne. One patient had recurrent hematuria where no germ was documented. Three patients have nutritional impairment even after IVIG. Chronic pulmonary disease is present in five patients (31,25%) and bronchiectasis in four (25%) patients. One patient has a degenerative neurological condition where infections have been excluded. Malignancy, autoimmunity and mortality were not reported as complications in our patients during the study period.

Mutations in patients diagnosed with XLA are presented in Table 3. Nive mutations were previously described, including single nucleotide substitutions, intronic variants and deletions (3, 8, 13–16). Three novel mutations were found according to our knowledge: in exon 17 c.1738 A>T, in exon 14 c.1235 C>A and a duplication in exon 2 c.99dupC. Unfortunately, functional studies to prove abnormal protein function are not available in our country.

Table 3
Genetic findings of XLA patients in Costa Rica

Patient	Nucleotide Variation[Ŧ]	Protein variation[Ŧ]	ACMG Classification and Criteria[#]	Previously described[&]
1	Exon 9 c.837T>G	p.Tyr279Ter	Pathogenic (PVS1, PM2, PP3, PP5)	31
2, 3	Exon 15 c.1480C>T	p.Gln494Ter	Pathogenic (PVS1, PM1, PM2, PP3, PP5)	16
4, 5	Exon 14 c.1420A>G	p.Lys430Glu	Likely Pathogenic (PM1, PM2, PP3, PP4, PP5)	8
6,7,8	Intron 9 c.840-1G>A	?	Pathogenic (PVS1, PM2, PP3)	44
9	Exon 17 c.1738 A>T	p.Ile580Phe	Likely Pathogenic (PM1, PM2, PP2, PP4)	Novel
10	Exon 14 c.1235 C>A	p.Gln412Pro	Likely Pathogenic (PM1, PM2, PP2, PP4)	Novel
11	Exon 14 c.1255 G>C	p.Gly419Arg	Likely Pathogenic (PM1, PM2, PP2, PP4, PP5)	3
12	Exon13 c.1105C>T	p.Leu369Phe	Pathogenic (PS3, PM1, PM2, PM5, PP3)	32, 33
13	Exon 2 c.117-119delCTA	p.Tyr40del	Likely Pathogenic (PM1, PM2, PM4, PP5)	34, 35
14	Exon 10 c.965G>A	p.Arg288Gln	Pathogenic (PS3, PM1, PM2, PP3)	36, 37
15	Exon 2 c.99dupC	p.Val34ArgfsTer8	Pathogenic (PVS1, PM2, PP3, PP5)	Novel
16	Deletion of exons 6 to 10	?	Pathogenic (PVS1, PM2, PP3, PP5)	38

[Ŧ] Based on the canonical transcript NM_000061.3

[#]PVS=Pathogenic Very Strong, PS= Pathogenic Strong, PM= Pathogenic Moderate, PP= Pathogenic Supporting (45)

[&] Revised on ClinVar, PubMed, HGMD and LOVD-BTK databases

Discussion

It has been reported that XLA corresponds to 6-11% of all patients with PIDs, that the incidence is approximately 1 in 100,000 to 200,000 live births and the prevalence is 1 in 10,000 (2, 17). Table 4 summarizes some studies with the incidence and prevalence according to the country that reports cases of XLA. In a report from Latin America, the minimal calculated incidence for XLA is reported in Honduras: 0.11 per 100,000 live births and the highest in Argentina: 1.68 per 100,000 live births (1). In a previous study, Costa Rica reports 9 patients with hypogammaglobulinemia and absence of B cells, but not characterized as XLA (1). Our country has a different distribution of PIDs than the reported worldwide (1, 18–20), where 60% of PIDs correspond to well - defined syndromes, where 55% pf patients have Ataxia Telangiectasia (1, 21). A 17% are combined deficiencies and thirdly 11% are antibody deficiencies (21), where XLA corresponds to 5.5% of all IDPs based on the present study.

Table 4
Summary of incidence and prevalence of XLA reported in different countries or regions.

Country or Region	Incidence (x 100.000 born alive)	Prevalence (x 100.000 inhabitants)	Number of Patients	Reference
Costa Rica	0.78–1.41	NR	9	1
Argentina	1.68	NR	94	47
Latin America	0.11-1.68[1]	NR	234[2]	1
Spain	0.96-1.20	NR	49	18
Norway	5.40	0.35	15	48
Central and East Europe	NR	0.07	104	22
West Europe	NR	0.11	359	22
USA	0.26	0.07	201	2
China	80 cases/ year for 16.87 million inhabitants.	NR	174	30
It corresponds to patients with hypogammaglobulinemia and absence of B cells.				
[2] It corresponds to the minimum incidence in Honduras and the maximum in Argentina.				

According to the literature, 50% of patients are diagnosed with a/hypogammaglobulinemia near 2 years of age although the specific diagnosis of XLA is made several years afterwards. The problem in Costa Rica is that immunoglobulin quantification is mainly done in central hospitals and many doctors from rural or remote areas do not routinely use this laboratory test.

In other series, patients with a family history of XLA are diagnosed significantly earlier (around 2 years) compared to those without a family history (around 5 years), and only 35% of patients who have a family member with XLA were diagnosed before the development of symptoms attributable to their immunodeficiency (2, 19, 22).

According to a study done in the United States, more than 40% of patients with PIDs are not diagnosed until late stages, even though many report serious and chronic health conditions before diagnosis such as: sinusitis, bronchitis and pneumonia (2). The importance of the recognition and management of PIDs is further accentuated by analyzing the rate of pre- and post-diagnosis hospitalization. It is reported that 70% of patients have hospitalizations before diagnosis and only 48% after diagnosis. Multiple reviews emphasize the importance of early diagnosis of PIDs to prevent disease-related morbidity and hence mortality (23–25).

Recurrent infection or infection was presented as the pivot sign in these patients, and it is also the most important characteristic described in most studies: the presence of recurrent bacterial infections in the first years of life (2, 19, 26–27). They also presented arthritis as an initial manifestation that led to the diagnosis. It is striking that neutropenia was not a guiding fact in the Costa Rican population.

The most common cause of morbidity is respiratory infections. Since 1956 there is a report of patients with agammaglobulinemia (both congenital and acquired) and lung disease. It is important to note that many of these manifestations are prior to the onset of IVIG in these patients and in patients where diagnosis is made more than 5 years after onset of symptoms. These patients have recurrent lung disease with pneumonia due to encapsulated bacteria. Bronchiectasis and pulmonary fibrosis develop as long-term complications. It is not possible to establish a relationship between XLA mutation and lung injury because it does not correlate with what is described in the literature (2, 11, 19, 28–30).

The most frequent complications observed were: recurrent acute and / or chronic otitis media, sinusitis, chronic diarrhea; clearly the most common reported in the literature. They are not quantified, nor documented in a standardized way that allows their statistical analysis. With the data, available in the patient files it is impossible to perform this analysis in the population studied. This is a clear limitation of retrospective studies, which suggests that prospective studies of patients with PID should be started to determine the most common infections and agents in this population, especially to improve early clinical care.

It has been shown in multiple studies that the gammaglobulin replacement decreases the frequency and severity of bacterial and viral infections. It also improves morbidity and mortality and decreases the incidence of pneumonia and hospital admissions due to infection (2).

So far, there are more than 500 *BTK* mutations reported in BTKbase. Mutations are distributed throughout the gene in coding and non-coding regions. Point mutations (~ 65%), small insertions, deletions (~ 25%), and large genomic DNA alterations (~ 10%) have been described. They have also been described along the BTK domains (3, 31). Table 4 describes the mutations found in our patients.

Except by three cases, all pathogenic/likely pathogenic variants have been previously described by other clinical studies (3, 8, 16, 32–38) or specific databases (31). Most of these mutations are missense variants (n=6), followed by nonsense (n=2), and one variant for splicing site, indels, in-frame deletion, frameshift deletion and large deletion. Seven out of 16 (43.7%) patients showed mutations in the kinase domain of BTK (BTK-KD) or SH1 domain, in concordance with previous studies (2, 3, 16, 39). It has been reported that the BTK-KD is highly susceptible to mutation, due to several conserved amino acids residues, such as K430, E445, R641 and motifs that are tightly conserved throughout the eukaryotic protein kinase superfamily. Around 67% of single nucleotide amino acid variations in this region are predicted to be harmful mutations, associated with the complete phenotype of XLA (31, 40).

The novel mutations described in this paper cannot be proved by functional assays because they are not available in Costa Rica. Patient 9 has changes an isoleucine for a phenylalanine in exon 17. This specific site was not previously described but in this exon patients with atypical presentations such as HLH (41) and *Pseudomona* infections (6) have been published. This could potentially affect the structure of the protein by the introduction of a benzene group, with differential physical-chemical properties. In addition, the nearby residues W581 and A582 are important in the structure and stability of protein binding to the catalytic site (42, 43). The BTK Base review shows mutations at position 579 and 581, but not at this specific site. At site 579 Tóth (16) describes a Macedonian patient. It is proposed that nearby sites (residue 572 to 582) correspond to a cluster of amino acids critical for protein binding. In addition, the nearby sites: 581 and 582 are important in the structure and stability of protein binding to the catalytic site (43). We speculate that this novel mutation causes structural and binding anomalies.

Multiples mutations in exon 14 as in patient 10, have been described to disrupts a highly conserved glycine- rich motif of the ATP binding site (43). Patient 10 presented a modification of an original glutamine by a proline in the exon 14 (p.Gln412Pro), which is an uncharged polar amino acid instead of hydrophobic basic one. Both mutations are in the SH1 domain that, as it is mentioned above, is highly susceptible to missense variants, and a common mechanism of disease. Although this mutation has not been previously described, the site already has two patients with the W421X mutation (31). The effects of these have not been greatly described either in the BTK expression or its function.

In patient 15, a cysteine duplication was detected in nucleotide 99 of exon 2, generating a frameshift modification with a premature stop signal 8 residues downstream. Therefore, it is expected to result in an absent or interrupted protein product at amino acid 42 of 659.

This is the first study of genetic studies conducted in our country for the diagnosis of IDPs, all other tests were performed abroad. It is also the first in Central America. This pioneering study provide relevant information and should be the principle of the detection of other IDPs by molecular methodologies. Moreover, 3 new likely pathogenic/pathogenic variants in *BTK* gene have been described in our population.

Declarations

Ethical Approval: The study was approved by the HNN Bioethics and Research Unit: code CLOBI-HNN-003-2012 for the first part of the study where and CEC-HNN-024-2017 for the second part where the molecular diagnosis of XLA is now implemented as part of the regular diagnosis in our hospital. The approval letters are available upon request if needed.

Consent to Participate: patients that met the criteria for XLA signed the informed consent (if older than 18 years of age), parents or guardians signed the informed consent in case of participants younger than 18 years old. An informed assent was signed by patients less than 18 years-old but older than 12 years-old as the Costa Rican law dictates. The consent forms are available upon request if needed.

Consent to Publish: the informed consent/assent asked the participants their permission to publish data and specifies the respect of confidentiality. The permission to publish was obtained by the corresponding Research Subarea, at “Centro de Desarrollo Estratégico e Información en Salud y Seguridad Social in Caja Costarricense de Seguro Social” according to the hospital´s regulations.

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Authorship Contributions: Dra. Ivankovich developed this project as a thesis to graduate as Master, she participated in all parts of the study. Dra. Danielian helped in developing the molecular analysis. Dra. Atmella and Dra. Silva helped perform initial molecular analyses at the University of Costa Rica and Dr. Morera, Dr. Santamaria and Dra. Barboza developed the molecular analyses for the clinical setting at the Children´s Hospital. Dr. Porras was the mentor for the thesis and revised all the project. All author approved the manuscript.

Competing Interests and Disclosure of Conflicts of Interest: the authors have no conflicts of interest.

Availability of data and materials: the protocol states that the patients’ charts were revised by the principal investigator and data sheet with all clinical information was filled. Then it was analyzed in an electronic sheet. These information is kept by the principal investigator and available upon request.

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