The dilemma of X-linked agammaglobulinemia carriers

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Background: Many patients with X-linked agammaglobulinemia (XLA) nowadays have reached adulthood, as well as their sisters, possibly carriers of a deleterious Bruton tyrosine kinase variant. Studies on motherhood outcomes in families with XLA are lacking.

Objective: We sought to investigate adherence to carrier status screening, interest in preconception and prenatal genetic counseling, and reproductive decisions in relatives with XLA.

Methods: In this multicenter, retrospective cohort study, we collected a 3-generation pedigree and data on mothers and sisters of patients with XLA, including carrier status and pregnancy outcome.

Results: Data on 53 adults with XLA, 52 mothers, and 33 sisters were collected. All XLA sisters received genetic counseling. Forty percent of the sisters chose to undergo carrier status determination, and 60% of them chose invasive prenatal testing. The main reasons for the sisters to decide not to undergo genetic testing were their young age and the willingness to carry on with the pregnancy regardless of the outcome of the genetic test, followed by the willingness to postpone the decision at the time of pregnancy and the decision to not have children. Prenatal testing resulted in 5 XLA diagnoses, with 2 pregnancy terminations, 1 miscarriage, and 2 XLA live births. Three carriers refused prenatal testing and had 6 live births, including 3 XLA-affected sons. One sister was diagnosed as a carrier after the birth of an XLA-affected son. In total, 9 XLA diagnoses were made, including 6 live births.

Conclusions: A number of XLA sister carriers decided to carry on with their pregnancy after receiving the diagnosis of an affected fetus or after refusing prenatal testing. We propose to initiate a more extensive collaborative study to verify the effect of genetic counseling on families with XLA in other cohorts from different countries. (J Allergy Clin Immunol Global 2025;4:100384.)

Key words: *XLA, Bruton, carrier, genetic counseling, prenatal testing, pregnancies, inborn errors of immunity, genetic diagnosis, X-linked agammaglobulinemia*

Inborn errors of immunity (IEIs) are a heterogeneous group of disorders caused by genetic defects that affect the function or development of the innate and adaptive immune system. To date, more than 500 IEIs have been described, with different genetic defects and clinical phenotypes, even within the same disease.[1] Early diagnosis, immunoglobulin replacement therapy, antibiotic prophylaxis, hematopoietic stem cell transplantation, gene therapy, and targeted therapies have led to a significant reduction in morbidity and mortality, allowing many patients with IEIs to reach adulthood and reproductive age.[1-3] As a result, pregnancy is achievable in a large group of women with IEIs, particularly those with prevalent antibody deficiencies, who account for half of all patients with IEIs.[4] However, studies of maternal outcomes in families with IEIs remain scarce. A recent report shows an increase in neonatal prematurity and an association between severe infection and pregnancy loss due to fetal death.[5]

Genomic testing is increasingly becoming an integral part of the diagnosis and management of patients with IEIs, and according to legislation in many European countries, genetic counseling should always accompany it.[6] Moreover, patients with IEIs and their relatives should have access to preconception and prenatal genetic counseling to have the opportunity to make informed and autonomous decisions that are consistent with their personal needs and values.

The American College of Medical Genetics and Genomics regularly updates its guidelines for screening for autosomal-recessive and X-linked disorders during pregnancy.[7] In

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Abbreviations used
BTK: Bruton tyrosine kinase
CVS: Chorionic villus sampling
IEI: Inborn error of immunity
IQR: Interquartile range
MLPA: Multiplex ligation-dependent probe amplification
XLA: X-linked agammaglobulinemia

addition, genetic counseling for prenatal diagnosis of X-linked genetic disorders includes female carrier screening to identify those heterozygous for a pathogenic or likely pathogenic variant who are at risk of having an affected child.[8,9]

X-linked agammaglobulinemia (XLA) is an inherited immunodeficiency caused by a block in early B-cell differentiation due to deleterious variants in the *BTK* gene encoding for Bruton tyrosine kinase (BTK).[10] The disease prevalence has been estimated between 1:100,000 and 1:200,000. However, the actual prevalence remains unknown because of the lack of an extended implementation of newborn screening programs in many countries, including Italy.[11] Recurrent infections by encapsulated bacteria, *Pseudomonas* spp, enteroviruses, and *Giardia lamblia* are common because of antibody deficiency. The onset of infections typically occurs after the age of 6 months when passively transferred maternal antibodies have been weakened. However, late-onset forms of XLA have also been described.[10,12] Treatment consists of antimicrobial therapy and lifelong IgG replacement therapy, which reduces the recurrence and severity of infections and allows patients to survive into adulthood.[13] However, despite treatment, most patients with XLA still experience invasive respiratory infections and might develop permanent lung damage, ranging from bronchiectasis to chronic obstructive lung disease or end-organ damage.[11,14] In addition, noninfectious complications such as autoimmunity, inflammatory bowel disease, and cancer are being recognized, often without effective therapies, with a negative impact on survival and quality of life.[11]

Decades ago, for most mothers who gave birth to children with XLA, accurate prenatal diagnosis was not possible, whereas nowadays molecular genetic analysis constitutes a decisive tool for the definitive diagnosis of XLA in males and allows the diagnosis of carrier status in females and prenatal diagnosis. Because of the proliferation and maturation of B cells expressing the normal *BTK* allele, female carriers are healthy and immunologically normal. For most mothers who gave birth to children with XLA, accurate prenatal diagnosis was not possible decades ago. However, many of these children with XLA have now reached adulthood,[14] and so have their sisters, who are possible XLA carriers and in their reproductive age.[15]

The idea of this observational study on Italian families with XLA was suggested by a complex clinical case of a patient suffering from 2 X-linked diseases, XLA and hemophilia A, whose sister came to our observation for prenatal counseling. Following the genetic consultation, she decided to carry the pregnancy to term regardless of the results of the genetic test.

To our knowledge, there is a lack of a clear figure on the carrier status of the sisters of patients with XLA, their interest in preconception and prenatal genetic counseling, and their consequent reproductive choices. Here, we report on the adherence to screening for carrier status, the interest in preconception and prenatal genetic counseling, and the reproductive decisions of female relatives of adult patients with XLA belonging to 52 Italian families.

METHODS

Study design

We conducted a multicenter retrospective cohort study on adults with XLA (>18 years) with a molecularly confirmed diagnosis (see the next section). Patients were regularly followed at 7 Italian referral centers for IEIs belonging to the ImmunITA network in Rome (AOU Policlinico Umberto I, IRCCS Bambino Gesù Children's Hospital), Milan (IRCCS Ca' Granda Ospedale Maggiore Policlinico), Padua (Ospedale Ca' Foncello Treviso), Florence (IRCCS Meyer Children Hospital), Naples (Policlinico Federico II), and Barletta (Dimiccoli Hospital). Eligible patients were invited to participate during their routine clinical visit. The sisters of the patients with XLA were also recruited. Informed consent was obtained from the patients and their sisters for participation. For those who agreed, we collected the 3-generation pedigree and data on the pregnancies of mothers with XLA, including number, outcome, age at conception, children's sex, number of XLA-affected children, and data on sisters of female participants with XLA, including demographic characteristics, XLA carrier status, and pregnancy outcome.

Semistructured interview

Data from sisters' genetic counseling were collected using a semistructured script based on open questions by clinicians involved in the study. The semistructured script consisted of 3 open-ended groups of questions, which can be found in full in the Online Repository at www.jaci-global.org. The Ethical Committee of the Sapienza University of Rome (Protocol 3525, 27.3.2019) approved the study. The study was performed in accordance with the Good Clinical Practice guidelines, the International Conference on Harmonization guidelines, and the most recent version of the Declaration of Helsinki.

BTK genetic test

Genomic DNA was extracted from peripheral blood leukocytes using the semiautomatic Maxwell 16 instrument (Promega Corp, Madison, Wis) and quantified using the NanoDrop 1000 spectrophotometer. Next-generation sequencing was carried out on an Ion Gene Studio S5 system (Ion Torrent, Thermo Fisher Scientific, Waltham, Mass) using a custom Ion AmpliSeq On-Demand panel (Thermo Fisher Scientific), designed to detect single nucleotide changes and small indel variants in genes associated with antibody deficiency and common variable immunodeficiency, including the *BTK* gene. DNA analysis was performed under standard conditions. Briefly, 20 ng of DNA was used to prepare libraries using the Ion AmpliSeq Kit for Chef DL8 and the IonXpress Barcode Adapter Kit. The barcoded and purified libraries were quantified using the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific) and pooled in an equimolar manner (30 pM). A template was prepared using the Ion 510, Ion 520, and Ion 530 Kit—Chef (Thermo Fisher

TABLE I. Data on pregnancy outcomes in 8 *BTK*-carrier sisters

Carrier sister ID	Familiarity	No. of pregnancies	Mother's pregnancy outcome	*BTK* variant in mother and affected son (NM_000061.3)
1	An XLA brother alive with coexistent hemophilia A	1	1. Prenatal diagnosis: XLA fetus; negative for hemophilia A Pregnancy carried to term	Exon 8-10 deletion
2	An XLA brother alive with respiratory failure	3	1. XLA live birth (no prenatal diagnosis; mother carrier status unknown for refusal) 2. Prenatal diagnosis: XLA fetus; pregnancy termination 3. Prenatal diagnosis: healthy boy	c.1517G>A; p.(Cys506Tyr)
3	An XLA brother alive with postimmunization polio paresis	2	Refusal to undergo prenatal diagnosis 1. XLA-affected boy 2. Girl	c.82C>T; p.(Arg28Cys)
4	An XLA brother alive	2	Refusal to undergo prenatal diagnosis 1. XLA-affected boy 2. Girl	c.83G>A; p.(Arg28His)
5	An XLA brother alive	2	Refusal to undergo prenatal diagnosis 1. Healthy boy 2. XLA-affected boy	c.746T>G; p.(Leu249Ter)
6	An XLA brother deceased at 26 y (respiratory failure)	3	1. Prenatal diagnosis: XLA fetus; pregnancy termination 2. Prenatal diagnosis: healthy boy 3. Prenatal diagnosis: girl	c.1102+1G>A; p.?
7	An XLA brother alive	1	1. Prenatal diagnosis: XLA fetus; pregnancy carried to term Spontaneous abortion at 14 wk of gestation	c.1921C>T; p.(Arg641Cys)
8	An XLA brother alive	1	1. Prenatal diagnosis: XLA fetus; pregnancy carried to term	c.572G>A; p.(Trp147Ter)

Scientific). Up to 8 samples were loaded on Ion 520 chips. The number of samples per chip was calculated to obtain an average cover of at least 500×. Next-generation sequencing data were analyzed using the Ion Reporter software version 5.18 (Thermo Fisher Scientific).

Cascade BTK testing

Deletions and duplications affecting the *BTK* gene were evaluated by multiplex ligation-dependent probe amplification (MLPA). The commercial kit P210-BTK (MRC Holland) was used to assess the copy number of each *BTK* exon as per the manufacturer's instructions. Four reference DNAs from nonaffected individuals of the same sex were processed in parallel. Results were analyzed and visualized using the Coffalyser.Net software version 220513.1739.

Cascade F8 testing

DNA region spanning exon 23 of the *F8* gene, coding for the coagulation factor VIII, was amplified by PCR using KAPA Taq HotStart DNA Polymerase (Roche) under standard conditions. Purified PCR products were sequenced on both strands using the BigDye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems) and were run on the ABI 3730 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Continuous variables were described using median and interquartile range (IQR). Categorical variables were described using frequencies and percentages. All statistical analyses were performed using SPSS software, version 22 (SPSS, Inc, Chicago, Ill). Images were created with BioRender.com.

RESULTS

Case report

A pregnant woman (Table I, patient 1) came to our attention during prenatal counseling. Her brother was affected by hemophilia A, caused by the c.6559G>A, p.(Gly2187Ser) likely pathogenic variant in exon 23 of the *F8* gene (NM_000132.4), and by XLA (Table I, patient 3). Targeted *BTK* gene analysis showed the complete absence of reads covering exons 8 to 10. The hemizygous deletion of exons 8 to 10 was confirmed using MLPA specific for the *BTK* region. The patient suffered from recurrent bleeding episodes and severe infections with hypogammaglobulinemia (IgG, 100 mg/dL; IgA and IgM, undetectable) with low

TABLE II. Characteristics of the enrolled population

Characteristics	Value
Patients with XLA (n = 53)	
Age (y) at baseline, median (IQR)	34 (25-43)
Composition of the family, n (%)	
No siblings	13 (25)
Born after ≥1 sister or unaffected brother	23 (43)
Firstborn	16 (30)
Born after an XLA-affected brother	1 (2)
Familiarity, n (%)	21 (40)
Having children, n (%)	8 (15)
Age (y) of daughters, median (IQR)	10 (5-18.5)
Mothers with XLA (n = 52)	
Age (y) at first pregnancy, median (IQR)	28 (24-32)
Number of pregnancies, median (IQR)	2 (1-3)
Number of sons/daughters	81/33
Sisters with XLA (n = 33)	
Age (y) at the study time, median (IQR)	32 (23-49)
Having children, n (%)	13 (39)
Agreed to screening before pregnancy, n (%)	12 (36)

peripheral CD19-positive cells (<1%). The patient's sister was referred as a carrier during her first pregnancy, but no report was available. She was then tested in parallel with MLPA specific for the *BTK* region and was found to carry the exon 8-10 deletion in heterozygosity. Her genomic DNA was analyzed by PCR amplification and direct sequencing of exon 23 of the *F8* gene. Because the c.6559G>A *F8* gene variant was not present in the sister, and because *BTK* and *F8* genes are both located on chromosome X, in Xq22.1 and Xq28, respectively, different scenarios are possible: (1) the *F8* mutation is *de novo* in the male patient with XLA and (2) the mother of the 2 siblings carried both *BTK* and *F8* gene alterations, but a meiotic recombination event occurred. Given that *BTK* and *F8* genes are distant (about 53 Mb), a crossing-over might happen during the gamete formation in 1 of the 2 siblings. The possibility of an XXY karyotype in the patient with XLA was excluded. Prenatal testing performed on chorionic villus sampling (CVS) identified a male fetus carrying the exon 8-10 deletion of the *BTK gene*. Following the genetic counseling, the sister chose to continue with the pregnancy.

Observational cohort study on patients with XLA and their families

We enrolled 53 adults with XLA (median age, 34 years; IQR, 25-43 years; range, 18-66 years) and their 33 sisters (median age, 28 years; range, 22-34 years) (Table II). All patients had a molecular diagnosis of XLA confirmed by identification of the *BTK* variant. Three-generation pedigree analysis revealed that 32 patients (60%) harbored a sporadic form and 21 patients (40%) harbored a familial form.

The 52 mothers of the patients with XLA have had a total of 112 pregnancies and 114 live births: 81 males (71%) (including 28 unaffected sons [34%]) and 33 females (29%) (Fig 1). At the time of the birth of their XLA-affected son, the mean age of the mother was 28 years (range, 19-41 years) (Table II). Among the 53 patients with XLA, 13 patients had no siblings, 16 were firstborns, 23 were born after sisters or unaffected brothers, and 1 was born after a late diagnosis in an affected brother. Eight patients with XLA had children: 4 females (obligate carriers) and 4 males. The 4 daughters were not yet pregnant (median age, 10 years; IQR, 5-18.5 years) (Table II).

All 33 sisters with XLA underwent genetic counseling according to the guidelines of the American College of Medical Genetics and Genomics[8] and the European Society of Human Genetics.[9] Twenty-one sisters (64%) decided not to undergo genetic testing. The reasons related to the refusal to be tested were revealed by the semistructured interview. These included young age (<20 years [38%]), the decision to postpone the testing at the time of pregnancy (14%), the personal reproductive choice to continue the pregnancy regardless of the outcome (24%), and the decision to not have children (10%). The remaining 14% did not declare the reason for not being tested. A woman who refused to be tested (Table I, patient 2) discovered her carrier status after the birth of her first child, who was diagnosed with XLA in the neonatal period. In her subsequent pregnancies, she underwent prenatal testing (see below), which resulted in an XLA fetus, whose pregnancy was terminated, and a healthy daughter. The remaining 4 sisters who refused testing had 6 newborns: 5 healthy boys and 1 daughter.

Twelve sisters (36%) underwent carrier status determination. Seven (58%) were diagnosed as XLA carriers and 5 (42%) were diagnosed as noncarriers. The 5 noncarriers had 7 pregnancies, with 2 male and 5 female children.

Including the sister who discovered her carrier status after the birth of her firstborn XLA-affected son (patient 2), we recorded a total of 15 pregnancies in the 8 XLA carriers (Table I) who were referred for genetic counseling at a median gestational age of 5 weeks. Three carriers (Table I, patients 3, 4, and 5) refused prenatal testing by diagnostic CVS or amniocentesis and had 6 live births: 3 XLA-affected sons, 1 healthy son, and 2 daughters.

Invasive prenatal diagnosis performed in 8 singleton pregnancies of 5 carriers (patients 1, 2, 6, 7, and 8) yielded the following results: 5 fetuses positive for the XLA variant, 1 male fetus negative for the variant, and 1 female fetus. Two mothers (patients 2 and 6) with an affected fetus decided to terminate the pregnancy. Three mothers with an XLA-affected fetus decided to carry the pregnancy to term, claiming that the desire to have a child outweighed the fear of having an affected child. One of them (patient 7) had a spontaneous miscarriage at 14 weeks of gestation.

In summary, in our cohort of 33 sisters of adults with XLA, in more than 28 pregnancies, the XLA status was diagnosed in 5 fetuses and 4 newborns, for a total of 6 XLA live births, 2 voluntary termination of pregnancy and 1 miscarriage (Table II; see also Fig 1).

DISCUSSION

Genetic counseling influences decision-making and reproductive options by taking advantage of carrier screening programs for genetic disorders targeted at high-risk individuals.[16] The management of high-risk pregnancies has not changed over time, although the technology used to identify the risk of having an affected child has improved.[17]

Decades ago, diagnosis of carrier status in families with XLA was based on evidence of complete X-chromosome inactivation in the B-cell lineage,[10] and an accurate prenatal diagnosis was not possible for most mothers, who were therefore unaware of their

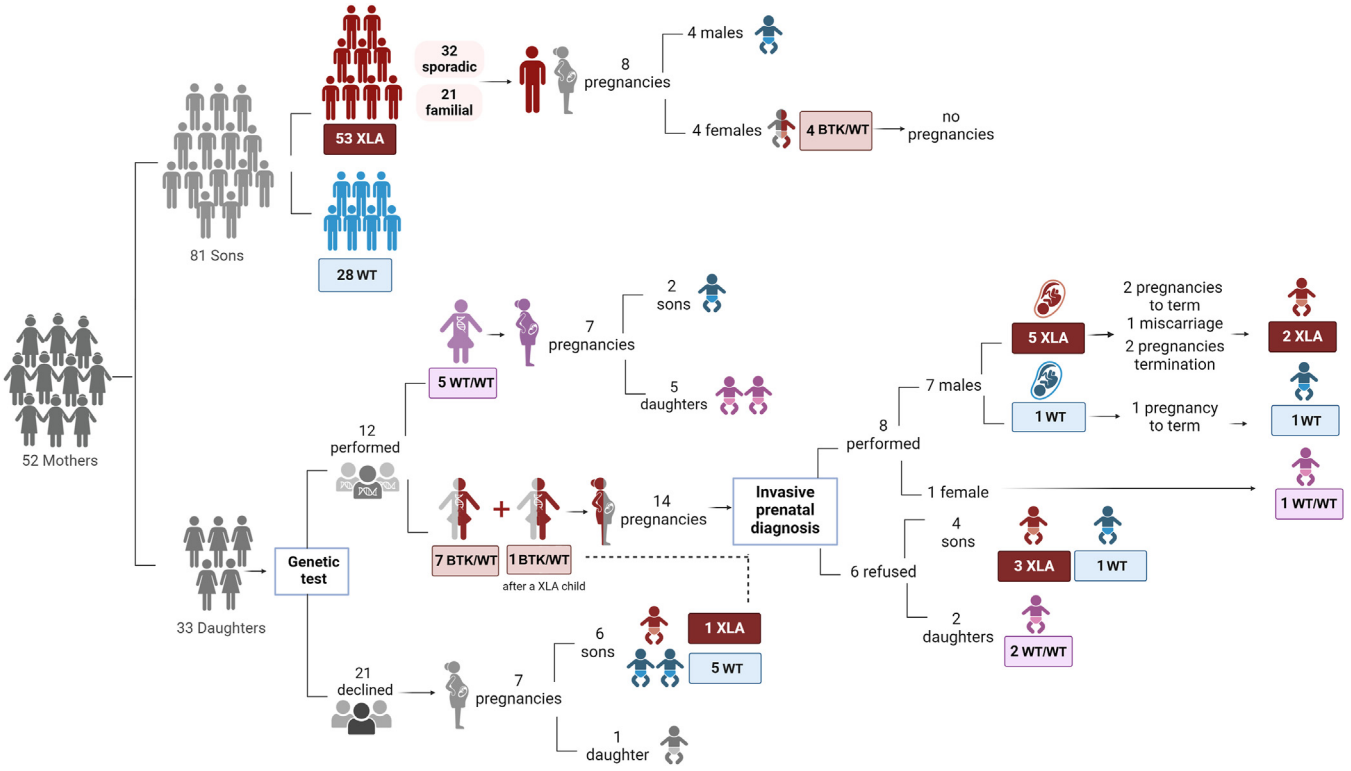

FIG 1. Reproductive outcomes of the family members of patients with XLA, who are represented by *red* shapes. Female patients carrying a pathogenic *BTK* variant (carriers) are represented by *red*/*gray* shapes; female and male patients carrying wild-type (WT) *BTK* variants are represented by *pink* and *blue* shapes, respectively.

reproductive risk unless suspected because of an affected family member.

With the advances in prenatal diagnosis, couples with a fetus affected by a genetic disorder are faced with the dilemma of whether to terminate the pregnancy.[18] In the pioneering study by Conley et al[10] of the 49 mothers of sporadic patients with XLA with proven mutations in the *BTK* gene, 84% were carriers of the mutation found in their sons. Nowadays, sisters of patients with XLA can be tested for their carrier status by genomic sequencing, which can have an impact on their reproductive outcomes. The detection of carrier status is part of genetic counseling,[19,20] together with updated information on the natural history of XLA, its associated conditions, medical and therapeutic management options, and clinical outcomes. Recent reports from national and international cohorts have confirmed a reduced incidence of severe infections and mortality in patients with XLA with early diagnosis, possibly following newborn screening programs and early diagnosis in families with XLA.[21]

Our study aimed to analyze the family history of patients with XLA, including the adherence to carrier status screening of sisters of patients with XLA, their interest in preconception and prenatal genetic counseling, and their reproductive choices. It was inspired by a clinical case of a complex adult contemporary affected by XLA and hemophilia A whose carrier sister chose to continue her pregnancy regardless of an XLA prenatal diagnosis.

We included an extensive series of Italian patients with XLA who had reached adulthood. Most of them harbored a sporadic form, in line with the national registry data.[14,22] As described in 2 extensive Italian studies published 20 years apart,[14,22] the overall survival of patients with XLA at age 40 years increased from 40% to 60% thanks to the shortening diagnostic delay, the shared use of updated recommendations for monitoring, and therapy.[14]

In our cohort, all sisters of patients with XLA underwent genetic counseling, and 36% of them decided to undergo carrier status testing. Thus, more than half of them declined genetic testing because of their young age, their will to postpone testing, their decision not to have children, or their determination to carry the pregnancy to term regardless of the outcome. Sixty percent of carriers underwent prenatal testing by diagnostic CVS or amniocentesis at the beginning of their pregnancy. The 2 carrier sisters who terminated their pregnancies had brothers with XLA who died at a young age from end-stage respiratory failure. Both declared that the poor outcome of their brothers influenced their decision. To note, their pregnancies were terminated more than 10 years ago, in a different context in terms of XLA prognosis.[14]

The remaining carriers decided to take the risk of giving birth to an XLA-affected child because their desire to have offspring outweighed the fear of having an affected child. This decision might have been influenced by their perception of XLA as an affordable disease. This scenario is different in carriers of "severe" X-linked conditions, who intend to make reproductive decisions to avoid having an affected child.[23]

Our data raise the question of what information we provide at the time of genetic counseling. We communicate the most recent data, showing an improved overall survival and a reduction in the number and severity of infections,[14] with many patients now

reaching adulthood and old age. Most adults with XLA are moderately healthy and lead productive lives.[13] However, despite current therapies reducing early mortality, patients continue to experience complications that might affect organ function. Thus, more efforts are still needed to improve management of permanent disabilities and quality of life.[11] To increase patients' and families' empowerment, we suggest that genetic counseling should include other parameters such as healthy life expectancy[24] and information on health-related quality of life reported by people living with XLA.[25,26] Finally, the impact of the system of care on patient outcomes should be considered, as recently shown.[27]

We are aware that the main limitation of our study is the few number of sisters of patients with XLA who decided to undergo carrier status detection and prenatal testing. However, this limitation is also the strength of the message of this preliminary study, as highlighted, that a number of XLA sister carriers decided to carry on their pregnancy despite receiving the diagnosis of an affected fetus or after refusing prenatal testing.

We hope to initiate a more extensive collaborative study to understand the decisions of carriers in other countries and to discuss and share information provided at the time of genetic counseling in families with XLA.

DISCLOSURE STATEMENT

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The data sets generated and/or analyzed during the present study are available from the corresponding author on reasonable request to researchers who provide a methodologically sound proposal. The data will be provided after de-identification in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization.

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