

Extreme variation in X-linked agammaglobulinemia phenotype in a three-generation family

Stephen J. Kornfeld, MD,^a Robert N. Haire, PhD,^a Scott J. Strong, PhD,^a
Emerita N. Brigino, MD,^a Huayang Tang, MD,^b Sun-Sang J. Sung, PhD,^b
Shu Man Fu, MD, PhD,^b and Gary W. Litman, PhD^a
St. Petersburg, Fla., and Charlottesville, Va.

Background: X-linked agammaglobulinemia is typically a severe life-threatening disease characterized by the failure of B-cell differentiation and antibody production, which manifests in infancy and early childhood. Recently, we reported a novel mutation (Cys¹⁴⁵→STOP) in Bruton's tyrosine kinase in a 51-year-old man who was referred for evaluation because of chronic nasal congestion, recurrent sinusitis, sporadic pneumonia, and a family history suggestive of an X-linked immunodeficiency disease. He had not been treated with gammaglobulin.

Objective: This study was performed to investigate the clinical and immunologic phenotypes of this patient's other affected male family members.

Methods: A detailed family history and comprehensive review of medical records was carried out. Genetic mutation analysis of the gene encoding Bruton's tyrosine kinase was carried out in the proband's brother and nephew.

Results: Clinically affected male family members exhibit marked phenotypic variation with manifestations ranging from extremely mild to severe recurrent infections. Immunologic evaluation revealed extreme variation in immunoglobulin levels, B-cell numbers, and functional antibody titers. Genetic analysis documented a novel mutation in the gene encoding Bruton's tyrosine kinase in the proband, his brother, and his nephew.

Conclusions: Despite their sharing the same genetic abnormality, extreme variation was noted in the immunologic findings and phenotypic expression of affected family members. This family study is extraordinary in that clinically affected male members who did not receive aggressive medical treatment died of the disease in childhood or survived into late adulthood. (*J Allergy Clin Immunol* 1997;100:702-6.)

Key words: Bruton's tyrosine kinase, DNA sequencing, primary immunodeficiency disease, protein tyrosine kinase, X-linked agammaglobulinemia

Abbreviations used

BTK:	Bruton's tyrosine kinase
IVIG:	Intravenous gammaglobulin
NR:	Normal range
XLA:	X-linked agammaglobulinemia
YOB:	Year of birth

X-linked agammaglobulinemia (XLA) is an X-linked, severe life-threatening disease characterized by the absence of B lymphocytes, the total absence or severe deficiency of all immunoglobulin classes, and recurrent bacterial infection with pyogenic pathogens.¹ Typically, the disease is first seen during infancy or early childhood and, unless treated aggressively with intravenous gammaglobulin (IVIG), antibiotics, or both, results in significant morbidity. In most cases, XLA will also lead to premature death caused by overwhelming infection or pulmonary insufficiency.²⁻⁵ Recently, it has been shown that XLA is associated with mutations and deletions in the gene encoding Bruton's tyrosine kinase (BTK).⁶⁻⁷ This discovery has made it possible to better define the phenotypic variations of the underlying genetic defect, thereby broadening significantly the clinical and laboratory spectrum of this disease.⁸⁻¹²

In this report we present a unique family with genetically documented XLA. The case source is a relatively healthy 52-year-old man who had never been treated with IVIG, had been treated only intermittently with antibiotics, and was proven to have an absence of BTK production associated with a mutation in the gene encoding *Btk*.¹³ His brother remained similarly well until age 41, whereas several other male family members followed the more typical disease pattern including early death caused by overwhelming infection. This study extends the clinical spectrum of XLA including the late diagnosis of affected individuals and survival into adult life without aggressive medical management. Furthermore, the findings presented here underscore the extreme clinical and immunologic variability that can be seen in patients sharing the same underlying genetic defect.

From ^athe Department of Pediatrics, University of South Florida College of Medicine, All Children's Hospital, St. Petersburg; and ^bthe Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville.

Supported in part by a grant from the Eleanor Naylor Dana Charitable Trust to Dr. Litman.

Received for publication Apr. 23, 1997; revised June 24, 1997; accepted for publication June 27, 1997.

Reprint requests: Gary W. Litman, PhD, Department of Pediatrics, University of South Florida College of Medicine, All Children's Hospital, 801 Sixth St. South, St. Petersburg, FL 33701.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/184499

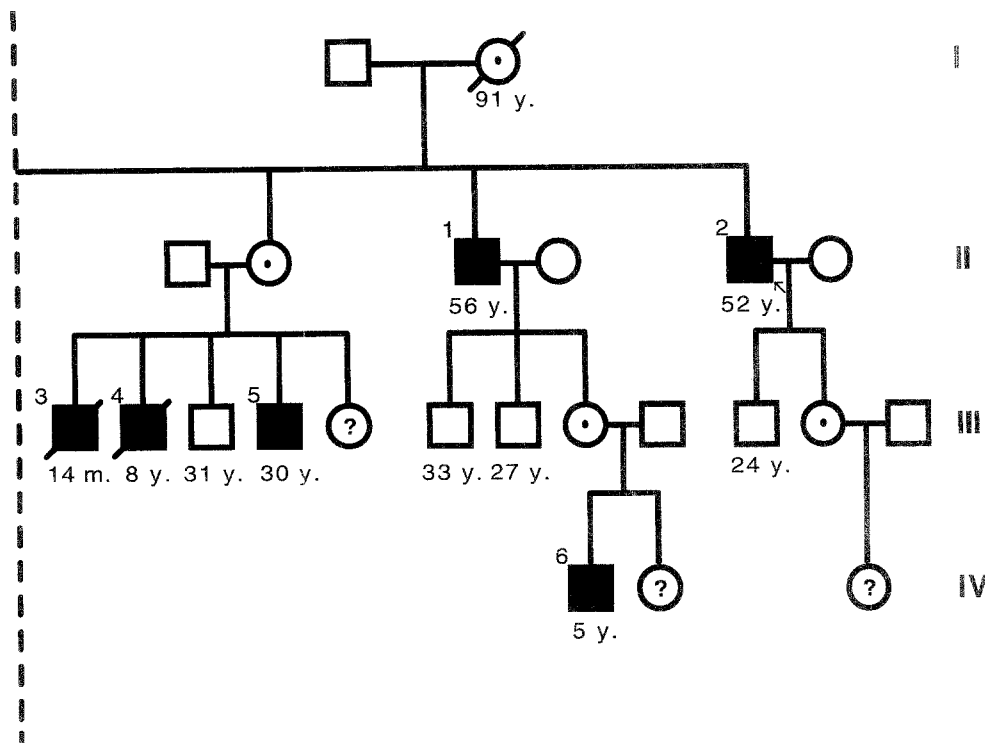


FIG. 1. Family pedigree. *Filled squares* indicate affected male subjects; *diagonal slashes* indicate deceased members; *open squares and circles* indicate unaffected members; *circles with black central points* indicate obligate carriers; and *circles with question marks* indicate female subjects of unknown carrier status. Individual identification numbers are indicated at upper left of symbols. Current age or age at time of death is in years (y) or months (m). Roman numerals to right indicate generation. *Dashed vertical line* indicates additional, uninformative family members. Proband (II-2) is designated by arrow.

METHODS

A detailed family history and, when possible, medical records were obtained. Immunologic parameters and quantitation of BTK protein had been reported for the proband.¹³ Analysis for mutations in the gene encoding *Btk* had been performed for both the proband (II-2) and his nephew (II-3).¹³ His brother, who also had a history suggestive of humoral immunodeficiency, underwent analysis for genetic mutations in the gene encoding *Btk*. These studies have ongoing approval by our Institutional Review Board.

RESULTS

Six male family members had clinical histories suggestive of humoral immunodeficiency (Fig. 1). Their case histories and immunologic evaluations are summarized when available (Table I).

Proband (II-2)

The proband, DS (year of birth [YOB] = 1944), is a 52-year-old man who was first seen for an allergy evaluation because of chronic nasal congestion. History revealed recurrent sinusitis and otitis media at age 9 years, for which a tonsillectomy and adenoidectomy were performed. He has had sporadic pneumonia since age 12 years, which typically has been responsive to oral antibiotics. Because of recurrent and chronic sinusitis, he underwent immunologic evaluation in 1980, which

revealed an IgG level of 501 mg/dl (normal range [NR], 639 to 1349 mg/dl), an IgA level of less than 7 mg/dl (NR, 90 to 410 mg/dl), and an IgM level of 17 mg/dl (NR, 55 to 300 mg/dl). He was tentatively diagnosed with selective IgA deficiency, and early antibiotic intervention was implemented on an as-needed basis. A bilateral Caldwell Luc sinus operation was performed after the immunologic evaluation. His last episode of pneumonia was in 1981; he was hospitalized and treated successfully with a 7-day course of intravenous antibiotics. Bouts of nasal congestion, occurring three to four times per year, were typically treated with 10-day courses of oral antibiotics.

His family history was suggestive of an X-linked immunodeficiency disease, which prompted additional immunologic evaluation and revealed a decreased IgG level of 401 mg/dl (NR, 723 to 1685 mg/dl), an IgA level of less than 7 mg/dl (NR, 69 to 382 mg/dl), and an IgM level of 15 mg/dl (NR, 63 to 277 mg/dl). IgG subclass quantitations showed a decreased IgG1 level of 244 mg/dl (NR, 450 to 900 mg/dl), an IgG2 level of 56 mg/dl (NR, 180 to 530 mg/dl), an IgG4 level of less than 8 mg/dl (NR, 8 to 100 mg/dl), and an elevated IgG3 level of 121 mg/dl (NR, 13 to 80 mg/dl). CD19⁺ B cells were markedly decreased (1%), but T-cell numbers were within normal limits. Although there were increases in

TABLE I. Clinical data for family members

Patient	Age of diagnosis	B cells (CD19)	T cells (CD3)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Proband (II-2)	51 yr (1995)	1%	87%	401	<7	15
Proband's brother (II-1)	41 yr (1982)	ND	ND	556	NT	50
Proband's nephew (III-3)	Undiagnosed	ND	ND	ND	ND	ND
Proband's nephew (III-4)†	10 mo (1964)	ND	ND	—	—	—
Proband's nephew (III-5)	Neonate (1966)	26%‡	55%§	117	NT	NT
Proband's grandson (IV-6)	19 mo (1993)	1%	normal	<9.7	<7.8	19.7

ND, Not determined; NT, not detected.

*Treated with IVIG.

†No gammaglobulin noted in serum protein electrophoresis.

‡EAC rosette assay.

§Sheep rosette assay.

postvaccination antibody titers to diphtheria 0.002 to 0.004 U/ml (NR, >0.010 U/ml) and tetanus 0.03 to 0.09 IU/ml (NR, >0.10 U/ml), they were not protective. Antibody to pneumococcus was undetectable and remained so after immunization. Anti-A isohemagglutinin titers were present at a dilution of 1:64 (NR, $\geq 1:32$), and anti-B titers were absent. Analyses of messenger RNA (complementary DNA) and genomic DNA confirmed a defect in the *Btk* gene (Cys¹⁴⁵→STOP).¹³

Proband's brother (II-1)

Subject TS (YOB = 1940) is a 56-year-old man who had severe impetigo at age 4 months and required hospitalization. At 8 months he had chronic diarrhea for which he was treated with a very simple diet. Growth and development remained normal. Recurrent chronic sinusitis, otitis media, and bronchitis were usually treated with penicillin or sulfamethoxazole. At age 22 he underwent mastoid surgery for treatment of chronic mastoiditis. Recurrent sinus drainage prompted an allergy consult during his early thirties. Allergy skin test results were negative, and quantitative immunoglobulin blood levels revealed hypogammaglobulinemia. Early antibiotic intervention was implemented on an as-needed basis. At age 41 he experienced a severe life-threatening infection with pneumococcal meningitis and pericarditis and was treated successively with intravenous penicillin for 30 days and IVIG was initiated. IVIG has been administered at a dosage of 10 gm every 4 to 8 weeks; notably, this dose is significantly below the recommended level for an adult weighing 86 kg. He continues to experience chronic sinus drainage and is usually treated for sinusitis twice yearly with 10- to 14-day courses of oral antibiotics. He was recently diagnosed with mild pneumonia and given a 10-day course of an oral cephalosporin. The ranges of his immunoglobulin levels during IVIG treatment over the past several years are IgG levels of 556 to 760 mg/dl (NR, 540 to 1480 mg/dl), IgA levels that are not detectable, and IgM levels of 50 to 60 mg/dl (NR, 45 to 260 mg/dl). Analysis of BTK mRNA (cDNA) revealed a defect (Cys¹⁴⁵→STOP) identical to that found in his brother (II-2).

Proband's nephew (III-3)

Subject MM (YOB = 1957) is the first son of five siblings. He was apparently well until a rash and fever developed at 5 months. He was hospitalized, and a low white blood cell count was noted. He remained essentially well with normal growth and development until age 14 months when he had cellulitis. He subsequently was hospitalized because of septicemia, treated unsuccessfully with antibiotics, and eventually died.

Proband's nephew (III-4)

Subject DM (YOB = 1963) is a brother of MM. He had *Pseudomonas* bacteremia at age 10 months. During his hospitalization, he was noted to have shotty cervical, axillary, and inguinal adenopathy. Serum protein electrophoresis showed no gammaglobulin. Isohemagglutinins were diminished (anti-A was 1:1 and anti-B was absent). He was treated with whole-blood infusions with marked improvement, diagnosed with Bruton's agammaglobulinemia, and given regular injections of intramuscular gammaglobulin. He remained in generally good health except for mild recurrent (monthly) upper respiratory tract infections and occasional diarrhea. No history of skin infections or pneumonia was noted. At age 8 he contracted encephalitis and eventually died.

Proband's nephew (III-5)

Subject KM (YOB = 1966) is another brother of MM. Given the family history suggestive of agammaglobulinemia in his first two brothers, the patient was evaluated. At 10 weeks of age, IgA and IgM levels were not detectable (lower range for normal: IgA = 6 mg/dl and IgM = 16 mg/dl); however, an IgG level of 300 mg/dl (NR, 272 to 762 mg/dl) was within normal limits, as expected. The patient began receiving intramuscular gammaglobulin at age 3 months. At age 1 year, the subject had an IgG level of 117 mg/dl (NR, 258 to 1393 mg/dl), and IgM and IgA levels remained undetectable. He had recurrent episodes of otitis media and was switched to plasma infusions at age 7 and later to IVIG treatment. Results of his physical examination were normal, with the exception of absent tonsils. Small

palpable lymph nodes were present. At age 7 he had normal numbers of B cells (26% erythrocyte antibody complement rosettes) and T cells (55% erythrocyte rosettes). Surface staining revealed that 4% of lymphocytes had IgG and 1% had IgM receptors while on intramuscular gammaglobulin. At age 28 he had painful right knee swelling, which was diagnosed as septic arthritis and was treated with a 2- to 3-week course of antibiotics. Otherwise, KM has remained essentially well experiencing only occasional mild upper respiratory tract infections and diarrhea. Analysis at the genomic DNA level revealed the same mutation (Cys¹⁴⁵→STOP) that was noted in the patient's uncles (II-1 and II-2).

Proband's brother's grandson (IV-6)

Subject ES (YOB = 1991) delivered at term by caesarean section after a pregnancy complicated by gestational diabetes. He was first seen with recurrent otitis media occurring every other week since the age of 3 weeks, but he has not had pneumonia, diarrhea, meningitis, fungal infections, or sepsis. Growth and development have been normal. At 13 months he was hospitalized for treatment of bronchitis. At 15 months he had chicken pox and recovered without any complications. At 18 months he was hospitalized for the second time when he had hand, foot, and mouth disease and streptococcal pharyngitis. At that time the subject had an IgG level of less than 9.7 mg/dl (NR, 825 to 1625 mg/dl), an IgA level of less than 7.8 mg/dl (NR, 80 to 410 mg/dl), an IgM level of 17.9 mg/dl (NR, 40 to 400 mg/dl), and an IgE level of 1.6 U/ml (NR, 0 to 180 U/ml). He was then referred to an immunologist. Physical examination revealed a well-developed, well-nourished boy in the 95th percentile for weight and height. Small tonsils and small, palpable lymph nodes were noted. Physical examination was otherwise normal. CD19⁺ B cells were 1% (NR, 5% to 11%). However, normal numbers of T cells were present, and the T-cell helper/suppressor ratio was normal. Isohemagglutinin titers were minimal, and his anti-A and anti B-levels were 1:2 and 1:1, respectively. IVIG infusions were initiated on a 3-week schedule at 400 mg/kg/dose, and the patient is currently doing well.

DISCUSSION

In this report we describe multiple family members with documented XLA who were first seen with marked phenotypic variation. From a clinical standpoint, this family is unique in that only minimal (uncharacteristic) morbidity has been experienced by the proband (II-2) and his brother (II-1), both of whom were born before the advent of antibiotic therapy and survived in generally good health until early in their sixth (proband) and fifth (sibling) decades without treatment with IVIG and with only intermittent antibiotic therapy. Detailed immunologic evaluation of the proband was consistent with the diagnosis of XLA, which was confirmed by genetic analysis, distinguishing him as the oldest patient diagnosed with this disease. Typically, patients with XLA of

this era were first seen with severe recurrent infections by 2½ years of age and died of infection early in childhood.³⁻⁵ The infectious illnesses sustained by these two patients (sinusitis, otitis media, pneumonia, and meningitis) are typical of humoral immunodeficiency. The frequency and severity of the illnesses were atypical in that they were relatively mild and sporadic and did not lead to significant morbidity. Patient II-1 continues to have extremely mild, rare infections despite receiving extremely small doses of IVIG. In contrast, the proband's two deceased nephews (III-3 and III-4), both of whom were born in the antibiotic era, had a much more typical course for XLA.

Analysis of *Btk* in the proband (II-2), his brother (II-1), and his nephew (III-5) revealed a novel defect in the gene.¹³ Quantitative analysis of BTK production in the proband (II-2) relative to production of other B-cell tyrosine kinases was consistent with the selective absence of this protein and corresponding kinase activities.¹³ Given these findings and the nature of the mutation (premature termination codon), it is unlikely that the genetic defect in the proband (II-2) is leaky and somehow allowed for the production of some BTK. Absence of BTK is known to affect B-cell differentiation.^{14,15} Variation in numbers of B cells, functional and quantitative antibody production (including normal isoagglutinin titers), and detection of significant amounts of IgG and IgM in the proband (II-2), his brother (II-1), and his nephew (III-5) may be partially responsible for their relatively benign clinical courses. Therefore it is reasonable to assume that mechanisms other than BTK can affect B-cell differentiation and can partially compensate for a lack of this enzyme. Although this issue is of great interest, we have not yet identified compensatory genes.

The range of phenotypic variation in this family is notable given the fact that the mutation introduces a premature stop codon, consistent with an inability to detect BTK protein or kinase activity. In an earlier report, male siblings exhibiting marked phenotypic variation involving a mutation in the *Btk* initiation codon (Met→Thr), in whom BTK expression could not be demonstrated, were shown to exhibit disparate clinical phenotypes.¹² In another case described previously by our group and for which no other affected family member has been identified, a mild phenotype was associated with a frameshift mutation and stop codon in the C-terminal region of *Btk*.^{8,11} Although interesting, these cases lack the broader significance of this study in that the proband (II-2) and his older brother (II-1) are 35 to 40 years older than these other patients. Although it could be argued that the exposure history of patients with confirmed mutations in XLA is the basis for variation in disease presentation and course, the evaluation of detailed clinical findings for the majority of family members makes this explanation highly unlikely, particularly given the lack of availability of antibiotics during the childhood and early adulthood of the proband (II-2) and his brother (II-1). Characterization of

differential X-chromosome inactivation in the female carrier members of this family in which the phenotypic variation is so extreme would be of considerable interest. However, such a study has not been conducted.

When comparing members of this family with the affected siblings in an earlier study,¹² it is notable that individuals with mild phenotypes produce significant amounts of IgG. The nonspecific nature of early symptoms and only marginally atypical histories of family members in later life emphasize the need to consider mild (atypical) forms of primary immunodeficiency disease in the absence of a significant family history in that as many as 20% to 25% of such cases arise spontaneously. Irrespective of disease history, aggressive management in adult patients with newly diagnosed primary immunodeficiency disease needs to be considered carefully because diminished immune function in later life places these individuals at particular risk.¹⁶

We thank the referring physicians (Dr. Ralph S. Shapiro and Dr. Patrick Mulroy) for their cooperation with obtaining pertinent medical records for these patients, and we thank Barbara Pryor for editorial assistance.

REFERENCES

1. Bruton OC. Agammaglobulinemia. *Pediatrics* 1952;9:722-8.
2. Leickly FE, Buckley R. Variability in B cell maturation and differentiation in X-linked agammaglobulinemia. *Clin Exp Immunol* 1986;65:90-9.
3. Lederman HM, Winkelstein JA. X-linked agammaglobulinemia: an analysis of 96 patients. *Medicine* 1985;64:145-56.
4. Hermaszewski RA, Webster ADB. Primary hypogammaglobulinemia: a survey of clinical manifestations and complications. *QJM* 1993;86:31-42.
5. Sideras P, Smith CIE. Molecular and cellular aspects of X-linked agammaglobulinemia. *Adv Immunol* 1995;59:135-223.
6. Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinemia is a member of the src family of protein-tyrosine kinases. *Nature* 1993;361:226-33.
7. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993;72:279-90.
8. Ohta Y, Haire RN, Litman RT, Fu SM, Nelson RP, Kratz J, et al. Genomic organization and structure of the Bruton agammaglobulinemia tyrosine kinase: Localization of mutations associated with varied clinical presentations and course in X chromosome-linked agammaglobulinemia. *Proc Natl Acad Sci U S A* 1994;91:9062-6.
9. Saffran DC, Parolini O, Fitch-Hilgenberg ME, Rawlings DJ, Afar DEH, Witte ON, et al. Brief report: a point mutation in the SH2 domain of Bruton's tyrosine kinase in atypical X-linked agammaglobulinemia. *N Engl J Med* 1994;330:1488-91.
10. Conley ME, Parolini O, Rohrer J, Campana D. X-linked agammaglobulinemia: new approaches to old questions based on the identification of the defective gene. *Immunol Rev* 1994;138:5-21.
11. Kornfeld SJ, Kratz J, Haire RN, Litman GW, Good RA. X-linked agammaglobulinemia presenting as transient hypogammaglobulinemia of infancy. *J Allergy Clin Immunol* 1995;95:915-7.
12. Bykowski MJ, Haire RN, Ohta Y, Tang H, Sung S-SJ, Veksler ES, et al. Discordant phenotype in siblings with X-linked agammaglobulinemia. *Am J Hum Genet* 1996;58:477-83.
13. Kornfeld SJ, Haire RN, Strong SJ, Tang H, Sung S-SJ, Fu SM, et al. Newly diagnosed X-linked agammaglobulinemia in a 51 year old man. *Mol Med* 1996;2:619-23.
14. Rawlings DJ, Witte ON. Bruton's tyrosine kinase is a key regulator in B-cell development. *Immunol Rev* 1994;138:105-19.
15. Rawlings DJ, Witte ON. The Btk subfamily of cytoplasmic tyrosine kinases: structure, regulation and function. *Semin Immunol* 1995;7:237-46.
16. Saltzman RL, Peterson PK. Immunodeficiency of the elderly. *Rev Inf Dis* 1987;9:1127-39.