



X-linked agammaglobulinemia

Twenty years of single-center experience from North West India

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ABSTRACT

Background: X-linked agammaglobulinemia (XLA) is an X-linked genetic defect in maturation of B lymphocytes that results in the absence of B lymphocytes in the peripheral blood and profound hypogammaglobulinemia. It is caused by a mutation in the *BTK* gene located on the X chromosome. There are no large series describing XLA from the developing world.

Objective: To analyze the clinical features, immunologic and genetic characteristics, and outcomes of 36 patients with XLA diagnosed and managed for a period of 2 decades.

Methods: Diagnosis of XLA was made on the basis of presence of *BTK* gene mutation or marked reduction of B lymphocytes in peripheral blood with a family history of an affected male relative. The diagnosis was confirmed by genetic mutation studies in 28 patients with 25 unique mutations in the *BTK* gene.

Results: There was a significant delay in diagnosis in most of the patients. The mean (SD) delay in the diagnosis was 4.2 (3.5) years. Point mutations were the most common mutations detected, accounting for 68% of all mutations. Deletions and insertions were also seen in a few cases. Four of the mutations are novel mutations that have not been previously reported. Seven of the 36 patients (19%) were dead at the time of analysis in the present cohort. The mean survival was 137 months (95% confidence interval, 13–163 months).

Conclusion: The present study is perhaps the largest series of patients with XLA from any developing country so far.

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Introduction

X-linked agammaglobulinemia (XLA) is a rare, inherited single-gene defect that primarily affects the humoral arm of the immune system. The disease is characterized by absence or low numbers of peripheral B lymphocytes and profound decrease in the levels of all immunoglobulin isotypes in the serum.^{1,2} It results from mutations in the *BTK* gene, which maps to the long arm of the chromosome X (Xq21.3 to Xq22).³ However, cases with normal levels of 1 or more

immunoglobulin isotypes have also been reported. These could be the result of so-called leaky mutations in the *BTK* gene.

This gene encodes for a tyrosine kinase Btk, which is indispensable for the normal development of B cells. Btk is required for the maturation of mature B cells from pre-B cells. XLA is transmitted as an X-linked recessive disease; males are primarily affected by the defect, and females are usually passive carriers. An occasional case of XLA has, however, been reported in females as a result of unfavorable lyonization.⁴

Most cases of XLA are familial in nature, and only one-third of cases are a consequence of de novo mutation in the *BTK* gene. Large series of XLA have been reported from the United States.^{2,5,6} Outside the United States, data are available from studies from United Kingdom, Europe, Latin America, and Asia.^{7–9} Data for Asia are from China, Japan, Taiwan, Hong Kong, Malaysia, and Iran.^{10–13}

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There are no large-scale studies available from India except for isolated case reports and small case series.^{14–16}

Gupta et al.¹⁷ have reported collated data regarding primary immunodeficiencies (PIDs) diagnosed in Chandigarh and Mumbai. Data regarding primary immunodeficiencies diagnosed in Mumbai has also been described in the literature.¹⁸ Data from both these reports indicate that there is marked difference in the type of PIDs diagnosed in Chandigarh and Mumbai. Antibody defects comprised only 15% of the total PID cases at Mumbai, whereas they were the most common PIDs diagnosed in Chandigarh, accounting for 42% of all PIDs. It could be debatable whether this actually represents a difference in the incidence of various PIDs in North and South India or whether it just reflects a referral bias. The differences could also be partly explained by the differences in the rates of consanguinity and the different genetic constitutions of North and South Indians.¹⁹

Children with XLA usually develop symptoms at 3 to 9 months of age when the maternally transferred cases have been catabolized. Rare cases of XLA diagnosed in adulthood have been reported.^{20,21} These cases probably represent milder forms of disease caused by leaky defects in the *BTK* gene. The common modes of clinical presentation include pneumonia, otitis media, diarrhea, skin infections, cellulitis, sepsis, and meningitis. Children with XLA are also exquisitely predisposed to enteroviral infections because humoral immunity is critical for dealing with enteroviral pathogens. Diarrhea is usually caused by common pathogens, such as *Campylobacter jejuni* and *Giardia lamblia*. Skin involvement is in the form of pyoderma gangrenosum, drug-induced Stevens-Johnson syndrome, and eczematous dermatitis that resembles atopic dermatitis.^{2,22}

Arthritis indistinguishable from juvenile rheumatoid arthritis may be the presenting manifestation in patients with XLA.²³ Persistent enteroviral infections may rarely lead to fatal encephalitis or a dermatomyositis-meningoencephalitis syndrome. Recurrent pulmonary infections frequently lead to bronchiectasis.

Methods

A comprehensive data review of all patients diagnosed as having XLA from April 1994 to September 2014 was undertaken for the purpose of the study. Data were obtained from the clinical case files and records of the Pediatric Immunodeficiency Clinic, Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India. The study was approved by a department review board as per the existing practice at our institute. Written informed consent was also required from parents for taking clinical photographs. Details recorded included age at onset and diagnosis, delay in diagnosis, initial clinical manifestation, laboratory results at diagnosis, therapy instituted, and outcome. Laboratory features analyzed included immunoglobulin levels at diagnosis, B-lymphocyte numbers, Btk expression on monocytes by flow cytometry, and details regarding *BTK* gene mutation analysis.

Serum Immunoglobulin Estimation

Serum immunoglobulins were estimated by single radial immunodiffusion in cases diagnosed before 2009. End point nephelometry using a semiautomated nephelometer (MININeph, The Binding Site, Birmingham, United Kingdom) was used for serum immunoglobulin estimation in cases diagnosed after 2009. Our laboratory has been participating in the UK National External Quality Assurance Scheme since 2011 for immunoglobulins and complements to ensure the reliability and accuracy of our results.

Lymphocyte Subset Analysis

Lymphocyte subset analysis was performed using either BD Tritest CD3 fluorescein isothiocyanate (FITC), CD19 phycoerythrin

(PE), and CD45 peridinin chlorophyll protein complex (PerCP) reagent kit (catalog No. 340381; BD Biosciences, San Jose, California) or the BD Multitest CD3 FITC, CD16⁺CD56 PE, CD45 PerCP, and CD19 allophycocyanin reagent (catalog No. 340500) using a lyse–no wash protocol. Briefly, 20 μ L of either of the 2 antibody cocktails was added to 50 μ L of EDTA anticoagulated blood in a FACS tube, mixed, vortexed, and incubated in dark for 20 minutes. After 20 minutes, 450 μ L of FACS lysing solution was added to the tubes, and the tubes were incubated for an additional 10 minutes. Finally, the tubes were vortexed, and the acquisition was performed on either BD FACSCalibur or BD FACSCanto flow cytometer. Lymphocytes were gated on CD45 vs SSC for analysis.

A complete blood cell count was also performed in each case using a 5-part automated hematology analyzer. The blood cell count included a total and differential leukocyte count, platelet count, absolute lymphocyte count, absolute neutrophil count, and an absolute eosinophil count.

Btk Estimation on Peripheral Blood Monocytes by Flow Cytometry

Btk protein estimation was also performed in some of the cases by flow cytometry. Peripheral blood mononuclear cells were isolated from test and control samples. Three tubes labeled as isotype, CD14, and CD14/*BTK* were used for both control and test samples. Surface staining was performed using 10 μ L of CD14 FITC antibody, which was added to the CD14 and CD14/*BTK* tubes. Isotype-specific antibodies were added to the isotype tubes. After incubation, for 15 minutes the tubes were washed twice with wash buffer, and 250 μ L of cold permeabilization buffer was added to the tubes while continuously vortexing the tubes. Tubes were then incubated at 4°C for 30 minutes. After this, the tubes were centrifuged at 1,000 rpm for 5 minutes. The supernatant was carefully pipetted off, leaving 100 μ L of permeabilization buffer in each tube. A total of 10 μ L of *BTK* PE antibody was then added to the CD14/*BTK* tubes and incubated for an additional 20 minutes. Finally, the acquisition was performed on FACSCalibur or FACSCanto, and Btk estimation was sought on gated CD14-positive monocytes.

BTK Gene Mutation Analysis

BTK gene mutation analysis was conducted at the National Defense Medical College, Saitama, Japan, and at the Department of Pediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, after obtaining written informed consent from the parents.

Diagnostic Criteria

A diagnosis of XLA was proffered in a given case if any of the following criteria were met: (1) a mutation in *BTK* gene ($n = 28$) classified as definite XLA and/or (2) a positive family history (an affected male sibling or maternal uncle or maternal cousin or maternal nephew) with hypogammaglobulinemia and markedly reduced B lymphocytes in peripheral blood ($<2\%$). These cases were diagnosed as probable XLA ($n = 8$). In addition, we also had 13 children with hypogammaglobulinemia and reduced B cell counts ($<2\%$). However, in the absence of a family history, these children were excluded from further analysis.

Six patients with normal IgA and 4 patients with normal IgM at time of diagnosis were also included in this study. Five of the 6 patients with normal IgA had a mutation in the *BTK* gene. Two of the 4 patients with normal IgM also had a mutation in the *BTK* gene. One patient with normal IgM and no mutation in the *BTK* gene had a lateral male relative (maternal uncle) afflicted with similar illness. Another patient with normal levels of IgM and IgA and no *BTK* gene mutation also had an affected male sibling. These cases were also included in the study.

Genotype-Phenotype Correlation

Nonsense mutations, frameshift mutations, deletions, and splice-site mutations within splice consensus sequence were classified as severe. Missense mutations that involved conserved residues or within highly conserved domains were designated as severe, whereas those that affected nonconserved residues were called less severe. Similarly, splice-site mutations at noninvariant sites were classified as less severe.

Results

Age at Onset and Diagnosis

The mean (SD) age at onset of symptoms was 1.6 (1.5), with a median age at onset of 1 year (range, 0.6–6 years). A diagnosis of XLA was established at the mean (SD) age of 5.9 (3.8) years, with a median age at the time of diagnosis of 5 years (range, 1–18 years) (Table 1).

Delay in Diagnosis

There was a significant interval between the first onset of symptoms and the establishment of diagnosis in most of the patients. The mean (SD) delay in diagnosis was 4.2 (3.5) years, with a median delay of 3.25 years (range, 0.16–14 years) (Table 1).

Immunoglobulin Levels at Diagnosis

Most of the children in our cohort had reduced levels of all 3 major immunoglobulin isotypes (ie, IgG, IgA, and IgM). The mean levels of various immunoglobulin isotypes are summarized in Table 2.

B-Cell Numbers

The percentage of B cells was markedly reduced (<2%) in all patients (Table 2).

Btk Protein Estimation on Monocytes by Flow Cytometry

Btk protein estimation on CD14-gated monocytes could be performed only in 5 patients.

Clinical Manifestations

Pneumonia and other respiratory tract infections (upper respiratory tract infection and empyema) were the most common manifestation present in 31 of 36 patients (86%) (Fig 1). Gastrointestinal symptoms in the form of diarrhea, vomiting and malabsorption were noted in 16 of 36 children (44%). Recurrent otitis media was another common manifestation seen in 50% of patients. Joint involvement in the form of arthritis was evident 42% of patients. Skin infections, such as cellulitis, pyoderma gangrenosum, and recurrent boils and abscesses, were observed in 25% of the patients. Meningitis and meningoencephalitis were also not uncommon, and the major ailment in 25% of the patients in the present cohort.

Table 1
Age at Onset and Diagnosis in Children With X-linked Agammaglobulinemia

Variable	Age, Mean (SD), y	Age, Median (Range), y
Present age of children	6.9 (4.0)	6 (1–18)
Age at onset of symptoms	1.6 (1.5)	1 (0.6–6)
Age at diagnosis	5.9 (3.8)	5 (1–18)
Delay in diagnosis	4.2 (3.5)	3.25 (0.16–14)

Table 2
Immunoglobulin Levels and B-Cell Counts at Diagnosis

	Mean (SD)	Median (Range)
Serum immunoglobulins at diagnosis (mg/dL)		
IgG	198 ± 137	204 (16–555)
IgA	28 ± 16	34 (4–57)
IgM	26 ± 26	20 (4–150)
B-cell percentage at diagnosis	0.2% (0.4%)	0.04% (0–2%)
Btk protein estimation by flow cytometry (n = 5)	Decreased expression in 3; normal in 2	

Neutropenia

Neutropenia, defined as an absolute neutrophil count of less than 15,00/mm³ was an uncommon manifestation observed in only 4 of 36 patients (11%). Infections in the neutropenic patients were similar to the rest of cohort, and there were no unique or atypical organisms. However, 1 patient had sepsis attributable *Pseudomonas aeruginosa*.

Family History of Disease

A positive family history of disease could be elicited in 20 of 35 patients (57%). History of similar illness in a male sibling or death of a male sibling was the most frequent mode of familial involvement, evident in 15 of 35 children (43%). The affliction of a maternally related lateral male relative, such as a maternal uncle or maternal cousin, was found in 12 of 35 cases (34%) and 2 of 35 cases (6%), respectively.

Meningitis and Meningoencephalitis

Meningitis and meningoencephalitis were present in 9 of 36 patients (25%) at analysis. It was associated with poor outcome when it was the initial mode of presentation. Four of the 9 children (44%) in whom meningitis was the predominant clinical presentation had succumbed to their illness. Although the cause of these cases of meningitis could not be conclusively established, it was likely enteroviral pathogens in some of these patients.

Unusual Clinical Manifestations

Unusual clinical manifestations that have not been reported earlier were encountered in the present cohort. Patient 27 presented with right orbital cellulitis after an extension from the right maxillary and ethmoid sinusitis (Fig 2B & 2C). Although sinusitis is a common manifestation in XLA, orbital extension of the same resulting in orbital cellulitis has not been reported earlier.

Molecular Analysis

Mutations in the *BTK* gene were identified in 28 of 36 patients (78%) in the present cohort. The most common mutations detected were point mutations, found in 19 of 28 patients (68%) in whom mutations were detected. Missense mutations accounted for 8 of 19 (42%), whereas nonsense mutations comprised 6 of 19 (32%) of these point mutations. Intronic and splice site mutations resulting in altered splice sites and exon skipping were found in 5 of 19 (26%) of all point mutations. Deletions were observed in 7 of 28 cases (25%); 2 of these deletions were large deletions that involved 2 or more exons and introns. Insertions resulting in altered reading frame were detected in 2 patients. Mutations were spread across the whole gene, affecting exons 1 to 19 without any predilection for any particular exon. Twenty-five unique mutations were identified; 2 sibpairs and 1 pair of maternal uncle and his nephew shared a common mutation in the present cohort. Four novel mutations that

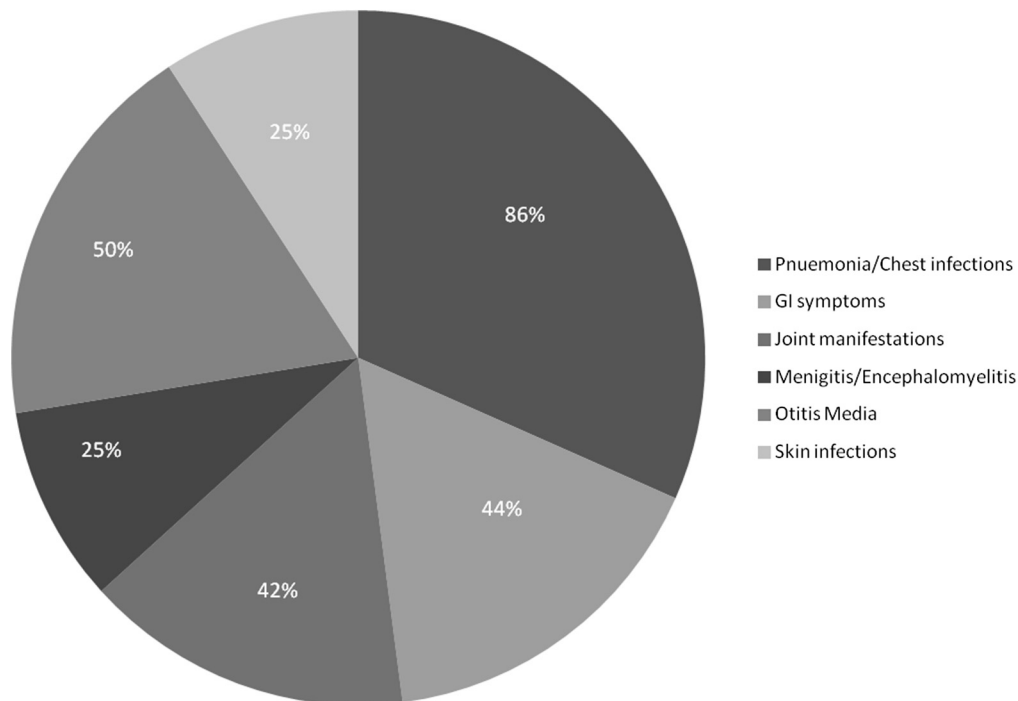


Figure 1. Predominant clinical manifestations in children with X-linked agammaglobulinemia in Chandigarh, India.

have not been previously described were also detected in our cohort of patients.

Genotype Phenotype Correlation

Nineteen mutations were classified as severe based on their predicted functional change, whereas 6 mutations were designated less severe. Five of the 6 less severe mutations were missense mutations

in nonconserved residues. One mutation in a noninvariant splice site was also designated as less severe. Severe clinical manifestations, such as meningitis and arthritis, respectively, were detected in 2 of 7 and 4 of 7 patients with less severe mutations. However, patient 3 with a less severe mutation had a delayed onset of symptoms at 4 years (median, 1 year; range, 0.6–6 years), a B-cell count of 0.8% (median, 0.04%; range, 0%–2%), and no severe infections, such as arthritis, meningitis, or gastrointestinal manifestations.



Figure 2. Different clinical manifestations in children with X-linked agammaglobulinemia in Chandigarh, India. (A) Arthritis of the right knee in patient 15. (B) Orbital cellulitis in patient 27. (C) Computed tomogram showing right maxillary and ethmoid sinusitis with orbital extension. (D) Radiograph showing consolidation in both lungs. (E) Radiograph showing arthritis of knee joint. (F) Failure to thrive and arthritis.

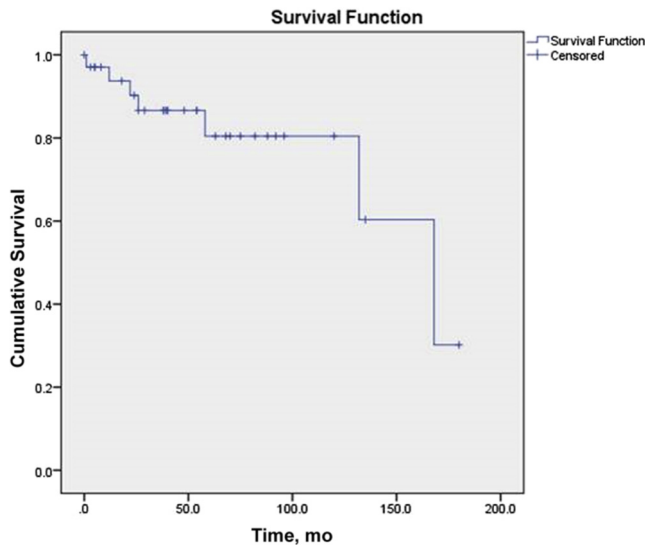


Figure 3. Kaplan-Meier survival plot in children with X-linked agammaglobulinemia.

Mortality and Outcome

Seven of the 36 patients (19%) had died at the time of analysis in our cohort of patients with XLA. Meningitis or meningoencephalitis was the presenting or predominant clinical manifestation in 4 of these 7 patients (57%). Mean survival in our cohort of children diagnosed as having XLA was 137 months (95% CI, 13–163 months) (Fig 3).

Discussion

XLA is an uncommon genetic defect; therefore, most of the reports published in the early years after its description included clinical and laboratory details of few patients. However, several large series have been reported from United States, United Kingdom, and Europe after the wider recognition of this disorder.^{5–9,22,24,25} Large case series have also been reported from the Asian subcontinent from China, Hong Kong, and Iran.^{10–12} These case series have helped us in gaining wider insight into the clinical and laboratory features and the outcome of this disorder.

There are no large clinical series of XLA published from the Indian subcontinent. This study describes the single largest cohort of patients with XLA from any developing country. Clinical and immunologic features and molecular defects in 36 patients with XLA have been detailed. Mutations in the *BTK* gene were detected in 28 of 36 patients (78%) in this study, validating the diagnosis of XLA in most of the patients.

Mean age at diagnosis in the current study was 5.9 years, which is higher than previously reported series from Europe, the United States, and the United Kingdom^{5,7,24} but similar to the series reported from Iran and lower than the series reported from China.^{10,12} A higher mean age at diagnosis in our series could be attributed to a lack of awareness about PIDs in general among practicing pediatricians in the country.

There was also a considerable delay in diagnosis, with a mean delay of 4.2 years and a maximum delay of 14 years. Patient 28 was diagnosed as having XLA at the age of 18 years, although he had an onset of illness at 4 years of age. A diagnosis of XLA was made in this patient after the diagnosis of XLA in his nephew at the age of 1½ years of age.

Respiratory tract infections were the most common presentation in our cohort, present in 86% of the patients, which is similar to the other series that have been previously reported. Respiratory tract infections were also the major cause of morbidity in this cohort and continued to occur intermittently in even those patients who were receiving intravenous immunoglobulin (IVIG) replacement therapy. Otitis media was the next common manifestation, present in half of the patients in this cohort. This is similar to large series and cohorts reported earlier (Table 3).

Joint involvement in the form of arthritis was another common manifestation in the present series, present in 42% of patients, which is significantly higher than most large series reported from the United States, the United Kingdom, and Italy (Fig 2A, 2E, and 2F).^{2,5,7,25} However, this higher predilection to arthritis is similar albeit still higher than cohorts and series reported from other Asian countries, such as China and Korea.^{10,11} The higher occurrence of arthritis in the present cohort could be related to a lower IgG level, even in patients undergoing IVIG replacement therapy. Arthritis as a manifestation of hypogammaglobulinemia has been reported earlier from North India.²³ It has suggested that the occurrence and recurrence of arthritis in XLA can be significantly decreased by maintaining trough IgG levels at higher than 500 mg/dL. Neutropenia was an uncommon clinical manifestation in our cohort, being present in only four patients (11%). This is lower than series of XLA reported from Iran, Japan, and USA.^{26–28}

Meningitis or meningoencephalitis was noted in 25% of patients in our cohort at the time of presentation or during the disease, which is similar to what has been reported in series reported earlier. It was invariably associated with a poor outcome and mortality.

IgA and IgM levels were normal in 6 and 4 patients, respectively. However, the *BTK* gene mutation was detected in 5 of 6 patients with normal IgA. Similarly, the *BTK* gene mutation was found in 2 of 4 patients with normal IgM levels. Patient with normal levels of IgM and IgA have been described in previously reported cohorts. It is also well described in literature that leaky phenotypes may not have classic features of the disease.

Table 3

Comparison of Types of Infections, Diagnostic Delay, and Mortality in the Cohort With Already Published Reports on X-linked Agammaglobulinemia

Variable	Present study (n = 36)	Plebani et al, ⁷ 2002 (Italian cohort; n = 73)	Aghamohammadi et al, ¹² 2006 (Iranian cohort; n = 37)	Winkelstein et al, ⁵ 2006 (US cohort; n = 201)	Basile et al, ⁹ 2009 (Argentinian cohort; n = 52)	Lee et al, ¹⁰ 2010 (Mainland China and Hong Kong; n = 62)
Pneumonia and respiratory tract infections, %	86	68.5	54	62	69	85.5
Otitis media, %	50	68.5	54	70	57	37
Recurrent diarrhea, %	44	13	49	23	27	29
Arthritis, %	42	10	16	7	8	29
Skin infections, %	25	27	16	18	31	25.8
Meningitis or encephalitis, %	25	4	22	12	16	12.9
Diagnostic delay, mean (SD) [range], y	4.2 (3.5) [0.16–14]	–	2 [0.08–13]	–	4.5 [0.7–17.5]	–
Mortality	19%	1.4%	14%	8.5%	6.1%	–

Mutations were detected in 28 of 36 patients in our cohort. Point mutations were the most common mutations detected and involved all the exons and domains. Although mutations were scattered throughout the *BTK* gene, the TK or SH1 domain was most commonly involved (13 [46%]) followed by the PH domain (9 [32%]), SH2 domain (3 [11%]), SH3 domain (2 [7%]), and 1 mutation was detected in the TH domain, which is similar to what has been reported earlier.^{24,25,29–31}

Missense mutations were the most frequent point mutations in our cohort, being found in 8 patients. The predicted effect of these 8 missense mutations was deduced using 4 different web-based computational tools. There was a fair degree of congruence in the predicted effects using the 4 different tools.

Four novel mutations were found in our cohort (Table 4). Three of these 4 novel mutations were point mutations, and 1 was deletion that resulted in a frameshift mutation. Other mutations are recurrent mutations, which have been reported previously in different series. Mutations often affect critical residues in the cytoplasmic *BTK* protein and are highly variable and uniformly dispersed throughout the *BTK* gene. Approximately, one-third of point mutations affect CGG sites, which usually code for arginine residues.

The high mortality rate of 20% and the mean survival of 137 months in our cohort of patients with XLA are much worse than reported from series reported from recent surveys from developed countries^{7,32} but similar to earlier series on XLA.^{8,22} A recent study from Iran has reported slightly higher mortality of 25.6% in their series of 41 patients with XLA.³³ This seemingly high mortality and poor outcome need to be understood in the context of the constraints of finances and resources in a developing country such as India. However, it is also encouraging to report that 2 of the patients in the present cohort have entered their third decade and are young adults. They are perhaps the longest survivors with XLA in our country.

There is no provision for the administration of IVIG to these patients either directly by the government or through any of its agencies. Most of the patients belong to lower- or middle-income groups with no health insurance. Provision for monthly doses of IVIG imposes a great financial burden on the parents and families of these children. It is an incessant struggle for the parents of these children and us to arrange finances for a monthly administration of IVIGs. Hence, most of the patients in our cohort receive less than the recommended doses of IVIG and often miss regular doses, resulting in low trough levels of IgG and breakthrough infections.

Table 4
Mutations in the *BTK* Gene in Children With X-linked Agammaglobulinemia in the Present Cohort^a

Patient no.	Exon/Intron	Domain	Nucleotide change	Codon change	Type of mutation	Predicted severity
1	Exon 10	SH2	842G>A	W281X	Point mutation (nonsense mutation)	Severe
2	Intron 5	PH	IVS5-1G>C c.392-1G>C		Point mutation (intronic/splice site mutation)	Severe
3	Exon 16	TK	1771C>A	N526K	Point mutation (missense mutation)	Less severe
4	Intron 8~9	SH3	Deletion		Deletion	Severe
5	Intron 17	TK	c.1750+2, T>C		Point mutation (intronic/splice site mutation)	Severe
6	Exon 1-3	PH	Deletion		Deletion	Severe
7	Exon 2	PH	83G>A	R28H	Point mutation (missense mutation)	Severe
8	Intron 18	TK	IVS18-8 G>A	p.53GlyfsX5	Point mutation (intronic/splice site)	Less severe
9	Intron 18	TK	IVS18-8 G>A	p.53GlyfsX5	Point mutation (intronic/splice site)	Less severe
10	Exon 15	TK	1514 T>A	V505D	Point mutation (missense mutation)	Severe
11	Exon 12	SH2	1064T>A	I355N	Point mutation (missense mutation)	Less severe
12	Exon 19	TK	1921C>T	R641C	Point mutation (missense mutation)	Severe
13	Exon 2	PH	126T>A	Y42X	Point mutation (nonsense mutation)	Severe
14	Exon 3	PH	158-159 insG	G53fsX5	Frameshift mutation (Insertion)	Severe
15	Exon 3	PH	158-159 insG	G53fsX5	Frameshift mutation (insertion)	Severe
16	Exon 2-3	PH	Deletion		Deletion	Severe
17	Exon 16	TK	1594C>T	Gln532>X	Point mutation (nonsense mutation)	Severe
18	Exon 12	SH2	1002T>G	Tyr(Y)334X	Point mutation (nonsense mutation)	Severe
19	Intron 1	PH	IVS1+1 G>A		Point mutation (splice-site mutation)	Severe
20	Exon 15	TK	1589T>C	L486P	Point mutation (missense mutation)	Less severe
21	Exon 17	TK	1828C>T	P566S	Point mutation (missense mutation)	Less severe
22	Exon 2	PH	169C>T	R13X	Point mutation (nonsense mutation)	Severe
23	Exon 18	TK	2013_2014delTA	Y627fsX635	Frameshift mutation (deletion)	Severe
24	Exon8	SH3	895C>T	R255X	Point mutation (nonsense mutation)	Severe
25	Exon 6	TH	c.461G>A	C154Y	Point mutation (missense mutation)	Less severe
26	Exon 17	TK	1863delA	C577fsX586	Frameshift mutation deletion	Severe
27	Exon 16	TK	1713_1716delTTTG	C527fsX528	Frameshift mutation (deletion)	Severe
28	Exon 16	TK	1713_1716delTTTG	C527fsX528	Frameshift mutation (deletion)	Severe

^aMutations highlighted in bold represent novel mutations.

However, there seems to be a ray of hope for these patients because Dr Sudhir Gupta, founder of the Foundation of Primary Immunodeficiencies, has dedicated himself to the cause of primary immunodeficiencies in India. It is also heartening to note that several local charitable organizations have also joined forces to help these children. They are providing financial assistance for the provision of IVIGs to some of the patients. Some of the state governments in the country have also started making provisions for providing IVIGs to these children under different health programs and schemes.

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