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Clinical characteristics and genetic profiles of 174 patients with X-linked agammaglobulinemia

Report from Shanghai, China (2000–2015)

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Abstract

X-linked agammaglobulinemia (XLA) is a humoral primary immunodeficiency. XLA patients typically present with very low numbers of peripheral B cells and a profound deficiency of all immunoglobulin isotypes. Most XLA patients carry mutations in Bruton tyrosine kinase (*BTK*) gene.

The genetic background and clinical features of 174 Chinese patients with XLA were investigated. The relationship between specific *BTK* gene mutations and severity of clinical manifestations was also examined. Mutations were graded from mild to severe based on structural and functional prediction through bioinformatics analysis.

One hundred twenty-seven mutations were identified in 142 patients from 124 families, including 45 novel mutations and 82 recurrent mutations that were distributed over the entire *BTK* gene sequence. Variation in phenotypes was observed, and there was a tendency of association between genotype and age of disease onset.

This report constitutes the largest group of patients with BTK mutations in China. A genotype–phenotype correlation was observed in this study. Early diagnosis of congenital agammaglobulinemia should be based on clinical symptoms, family history, and molecular analysis of the *BTK* gene.

Abbreviations: BTK = Bruton tyrosine kinase gene, ESID = European Society for Immunodeficiencies for primary immunodeficiency diseases, JIA = juvenile idiopathic arthritis, PAGID = Pan-American Group for Immunodeficiency, PCR = polymerase chain reaction, PH = Pleckstrin homology, PID = primary immunodeficiency, PolyPhen-2 = Polymorphism Phenotyping v2, SH1 = catalytic kinase, SH3 = Src homology, SIFT = Sorting Intolerant From Tolerant, TH = Tec homology, XLA = X-linked agammaglobulinemia.

Keywords: Bruton tyrosine kinase, Chinese, humoral immunodeficiency, X-linked agammaglobulinemia

1. Introduction

X-linked agammaglobulinemia (XLA, MIM# 300755) is a primary immunodeficiency due to human B-lymphocyte development disorder, thus resulting in antibody deficiency and recurrent bacterial infection.[1] The abnormal gene in XLA maps to q22 on the long arm of the X chromosome and encodes the B-cell protein tyrosine kinase BTK (Bruton tyrosine kinase).[2] BTK is composed of 5 distinct structural domains, namely, Pleckstrin homology (PH), Tec homology (TH), Src homology (SH3), SH2, and catalytic kinase (SH1) domains.[3–7] The human *BTK* gene is located in the Xq22 region of the X chromosome. It encompass 37.5 kb including 19 exons. Eighteen of them encode protein. A cluster of transcriptional start sites has been identified upstream of exon 1. Both in vivo and in vitro studies have proved that BTK protein is essential for the survival, cell cycle progression, and proliferation of B cells in response to surface Ag receptor stimulation.[8] The defective BTK in XLA impairs early B-cell development, resulting in a marked reduction of mature B cells in peripheral blood. Reports from different countries and ethnic groups have demonstrated that approximately 90% of males with presumed XLA have mutations in *BTK*. Online BTK database contain 1155 entries compiled from 974 unrelated families with 602 unique molecular events, indicating majority of *BTK* mutations being random. Results from several large cohort studies added valuable knowledge to our understanding of the spectrum of clinical features of XLA.[9–13] Two recent reports suggest the presence of genotype–phenotype correlations[14,15]; this is in contrast to earlier

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publications.[16] The genetic and epidemiological characteristics of XLA remain largely unexplored in the mainland of China. The current study provides clinical presentation and *BTK* mutation profile of 174 Chinese XLA patients.

2. Materials and methods

2.1. Patients

One hundred seventy-four patients were included for this retrospective analysis. They were evaluated in the immunodeficiency clinic at the Shanghai Jiao Tong University School of Medicine from 2000 to 2015. The initial diagnosis of XLA of majority of patients was made in our clinic. A few patients were referred for genetic counseling and molecular diagnostic analysis after clinical diagnosis made in other areas of China. The age of diagnosis is defined as the age at first clinical visit. The diagnosis of XLA was made based on the criteria of Pan-American Group for Immunodeficiency (PAGID, 1999) and European Society for Immunodeficiencies for primary immunodeficiency diseases (ESID).[17]

2.1.1. Definitive diagnosis. Male patient with less than 2% CD19 B cells and at least one of the following:

1. Mutation in Btk.
2. Absent Btk mRNA on Northern blot analysis of neutrophils or monocytes.
3. Absent Btk protein in monocytes or platelets.
4. Maternal cousins, uncles, or nephews with less than 2% CD19 B cells.

2.1.2. Probable diagnosis. Male patient with less than 2% CD19 B cells with all of the following:

1. Onset of recurrent bacterial infections in the first 5 years of life.
2. Serum IgG, IgM, and IgA more than 2 SD below normal range for age.
3. Absent isohemagglutinins and/or poor response to vaccines.
4. Other causes of hypogammaglobulinemia have been excluded.

2.1.3. Possible diagnosis. Male patient with less than 2% CD191 B cells in whom other causes of hypogammaglobulinemia have been excluded and with at least one of the following:

1. Onset of recurrent bacterial infections in the first 5 years of life.
2. Serum IgG, IgM, and IgA more than 2 SD below normal range for age.
3. Absent isohemagglutinins.

The informed consent for genetic testing was obtained from parents. This study was approved by Shanghai Children's Medical Center Investigation Committee.

2.2. BTK mutation detection

2.2.1. BTK gene analysis. Genomic DNA of study patients was extracted from blood leukocytes according to standard protocols. The *BTK* gene was amplified from cDNA by using a set of specific primers with a single annealing temperature and the same conditions for all the segments as previously described.[18,19] Mutations were detected by sequencing from the opposite direction with the National Center for Biotechnology Information program Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/).

2.3. Genotype–phenotype correlation

Mutations were classified into "severe" or "less severe" as previously described.[14,15,20] Frameshift and nonsense mutations leading to protein truncation were considered as severe mutations. We used Polymorphism Phenotyping v2 (PolyPhen-2),[21] Sorting Intolerant From Tolerant (SIFT),[22] and MutationTaster[23] to predict the severity of missense mutation, whether an amino acid substitution affects protein function. Disease severity was gauged by the types of infections before the diagnosis.

2.4. Statistical analysis

The demographics, immunological data, and clinical characteristics were depicted by descriptive statistics. Chi-square tests were performed to test for significant differences between genotypes and phenotypes. Student unpaired *t* test was used to compare other variables between groups. SPSS 17.0 statistical software was used to perform analyses.

3. Results

3.1. Demographic data

The 174 XLA patients were from 22 provinces and municipalities throughout China. Among them, 110 patients (63.22%) came from 6 provinces and 1 municipality in East China, whereas the other 64 patients (36.78%) were from other 15 provinces. One hundred forty patients (80.46%) were diagnosed from 2007 to 2015, whereas only 20 patients (11.49%) were diagnosed from 2001 to 2007 (Table 1).

The 174 patients included 127 cases with definite diagnosis, 31 cases with probable diagnosis, 12 cases with possible diagnosis, and 3 cases with clinical suspicion.

The age of onset of those patients was 2.15 ± 2.16 years with median of 1. One hundred thirty-five patients (77.59%) had onset before 3 years of age. Only 1 patient manifested symptoms at 13 years of age.

The average age of diagnosis was 7.09 ± 3.98 years, with range from 0.17 to 19. Eighty-seven patients (50.58%) were diagnosed during the first 6 years. However, only in 5 patients (2.91%), the diagnosis was made before 1 year of age.

Family histories were available from 170 of the 174 patients. Fifty-nine patients (34.71%) had a positive family history (i.e., early male abortion within 3 generations in the family), whereas the other 111 patients (65.29%) had no family history of immunodeficiency. Patients 38, 39, and 40 were cousins. Patients 169 and 170 were twins (Fig. 1).

3.2. The level of serum immunoglobulin and the percentage of CD19+ B Cells

Peripheral CD19 + cells were tested in 168 of 174 patients at the time of diagnosis. The percentage of CD19+B cells was less than 2% in 165 patients, 2% in 1 patient (P1), and 3% in another (P7). Serum IgG levels were tested in 171 patients with IgG <2 g/L in 142 patients. Among the 29 patients with IgG >2 g/L, 11 received IVIG 1 month before the testing was performed. However, CD19 +B cells were below 2% in all 29 patients.

Serum IgA was measured in 168 of 174 XLA patients, and IgM was measured in 169. Compared with the healthy counterparts,[24] 166 patients had IgA level below low limit of normal, ranging from 0.00 to 0.76 g/L. Meanwhile, 166 (98.22%) patients showed IgM level from 0.01 to 0.71 g/L.

Table 1

Characteristics features of 174 XLA patients.

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
1	+	0.75	8	12	2	1			Pneumonia	Guillian–Barre syndrome, head injury
2	+	0.83	3.5	6	0.2	1.5	0.1	0.2	pneumonia, otitis media, persisting diarrhea, skin infection	Arthritis
3	–	3	4	7	0.28	0	0.03	0.01	Purulent arthritis, persisting diarrhea	
4	–	3	17	25	0.1	2.67*	<0.1	<0.1	Pneumonia, pulmonary abscess, persisting upper respiratory tract infection	
5	–	0.5	9	13	0.1	0.39	0.07	0.05	Purulent meningitis, pneumonia, otitis media, persisting Diarrhea (protozoa infection), skin infection, tuberculous meningitis, measles, chicken pox	Subdural hemorrhage
6	–	0.08	2	7	0.06	4.34*	0.25	0.21	Pneumonia, otitis media	Arthritis, hypoferric anemia
7	–	0.25	1.5	6	2.07	0.33	0.06	0.45	Pneumonia, persisting upper respiratory tract infection, persisting diarrhea, shigellosis	
8	–	0.5	11	16	0.03	0.33	0.25	0.18	Pneumonia, persisting upper respiratory tract infection, otitis media	
9	–	1	8	10	0	0.33	0.67	1.02	Sepsis, pneumonia, persisting upper respiratory tract infection, persisting diarrhea, peritonitis	Thrombocytopenia
10	–	8	13	15	0.02	0.43	0.07	0.35	Pneumonia, persisting upper respiratory tract infection	Bronchiectasia
11	–	0.42	10	14	0.1	1.81	0.17	0.24	Pneumonia, otitis media	Arthritis, epidural hematoma, henia
12	–	0.08	10	17	1	1.49	0.22	0.17	Pneumonia/bronchitis	atelectasis, bronchiectasia
13	–	7	7	11	0.2	0.08	0.28	0.17	Pneumonia, persisting diarrhea	Arthritis
14	–	1	3.5	10	0	0.16	0.03	0.08	Pneumonia, persisting diarrhea, skin infection	
15	–	3	8.5	11	0.51	0.33	0.07	0.14	Pneumonia/bronchitis, conjunctivitis	Arthritis, thrombocytopenia
16	–	8	8	12	0.4	0.96	0.28	0.17	Purulent meningitis, pneumonia	
17	+	0.33	10	18	0	1.94	0.67	0.18	Suppurative pericarditis, pneumonia/bronchitis, persisting upper respiratory tract infection, otitis media	
18	–	0.5	10	12	0.1	0.07	0.25	0.18	Pneumonia, otitis media	Arthritis, neonatal hemolytic disease, cerebral hemorrhage
19	–	1	4	8	0.05	1.64	0.28	0.17	Pneumonia, persisting upper respiratory tract infection	Arthritis
20	–	2	6	10	0.13	0.31	0.28	0.18	Pneumonia, chicken pox	
21	+	1	8	13	0	0.08	0.28	0.17	Persisting upper respiratory tract infection, otitis media, impetigo, parotiditis, herpes zoster	Growth hormone deficiency
22	–	0.5	4	7	1.22	0	0.01	0.02	Persisting diarrhea, soft tissue infection	
23	–	1	3	22	0.16				pneumonia	Arthritis
24	+	7	11	14	0.52	1.46	0.22	0.16	Purulent meningitis, pneumonia, otitis media, nasosinusitis, tuberculosis	Arthritis
25	+	2	3	7	0.14	1.18	0.28	0.17	Pneumonia, persisting diarrhea	
26	–	2	8	14	0.1	1.46	0.03	0.57	Persisting upper respiratory tract infection, otitis media	Arthritis
27	–	1.5	6.5	12	0.41	1.99	0.25	0.18		Skin polyarteritis nodosa, hernia

(*continued*)

Table 1
(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
									Virus meningitis, persisting upper respiratory tract infection, EBV infection hand-foot-and-mouth disease conjunctivitis	
28	–	1	15	22	0.13	1.41	0.22	0.24	Purulent meningitis, pneumonia, persisting upper respiratory tract infection, otitis media	
29	–	0.5	3	5	0.2	0.43	0.22	0.3	Purulent meningitis, pneumonia, otitis media, persisting diarrhea	
30	–	4	4	10	0.05	11*	0.25	0.43	Pneumonia, persisting diarrhea	
31	–	1	12	17	0.13	2.17	0.28	0.17	Pneumonia, measles	
32	–	0.16	11	14	0.2	0.1	0.01	0.18	Pneumonia, persisting upper respiratory tract infection, otitis media, nasosinusitis, chicken pox	
33	+	4	6	10	0	0.81	0.07	0.1	pneumonia, otitis media	
34	–	2	2	7	0.1	0.39	0.28	0.21	Bronchitis, otitis media	Neutropenia
35	+	0.5	2	6	0.24	0.08	0.28	0.17	Pneumonia, otitis media, persisting diarrhea, chicken pox	
36	–	1	2	6	0	0.45	0.25	0.2	Purulent meningitis	ASD, cholecystolithiasis
37	–	3	13	22	0.1	0.74	0.07	0.16	Suppurative arthritis, bronchitis, nasosinusitis, persisting diarrhea, suppurative adenomesenteritis peritonitis	bronchiectasia
38	+	0.83	0.8	3	0.17	0.52	<0.25	0.19		
39	+	1	9	13	0.03				Sepsis, pneumonia, otitis media, nasosinusitis persisting diarrhea	Arthritis, epilepsy (38 39 40)
40	+	4	8	12	0.06	0.35	0.07	0.06	Pneumonia, persisting upper respiratory tract infection, otitis media, nasosinusitis	
41	–	0.25	6	8	0.17	1.43	0.25	0.18	Pneumonia, persisting upper respiratory tract infection	
42	–	0.25	19	29	0.59	0.33	0.07	0.08	Persisting upper respiratory tract infection, persisting diarrhea (protozoa infection), chronic colitis chronic pancreatitis	
43	–	6.5	7	15	0.2	1.41	0.22	0.18	Pneumonia	
44	–	5	11	18	1.52	0.23	1.41	0.18	Pneumonia, persisting upper respiratory tract infection, pleural effusion	
45	+	5	10	12	0.81	1.43	0.25	0.18	Pneumonia, persisting diarrhea, chronic colitis	Arthritis
46	–	2	9	12	0.4	0.52	<0.17	<0.28	Pneumonia, otitis media, nasosinusitis, measles	Lymphadenectasis
47	–	7	16	19	0.52	6.99*	0.54	0.71	Pneumonia, persisting upper respiratory tract infection, persisting diarrhea, skin abscess toxic bacillary dysentery	Thrombopenia, erythronoclastic anemia, granulopenia
48	–	1.8	1.5	2	1	<1.43	0.25	0.24	Purulent arthritis	
49	+	4.5	5	6		0.43	<0.25	0.5	Persisting upper respiratory tract infection, otitis media, skin infection	Arthritis
50	–	1	9	11	0.6	0.1	0	0	Purulent meningitis, persisting upper respiratory tract infection, otitis media	

(*continued*)

Table 1

(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
51	+	4	12	12	0.14	<0.33	<0.0667	<0.0417	Purulent meningitis, pneumonia, otitis media, nasosinusitis, purulent lymphadenitis	Arthritis
52	+	2.5	13	16	0.1	0.333	<0.0667	0.05	Purulent meningitis, pneumonia, otitis media, hand-foot-and-mouth disease	
53	+	1	15	15	0.88	0.68	0.06	1.09	Pneumonia, otitis media, pleural effusion	
54	–	1	6	8	0	1	0.11	0.71	Pneumonia, otitis media, dental ulcer	
55	+	0.25	7	8	3	<0.33	<0.07	0.07	Purulent meningitis, pneumonia, otitis media, measles	Arthritis
56	–	3	3	4	0.01	<0.07	<0.259	<0.034	Purulent meningitis, sepsis, pneumonia, Abscess of gluteal region	
57	–	3	11	22	0	0.07	0	0.13	Sepsis, sacroiliac abscess, pneumonia, persisting upper Respiratory tract infection	
58	–	1	8	21	0.2	1.95	0.45	0.16	Pneumonia, persisting upper respiratory tract infection	
59	–	0.66	8	20	0.68	0.7	0.02	0.68	Pneumonia, persisting upper respiratory tract infection, Otitis media	
60	+	0.83	8	19	0.8	0.45	0.07	0	Suppurative pericarditis, pneumonia, persisting upper respiratory tract infection	
61	–	10	12	12	0.3	2.68	1.56	0.26	Pneumonia, pleural effusion	
62	+	0.58	2	2	1.5	0.34	<0.2	<0.17	Infectious purpura fulminans, pneumonia, persisting upper respiratory tract infection, otitis media	Thrombopenia
63	+	1.5	11	12	0	0.02	0.03	0.18	Purulent pleurisy	Guillian–Barre syndrome, pleural thickening, chest collapse
64	–	0.5	4	4	0.1	<0.33	<0.0667	0.16	Pneumonia, persisting upper respiratory tract infection, otitis media	Persisting arthritis, henia
65	+	3	5	5	1	1.72	<0.2	0.31	Purulent meningitis, pneumonia, persisting upper respiratory tract infection, purulent pleurisy	Atelectasis
66	+	1	11	15	0.7	1.29	<0.0667	<0.0417	Persisting upper respiratory tract infection, pneumonia, otitis media, osteomyelitis, persisting diarrhea	
67	+	2	6	9	0	0.333	0.07	0.08	Conjunctivitis, pneumonia, hand-foot-and-mouth disease , chicken pox	
68	+	3	8	11	1	<1.36	<0.243	<0.173	Pneumonia/bronchitis, persisting upper respiratory tract infection, otitis media, nasosinusitis urinary system infection, measles	JIA
69	+	4	5	10	0	0.07	<0.24	<0.18	Pneumonia, persisting upper respiratory tract infection, pleural effusion , otitis media, sepsis	
70	+	0.08	6	8	0	0.66	0.24	<0.20	Purulent skin infection , pneumonia, persisting upper respiratory tract infection, otitis media lymphadenitis, persisting diarrhea	
71	–	0.5	11	17	0.12	2.37*	0.45	0.35		Colitis gravis

(*continued*)

Table 1

(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
									Persisting upper respiratory tract infection, pneumonia, otitis media, encephalitis, chronic nasosinusitis chronic nasosinusitis	
72	+	6	10	16	0.16	0.54	0.24	0.16	Pneumonia, pleurisy, henia	
73	–	0.25	4	12	0.16	2.33*	0.02	0.33	Persisting upper respiratory tract infection, pneumonia, skin infection	Neutrophil deficiency
74	+	7	9	10	0.11	4.92		0.38	Persisting diarrhea, pneumonia/bronchitis, pleurisy, pleural effusion	Megaloblastic anemia , cacotrophy, skin sensibility
75	–	3	10	11	0	0.621	<0.063	<0.052	Persisting diarrhea, persisting upper respiratory tract infection, pneumonia/bronchitis, measles pharyngo-conjuctival fever, pleural effusion , parotiditis, encephalitis	Urticaria, granulopenia
76	–	3	14	15	0.28	2.4	0.24	0.47	Pneumonia, pleural effusion , nasosinusitis	
77	+	0.33	2	3	0	0.49	0.1	0.12	otitis media, severe pneumonia, persisting upper respiratory tract infection	
78	–	0.63	4	6	1	1.88	0.09	<0.0417	Persisting upper respiratory tract infection, persisting diarrhea, pneumonia	Dislocation of the hip joint, epilepsy
79	–	6	8	10	0.1	1.6	0.53	0.56	Otitis media, persisting upper respiratory tract infection, hand-foot-and-mouth disease , encephalitis	Cartilage injury
80	+	0.5	15	17		0.07	<0.024	<0.18	Otitis media, nasosinusitis, persisting upper respiratory tract infection, pneumonia, urinary-tract infection	
81	–	2	3	5	1	<1.36	<0.24	<0.17	eczema, pneumonia, rhinitis	Hernia
82	–	1	10	12		0.86	0.09	0.18	Persisting upper respiratory tract infection, pneumonia, eczema	
83	–	2	11	13	0.1	<0.33	<0.0667	<0.0520	Meningitis, sepsis, persisting upper respiratory tract infection, pneumonia, otitis media	
84	+	0.75	14	15	0	<0.33	<0.0667	<0.114	Perianal abscess, acute appendicitis, nasosinusitis, pneumonia/bronchitis persisting upper respiratory tract infection	Congenital laryngeal stridor
85	+	0.42	4	6	1	<0.33	<0.0667	<0.0417	Persisting diarrhea, pneumonia, hand-foot-and-mouth disease , persisting upper respiratory tract infection pleural Effussion , sepsis	Agranulocytopenia
86	+	0.58	8	10	0	0.11	<0.243	<0.160	Otitis media, persisting upper respiratory tract infection, appendicitis, pneumonia, synovitis	
87	+	5	9	11	0.01	2.44*	<0.224	<0.179	Pneumonia, persisting upper respiratory tract infection, otitis media	
88	+	2.5	4	6	0	0.16	0.01	0.06	Hand-foot-and-mouth disease , encephalitis, purulent gonarthritis, eyelid abscess	
89	–	2	2	4	1	0.33	0.07	0.11	Measles, pneumonia, cellulitis, laryngitis, persisting diarrhea, hand-foot-and-mouth	

(*continued*)

Table 1
(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
									disease encephalitis, persisting upper respiratory tract infection	
90	–	3	8	13	0	<0.33	<0.0667	<0.0417	Otitis media, pneumonia, persisting upper respiratory tract infection	
91	+	3	5	6	0	1	0.11	0.19	Pneumonia, persisting upper respiratory tract infection	
92	–	0.25	11	12	0	0.24	0.1	0.01	Persisting upper respiratory tract infection, lymphadenitis, otitis media	JIA
93	+	2	3	4	0.2	3.4*	0.01	0.04	Persisting upper respiratory tract infection, pneumonia	
94	+	0.5	4	5	0.13	0.15	0.04	0.08	Pneumonia, otitis media, dental ulcer	
95	–	4	5	6	0	0.256	0	0.04	Persisting upper respiratory tract infection, pneumonia	
96	–	2	3	6	0	19.4	0.46	0.32	Pneumonia, persisting upper respiratory tract infection	Secondary epilepsy, hydrocephalus
97	+	0.33	3	4	0.06	<0.07	<0.06	<0.17	Persisting upper respiratory tract infection, pneumonia, otitis media	
98	+	3	6	7	0.1	1.81	<0.06	0.28	Persisting upper respiratory tract infection, otitis media, purulent meningitis	
99	+	0.08	4	5	0.13	0.96	<0.06	0.04	Persisting upper respiratory tract infection, pneumonia	
100	–	1	3	4	0	3.6	<0.06	0.19	Persisting upper respiratory tract infection, otitis media, pneumonia	
101	+	0.67	2	3	0	0.21	0.05	0.09	Persisting upper respiratory tract infection, sepsis	
102	+	0.25	17	18	0.06	0.4	0.02	0.08	Persisting upper respiratory tract infection, amygdalitis, pneumonia, otitis media	
103	–	0.58	6	6	0	1.44	<0.06	0.16	Pneumonia, otitis media, persisting upper respiratory tract infection	
104	–	0.67	0.8	1	0.9	3.14*	<0.06	<0.17	Pneumonia	
105	–	1	1	1	0	0.32	<0.06	0.07	Liver function damage, pneumonia, Gastrointestinal hemorrhage	Cytopenia of 2 series
106	–	1	18	18	0.08	<0.33	<0.77	0.22	Pneumonia, nasosinusitis, parotiditis, persisting upper respiratory tract infection, persisting diarrhea	Arthritis
107	–	0.75	6	6	0.06	0.59	<0.06	<0.17	Persisting upper respiratory tract infection, pneumonia, nasosinusitis, chicken pox, otitis media	
108	+	0.5	14	14	0	0.61	0.22	0.24	Persisting upper respiratory tract infection, pneumonia, otitis media, bronchiectasia	Arthritis
109	–	5	7	7	0.08	0.73	<0.26	<0.17	Pneumonia, persisting upper respiratory tract infection, persisting diarrhea	
110	–	0.92	4	5	0	0.03	0.02	0.01		Gonarthritis

(*continued*)

Table 1
(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
									Persisting upper respiratory tract infection, meningitis, otitis media, nasosinusitis, bronchitis	
111	–	2	5	5	0	0.06	0.24	0.17	Synovitis, myringitis, persisting upper respiratory tract infection	Cheirarthritis
112	–	2	10	10	0.06	<1.34	<0.23	<0.169	Pneumonia, sepsis, purulent meningitis	
113	–	0.92	1.2	1	0.2	0.07	<0.25	<0.17	Persisting upper respiratory tract infection	Arthritis
114	+	0.58	5	5	0	0.31	0.01	0.14	Persisting upper respiratory tract infection, persisting skin infection , pneumonia/bronchitis parotiditis, bone tuberculosis	
115	–	1.75	3	3	0.5	0.07	<0.25	0.17	Pneumonia, dermatomyositis, persisting upper respiratory tract infection	
116	–	1	9	9	0.08	2.89	0.64	<0.17	Pneumonia, meningitis, synovitis, otitis media, hand-foot-and-mouth disease , asthma persisting upper respiratory tract infection	
117	–	3	8	8	0.1	0.4	<0.06	0.18	Pneumonia, meningitis hydrocephalus	
118	–	13	13	13	0	8.12*	0.76	0.6	Persisting upper respiratory tract infection	
119	–	0.5	2	2	0.1	0.18	0.08	0.13	otitis media, pneumonia, persisting upper respiratory tract infection, severe myocarditis central nervous infection, fungal infection	Demyelinating encephalopathy, hepatic lesion
120	–	1	5	5	0.04	0.46	<0.25	<0.18	Persisting upper respiratory tract infection, empyema	
121	–	3	7	13	0.1	3.28*	<0.0667	0.3	pneumonia/bronchitis, tuberculous pleurisy, tuberculous meningitis persisting upper respiratory tract infection, pleural effusion	Hernia
122	–	2	9	9	0.14	3.86	0.64	<0.17	Persisting upper respiratory tract infection, pneumonia, bronchitis	
123	+	1	1	1	0	<0.12	<0.06	<0.27	Pneumonia	
124	–	3	3	5	1	0.33	0.07	0.06	Persisting diarrhea, sepsis, persisting upper respiratory tract infection, bronchitis	
125	–	2	8	8	0.1	<1.45	<0.25	0.2	Pneumonia, otitis media	
126	–	4	6	6	0	1.59	<0.06	<0.04	Persisting upper respiratory tract infection, otitis media, pneumonia	
127	–	0.08	0.2	1	0.35	1.4	0.26	0.24	Pneumonia, eczema, virus hepatitis, virus enteritis;, viral nephritis	
128	–	2	2	4	0.3	1.22	<0.24	<0.18	Rash, persisting diarrhea, persisting upper respiratory tract infection, ichthyosis, pneumonia	
129	–	0.92	1	2	0	12.8	<0.063	<0.042	Pneumonia, infectious dermatitis	
130	–	0.5	15	15	1	1.77	<0.06	<0.08	Persisting diarrhea, persisting upper respiratory tract infection, pneumonia	Arthritis
131	–	1	11	11	0.02	0.251	<0.245	<0.156	Pneumonia, persisting upper respiratory tract infection, nasosinusitis, otitis media	

(*continued*)

Table 1

(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
132	–	0.08	6	7	0.02	2.22	<0.245	<0.156	Pneumonia, persisting upper respiratory tract infection, meningitis, otitis media	
133	+	3	17	17	0.04	4.67	<0.245	<0.382	Persisting upper respiratory tract infection, otitis media, nasosinusitis	
134	+	0.5	16	16	0.01	4.7	<0.245	<0.246	Pneumonia, persisting upper respiratory tract infection, encephalitis, nasosinusitis	
135	–	7	7	9	0.04	1.78	<0.06	<0.16	Pneumonia, persisting upper respiratory tract infection, nasosinusitis, otitis media, arthritis	
136	–	4	4	4	0.04	3.17	<0.245	<0.156	Pneumonia, hand-foot-and-mouth disease , secondary purulent encephalitis, mycotic stomatitis	Cardiac damage, anemia, gastroesophageal reflux, right pelvis, cystolith
137	–	1.42	3	3	0.14	2.21	<0.245	<0.156	Sepsis, hypoferric anemia, perianal abscess	rheumatoid arthritis
138	–	0.5	0.5	2	0	0.6	0.24	0.13	Pneumonia, enteritis	ASD, cholecystolithiasis
139	–	3	3	3	0.02	<0.06	<0.06	<0.20	Pneumonia, mastoiditis	Hernia
140	–	3	3	3	1	<1	<0.06	<0.08	Severe pneumonia, necrotizing fasciitis, otitis media, sepsis, mycotic stomatitis	
141	–	1	11	12	0.2	0.65	0.2	0.08	Pneumonia	PDA, hydropericardium, short stature, cardiac damage
142	–	1	1	1	0.02	<1.37	<0.25	<0.17	Sepsis, skin infection , acute purulent otitis media, diarrhea acute upper respiratory tract infection, monocytosis	Oral ulcer, temporal arachnoid cysts, hyperpyretic convulsion,
143	–	3	3	3	0	<2.43	<0.06	0.31	Sepsis, hyperpyretic convulsion	
144	–	1	6	6	1	0.08	<0.256	0.9	Nasosinusitis, mycotic stomatitis, sepsis	Kawasaki disease
145	–	0.83	0.8	2	0.13	<1.3	<1.245	<0.156	pneumonia	
146	+	2	2	Die	0.8	<0.33	<0.0667	<0.220	Pneumonia, meningitis, measles, otitis media, respiratory tract infection	
147	–	4	4	5	0.25	2.84	<0.0667	0.13	Pneumonia, otitis media, nasosinusitis, measles	
148	–	1.16	4	4	0.2	1.23	0.03	0.11	recurrent upper respiratory tract infection	
149	–	0.5	5	5	0.05	<0.33	<0.0667	<0.0417	Pneumonia, persisting upper respiratory tract infection, purulent arthritis, measles	Injury of sciatic nerve, thrombopenia purpura
150	–	1	8	9	0	<0.42	<0.2	<0.06	Pneumonia, bronchiectasia, parotiditis	Liver function damage, Subdural hematoma
151			5	5	0.02	<0.16	<0.16	<0.16	Pneumonia	duplication of the left kidney, Guillian-Barre syndrome
152	–	4	9	10	0	1.23	<0.06	<0.17	Pneumonia	Nasosinusitis
153	–	1	4	5	0	0.33	0.07	0.77	Pneumonia, persisting upper respiratory tract infection, sepsis, otitis media	Right knee synovitis
154	–	2	5	6	0	0.01	0.01	0.09	Pneumonia, persisting upper respiratory tract infection, otitis media	
155	+	0.42	5	5	0.04	1.34	0.24	0.17	Pneumonia, persisting upper respiratory tract infection	Right limb activity disorder
156	+	1.25	8	8	0.02	4.32	<0.06	<0.16	Pneumonia, rhinitis, otitis media, purulent skin	
157	–		13	25						

(*continued*)

Table 1
(continued).

Pts	Family history	Age at onset, y	Age at diagnosis, y	Present age	B cells, %	IgG, mg/dL	IgA, mg/dL	IgM, mg/dL	Clinical presentation	
									Nasosinusitis, suppurative adenomesenteritis, peritonitis, suppurative gonarthritis chronic tracheitis, bronchiectasis	
158					0e	14.5	<0.07	0.05		
159		7	7	15	0.1	1.53	0.24	3.66	Empyema , gangrenous appendicitis	
160	+	2	9	9	0	0.426	<0.245	<0.156	Pneumonia, persisting upper respiratory tract infection, hand-foot-and-mouth disease , virus meningitis,	
161	–			8	0.34	2.86	<0.0667	0.06	Acute purulent encephalitis, brain abscess, acute alveobronchiolitis, MODS pleural effusion, acute asthmatic attack	
162	+	3	4	8					Upper respiratory tract infection, pneumonia, facial paralysis	
163			12	13	1	0.8	0	0.1	Pneumonia, pleurisy, hydropericardium, pleural effusion	
164	+	4	5	5	0	0.13	<0.26	<0.17	sepsis, pneumonia, virus perichondroma	
165	–	2	5	5	0	0.52	0.12	0.11	Pneumonia, pleural effusion , enteritis;, sepsis, skin infection , otitis media, bronchitis	
166	+	2	4	4		0.07			Upper respiratory tract infection, pneumonia, diarrhea, otitis media	
167	+	1	6	11	0.1	<0.07	<0.23	<0.17	Pneumonia, synovitis, otitis media, parotiditis, pleural effusion , arthritis hand-foot-and-mouth disease , impetigo	
168	–	5	11	11	0.1	<0.07	<0.06	<0.17	Pneumonia, recurrent upper respiratory tract infection, dental ulcer, parotiditis hand-foot-and-mouth disease , purulent arthritis of right knee	
169	+	0.75	1	3	0.2	1.92	0.07	0.15	Diarrhea, pneumonia, recurrent upper respiratory tract infection, hyperpyretic convulsion	
170	–	0.75	1	3	0.4	2.05	0.07	0.05	Diarrhea, pneumonia, recurrent upper respiratory tract infection, hyperpyretic convulsion	
171	+	6	6	8	0.04	0.18	<0.06	<0.17	Knee joint hydrarthrosis;, pneumonia, atelectasis, bronchiectasia, tuberculosis	
172	–	0.25	10	10	0	<0.07	<0.06	<0.156	Pneumonia, otitis media, acute purulent arthritis, purulent skin	
173	–	7	7	9	0.02	<1.5	<0.25	<0.16	Hip joint synovitis, otitis media, gonarthritis, congenital dislocation of hip joint	
174	+	1	4	4	0.1	0.03	0.03	0.06	Recurrent upper respiratory tract infection, recurrent pneumonia, pleural effusion pulmonary abscess, sepsis	Anemia

EBV = Epstein-Barr virus, Pts = patients, XLA = X-linked agammaglobulinemia.

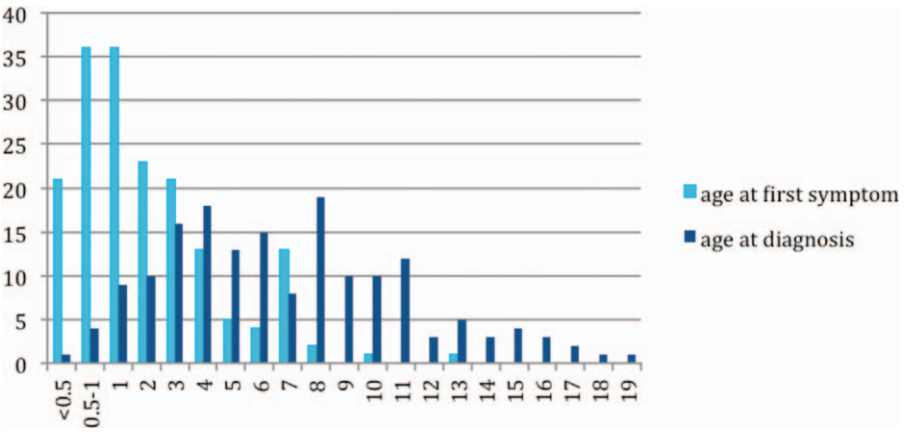

Figure 1. Distribution of the age of disease onset and diagnosis in patients with XLA.

Table 2

Btk mutation analysis in 142 children with XLA in this study.

Pts	Localization	domain	Nucleotide substitutions	Amino acid substitution	Type of mutation	Mother
1	Intron 1	PH	IVS1+1G>A	Splicing	Heterozygous	
2	Exon 2	PH	128–133delAAGCT	Deletion mutation	Heterozygous	
3	Exon 2	PH	134 T>C	M1T	Missense mutation	Heterozygous
4	Exon 2	PH	214C>T	R28C	Missense mutation	Heterozygous
5	Exon 2	PH	214C>A	R28S	Missense mutation	Heterozygous
6	Exon 2	PH	250–251delTA[a]	Y40X	Deletion mutation	NIL
7	Exon 2	PH	258delT[a]	Y42X	Deletion mutation	Heterozygous
8	Exon 2	PH	269–271delGTGinsTT[a]	R46fsX56	Indel mutation	Heterozygous
9	Exon 3	PH	305–307 delTTG	V58del	Deletion mutation	NE
10	Exon 5	PH	496A>T[a]	K122X	Nonsense mutation	NIL
11	Exon 6	TH	603–619del GACAGCCAAAAATGCTA ins ATGGTC[a]	Q157fsX189	Indel mutation	NIL
12	Exon 8	SH3	786–787delG	V219fsX228	Frameshift	NIL
13	Exon 8	SH3	887G>A	W252X	Nonsense mutation	NE
14	Intron 8	SH3	IVS8+1 G>A	splicing	Heterozygous	
15	Exon 10	SH3	994C>T	R288W	Missense mutation	Heterozygous
16	Exon 10	SH2	995G>A	R288Q	Missense mutation	NIL
17	Exon 10	SH2	989–999del TGACTCGGAGTinsGGTGGTATTCCAAA[a]	M286-S289delinsRWYSK	Indel mutation	Heterozygous
18	Exon 10	SH2	1021C>T	Q297X	Nonsense mutation	NE
19	Exon 11	SH2	1048–1049 delGT	V306fsX321	Frameshift	Heterozygous
20	Intron 11	SH2	IVS11–2A>C	Splicing	NE	
21	Exon 12	SH2	1114C>T	Q328X	Nonsense mutation	Heterozygous
22	Exon 14	TK	1366 C>T[a]	Q412X	Nonsense mutation	NE
23	Exon 14	TK	1420A>T	K430X	Nonsense mutation	heterozygous
24	Intron 14	TK	IVS14+5 G>A	Splicing	Heterozygous	
25	Intron 14	TK	IVS14+2T>C	Splicing	Heterozygous	
26	Exon 15	TK	1589T>C	L486P	Missense mutation	Heterozygous
27	Exon 15	TK	1658T>C	M509T	Missense mutation	Heterozygous
28	Exon 15	TK	1658T>C	M509T	Missense mutation	Heterozygous
29	Exon 15	TK	1690C>T	R520X	Nonsense mutation	Heterozygous
30	Exon 15	TK	1690C>T	R520X	Nonsense mutation	NIL
31	Exon 16	TK	1700C>T	A523V	Missense mutation	Heterozygous
32	Exon 16	TK	1705C>T	R525X	Nonsense mutation	Heterozygous
33	Exon 16	TK	1763G>A	R544K	Missense mutation	Heterozygous
34	Intron 16	TK	IVS16+5G>C	splicing	Heterozygous	
35	Exon 17	TK	1816C>T	R562W	Missense mutation	Heterozygous
36	Intron 17	TK	IVS17+1G>A	splicing	Heterozygous	
37	Intron 17	TK	IVS17–1G>C[a]	splicing	Heterozygous	
38	Exon 17	TK	1877C>A	A582D	Missense mutation	Heterozygous
39	Exon 17	TK	1877C>A	A582D	Missense mutation	NE
40	Exon 17	TK	1877C>A	A582D	Missense mutation	Heterozygous
41	Exon 19	TK	2054G>A	R641H	Missense mutation	Heterozygous
42			NIL			
43			NIL			
44			NIL			
45			NIL			
46			NIL			

(*continued*)

Table 2

(continued).

Pts	Localization	domain	Nucleotide substitutions	Amino acid substitution	Type of mutation	Mother
47			NIL			
48	Exon 10	SH3	1000C>T[a]	Q290X	Nonsense mutation	NE
49	Exon 2	PH	251A>G	Y40C	Missense mutation	Heterozygous
50	Exon 15	TK	1690C>T	R520X	Nonsense mutation	Heterozygous
51	Intron 15	TK	IVS15–12_9del TTTG[a]	splicing	Heterozygous	
52	Intron 9	SH3	IVS9+4_7del AGTA[a]	splicing	Heterozygous	
53	Exon 3	PH	314T>C	I61T	Missense mutation	Heterozygous
54			NIL			NE
55	Exon 15	TK	1522delT[a]	C464fsX483	Frameshift	NIL
56	Exon 14	TK	1472–1473delAA[a]	K447fsX454	Deletion mutation	NE
58	Exon 17	TK	1804A>T[a]	K558N	Missense mutation	NE
59	Exon 16	TK	1762A>T[a]	R544W	Missense mutation	NE
60	Exon 17	TK	1858 delA[a]	V585fsX595	Frameshift	NE
61	Exon 2	PH	215G>A	R28H	Missense mutation	Heterozygous
62	Exon 18	TK	2021T>A	M630K	Missense mutation	NE
63	Exon 17	TK	1764G>T	R544S	Missense mutation	Heterozygous
64	Intron 17	TK	IVS17–2A>G	splicing	NIL	
65	Exon 2	PH	242T>c	L37P	Missense mutation	Heterozygous
66	Intron 8	SH3	IVS8+5delG[a]	splicing	Heterozygous	
67	Exon 15	TK	1493A>T[a]	H454L	Missense mutation	Heterozygous
68	Exon 15	TK	1638C>A	C502X	Nonsense mutation	Heterozygous
69	Exon 11	SH2	1038–1040delAGG[a]	G303del	Deletion mutation	Heterozygous
70	Exon 17	TK	1816C>T	R562W	Missense mutation	Heterozygous
71	Exon 16	TK	1706G>A	R525Q	Missense mutation	Heterozygous
72	Exon 15	TK	1493A>G	H454R	Missense mutation	Heterozygous
73	Exon 9	SH3	949delG[a]	E273fsX276	Frameshift	NE
74	Exon 2	PH	215G>A	R28H	Missense mutation	NE
75	Exon 8	SH3	878delT[a]	L249fsX276	Frameshift	Heterozygous
76	Exon 15	TK	1487T>G[a]	L452R	Missense mutation	Heterozygous
77	Intron 17	TK	IVS17+1G>A	splicing	Heterozygous	
78	Exon 10	SH2	995G>A	R288Q	Missense mutation	NIL
79		TK	Deletion of exon 16 to 19	large deletion	NE	
80	Exon 6	TH	587–588insA[a]	Y152X	Frameshift	Heterozygous
81	Exon 8	SH3	859C>T	R255X	Nonsense mutation	Heterozygous
82			NIL			
83	Exon 19	TK	2054G>A	R641H	Missense mutation	NIL
84	Exon 15	TK	1691G>A	R520Q	Missense mutation	Heterozygous
85			NIL			
86	Exon 8	SH3	895C>T	R255X	Nonsense mutation	Heterozygous
87	Exon 15	TK	1588–1589insACC[a]	485–486insH	Frameshift	Heterozygous
88	Intron 9	SH3	IVS9+3A>T[a]	splicing	NE	
89	Exon 17	TK	1831G>C	E567Q	Missense mutation	Heterozygous
90	Exon 10	SH2	1080delA[a]	T316fsX330	Frameshift	NIL
91	Intron 17	TK	IVS17–2delA[a]	splicing	Heterozygous	
92		PH-TK	deletion of exon 2 to 16[a]	large deletion	NE	
93	Exon 2	PH	169C>T	R13X	Nonsense mutation	Heterozygous
94	Exon 14	TK	1427delT[a]	Y476fsX483	Frameshift	NE
95	Exon 17	TK	1877T>C[a]	V626A	Missense mutation	NE
96	Exon 18	TK	2016–2018delCAT[a]	T628del	Deletion mutation	NE
97	Exon 10	SH2	1004–1005insC[a]	A291fsX292	Frameshift	Heterozygous
98	Exon 19		3427bp deletion incl ex 5[a]	Indel mutation	NE	
99			NIL			
100	Exon 17	TK	1816C>T	R562W	Missense mutation	NE
101	Intron 17	TK	IVS17+1G>T	splicing	Heterozygous	
102	Exon 2	PH	164T>C	L11P	Missense mutation	Heterozygous
103	Exon 16	TK	1684C>T	R562W	Missense mutation	Heterozygous
104	Intron 11	SH2	IVS11–1G>T[a]	splicing	Heterozygous	
105	Exon 2	PH	215G>A	R28H	Missense mutation	Heterozygous
108	Exon 8	SH3	885delG	W251fsX276	Frameshift	Heterozygous
109	Exon 3	PH	424T>G	F98V	Missense mutation	Heterozygous
110	Exon 5	PH	502–503insT	W124fsX137	Frameshift	Heterozygous
111	Intron 10	SH2	1025–1026delAG	E298fsX321	Frameshift	NIL
112	Intron 10	SH2	1036G>A	G302R	Missense mutation	NE
113			NIL			
114			exon 19 deletion	Deletion mutation	NE	
115	Intron 3	PH	IVS3+1G>A	Splicing	NE	
116			exon 2–3 deletion	Deletion mutation	NE	
117	Intron 15	TK	IVS15–23A>C[a]	Splicing	NE	
118			NIL			
119			NIL			

(*continued*)

Table 2

(continued).

Pts	Localization	domain	Nucleotide substitutions	Amino acid substitution	Type of mutation	Mother
120	Exon 15	TK	1571delG	G480fsX483	Frameshift	Heterozygous
122	Exon 2	PH	215G>A	R28H	Missense mutation	Heterozygous
123	Exon 15	TK	1684C>T	R562W	Missense mutation	Heterozygous
125	Intron 11	SH2	IVS11–1G>T[a]	Splicing	Heterozygous	
127			NIL			
130	Exon 2	PH	258T>C	Y42X	Nonsense mutation	
144	Exon 15	TK	1855C>T	P619S	Missense mutation	Heterozygous
147	Exon 6	SH3	763C>T	R255X	Nonsense mutation	Heterozygous
149			NIL			
150	Exon 17	TK	1899C>T		Missense mutation	
151	Exon 2	PH	83G>T	R28L	Missense mutation	Heterozygous
152	Exon 12	TK	1387G>C	G419R	Missense mutation	Heterozygous
153	Exon 2	PH	37C>T	R13X	Nonsense mutation	NE
154	Exon 15	TK	1581–1584delTTTG	C527fsX528	Frameshift	NIL
161	Exon 15	TK	1684C>T	R562W	Missense mutation	
162	Exon 3	PH	469C>T[a]	Q157X	Nonsense mutation	
163	Exon 12	SH2	1120A>T[a]	K374X	Nonsense mutation	Heterozygous
164	Exon 8	SH3	843G>A	W281X	Nonsense mutation	Heterozygous
165	Exon 6	SH3	722_728delATTTTAT[a]	Y241SfsX34	Frameshift	NIL
166	Exon 2	PH	209delA[a]	N72IfsX49	Frameshift	NE
167	Intron 5	PH	IVS5–2A>G	Splicing	NE	
168	Intron 2	PH	IVS2+3_+6delAAGT[a]	Splicing	NE	
169	Exon 18	TK	2034G>T[a]	W634C	Missense mutation	NE
170	Exon 18	TK	2034G>T[a]	W634C	Missense mutation	NE
171	Exon 17	TK	1854delC[a]	F574fsX586	Frameshift	NE
174	Intron 11	SH2	IVS11–1G>C[a]	Splicing	NE	

BTK = Bruton tyrosine kinase, NE = not examined, NIL = no mutation detected, PH = Pleckstrin homology, Pts = patients, SH1 = Src homology 1, SH3 = Src homology 3, TH = Tec homology domain, TK = tyrosine kinase domain.

[a] Novel mutation.

3.3. Related infections and accompanied symptoms

Clinical manifestations of infection were identified in 173 patients before XLA was diagnosed, predominately respiratory tract infection, which accounted for 94.25% (164 patients) of the infections.

Lower respiratory tract infection (bronchitis/pneumonia) were found in 134 cases (77.01%), whereas otitis media was found in 70 cases (40.23%), persisting diarrhea in 33 cases (18.97%), nasosinusitis in 23cases (13.22%), and skin infections in 14 cases (8.05%).

Table 3

The frequency data of related infections and accompanied symptoms in the XLA patients.

Related infections	Incidence of disease
Lower respiratory tract infection (bronchitis/pneumonia)	77.01%
Otitis media	40.23%
Persisting diarrhea	18.97%
Nasosinusitis	13.22%
Skin infections	8.05%
Severe infection	
Central nervous system infection	19.65%
Deep-seated infections	19.8%
Sepsis	8.67%
Tuberculosis	3.47%
Noninfection diseases/accompanied symptoms	
Aseptic arthritis	18.75%
Neutropenia/thrombocytopenia	5.17%
Anemia	3.57%
Guillain–Barre syndrome	1.74%

XLA = X-linked agammaglobulinemia.

Fifty-eight patients (33.33%) suffered severe infections, such as central nervous system infection (34 cases), sepsis (15 cases), suppurative arthritis (6 cases), suppurative pericarditis (2 cases), bone tuberculosis (1 case), osteomyelitis (1 case), and purpura fulminans (1 case).

Upper respiratory tract infection and otitis media were the most common symptoms in this patient population. Individually, upper respiratory tract infection occurred 20 times a year, whereas otitis media occurred 10 times in 1 patient.

As illustrated in Table 2, all 174 patients had other complications such as arthritis (32 cases, 18.39%), neutropenia or thrombocytopenia (9 cases, 5.17%), polyarteritis nodosa (P27, 0.57%), growth hormone deficiency (P21, P141, 1.15%), megaloblastic anemia (P74, 0.57%), and infection after vaccination of poliovaccine (P31, 0.57%). The rate and distribution of related infections and accompanied symptoms in XLA patients were showed in Table 3.

3.4. Correlation analysis of clinical characteristics

There is significant difference in number of cases diagnosed before and after year 2007. The diagnosis of 154 patients was made after 2007, with the onset age of 2.21 ± 2.18 years, whereas only 20 patients were diagnosed before 2007, with the onset age of 1.76 ± 1.91 years. The age of diagnosis was not statistically different ($t = 0.804$, $P = 0.422$). But the interval between onset of symptoms and XLA diagnosis was significantly different in those 2 groups, with 4.36 ± 3.13 and 7.69 ± 3.32 years, respectively, for after and before 2007 groups ($t = -2.840$, $P = 0.005$).

The average age of XLA diagnosis in patients with nasosinusitis as complication was 10.63 ± 3.97 years, which is significantly later than those without nasosinusitis (6.41 ± 4.04 years; $t = 4.653$, $P < 0.0001$).

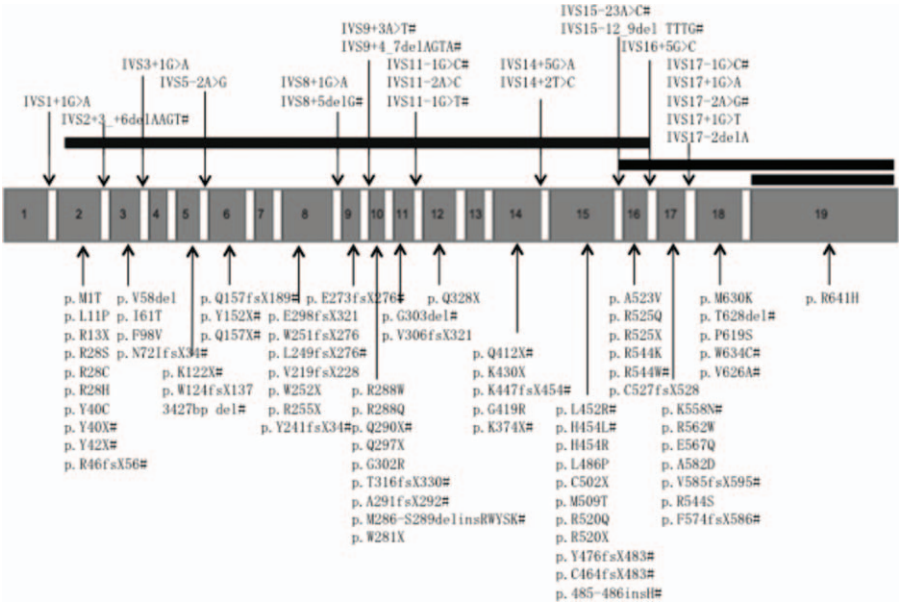

Figure 2. Schematic representation of BTK mutations detected in the present study.

No significant correlations were noticed between the severity of infections, family history, or region of the disease. Twenty-two of the 60 (36.67%) patients with positive family history were found to have severe infections, and only 36 (33.33%) in patients without family history ($\chi = 0.190$, $P = 0.753$).

3.5. BTK mutation analysis

The *BTK* gene analysis was performed in 142 of 174 XLA patients. One hundred twenty-seven patients, coming from 124 individual families, were found to have *BTK* gene mutation, including 45 novel mutations (Table 2). Forty-eight patients were found to have missense mutations (38.10%). Frameshift due to insertions or deletions accounted for 23.02% (29 patients). Splicing mutations were identified in 23 patients (18.25%), nonsense mutations in 21 (16.67%), and large deletions in 5 (3.97%). The locations of mutations were scattered throughout the *BTK* gene, with TK domain being most frequent (n = 64, 50.79%), followed by the PH domain (n = 28, 22.22%), the SH2 domain (n = 16, 12.70%), the SH3 domain (n = 15, 11.90%), and the TH domain (n = 3, 2.38%) (Fig. 2). We also identified 1 large deletion between PH domain and TK domain and another large deletion affecting region between exon 16 and exon 19 (patients 33 and 40, respectively). Forty-five new mutations were identified when comparing with the online BTK database (http://structure.bmc.lu.se/idbase/BTKbase/), including frameshift (20), missense (7), splicing (11), nonsense (5) mutations, and large deletions (2).

3.6. BTK genotype–phenotype correlation

The age of onset of the 93 patients with severe genotype mutation was 1.91 ± 1.84 years, which was significantly earlier than that of 27 patients with mild genotype mutation (2.83 ± 2.32 years; $t = -2.180$, $P = 0.031$). The average age of diagnosis in severe genotype patients was 6.34 ± 3.82 years, which was significantly earlier than that of mild genotype ($t = -2.751$, $P = 0.007$).

Thirty-three of the 50 (66.0%) patients with positive family history had severe genotype. However, this rate is much higher (60 of 72, 83.33%) in patients without family history ($\chi = 4.893$, $P = 0.032$). No correlations were found regarding the severity of infections, the severity of genotype, or arthritis as complication. Thirty-two of the 42 (76.19%) patients with severe infections had severe genotype, and those with mild infections was 63 of 82 (76.82%), not statistically different ($\chi = 0.006$, $P = 1.000$).

Twenty-one of the 26 (80.76%) patients with arthritis as complication had severe genotype, and the rate in those without arthritis was 65 of 88 (73.86%), which was not statistically significant ($\chi = 0.517$, $P = 0.607$).

4. Discussion

X-linked agammaglobulinemia accounts for 6% to 11% of primary immunodeficiency (PID). The reported incidence of XLA varies in different countries. It is 1/200,000 live births in Switzerland, 1/10,000,000 to 1/20,000,000 live births in Spain, 1/100,000 to 1/285,000 live births in Norway and 1/379,000 live births in the United States.[11,26–28] It is difficult to accurately access the incidence of XLA in mainland of China due to the lack of national or local PID registration. Based on the population census of 16.87 million new birth populations in 2014, using the incidence of Norway, the new cases of XLA would be above 80 annually, and the cumulative cases below 14 years of age should be above 1000. In our center, the number of XLA cased among total diagnosed PID is higher than that of other countries, that is, 174 diagnosed in 14 years. It is partially due to simple tested required for making the diagnosis and longer survival of XLA patients. However, further investigations are still needed to delineate the mechanism. Since we are one of the 2 major medical centers in the entire mainland of China managing those patients, our cohort is 174, much lower than the estimated 1000 cases, we predict that there are still significant number of patients who are not diagnosed. The fact that much more XLA cases were diagnosed after 2007 suggests physicians have increased index of suspicion on immunodeficiency patients in China.

There are 31 provinces and municipalities in mainland China. We noticed a skewed geographic distribution of XLA patients. Among the 174 XLA patients reported here of 22 provinces and municipalities, most of them were from the 6 provinces and 1

Table 4

Seventy-four patients with BTK mutations published in Chinese version.

Pts	Type of mutation	Localization	Nucleotide substitution	Amino acid substitution	Domain	Carrier	References
1.	Splicing	Intron 9	c. IVS9+2T>C		SH3	+	[36]
2.	Missense	Exon 17	1878T>C	F583L	TK	+	[36]
3.	Nonsense	Exon 8	895C>T	R255X	SH3	+	[36]
4.	Frameshift	Exon 6	537delC	N135fsX177	PH	+	[36]
5.	Nonsense	Exon 5	504delG	R123X	PH	+	[36]
6.	Missense	Exon 15	1637G>A	C502Y	TK	+	[36]
7.	Missense	Exon 17	1764G>T	R544S	TK	–	[36]
8.	Splicing	Intron 14	IVS14–2A>G			+	[37]
9.	Frameshift	Exon 14/18	1344delC/2031T>C			–	[37]
10.	Missense	Exon 14	1402C>T			–	[37]
11.	Deletion	Exon 16	1713–1716delTTTG			–	[37]
12.	Missense	Exon 16	1706G>A	R525Q	TK	+	[38]
13.	Insertion		546insACAGTGATCT	L138ins140X	PH	–	[38]
14.	Nonsense		975G>A	W281X	SH2	–	[38]
15.	Nonsense		832C>T	Q234X	SH3	–	[38]
16.	Missense		1900A>T	I590F	TK	–	[38]
17.	Deletion		472delTTCTCCCC	F114delX115	PH	+	[38]
18.	Missense		1904A>C	Y591S	TK	+	[38]
19.	Missense		164T>C	L11P	PH	+	[38]
20.	Frameshift	Exon 9	949delG			–	[39]
21.	Missense	Exon 17	1764G>T	R544S		+	[39]
22.	Missense	Exon 15	1637G>A	C502Y		–	[39]
23.	Missense			L11P			[40]
24.	Missense			R28C			[40]
25.	Missense			I355N			[40]
26.	Missense			I590F			[40]
27.	Missense			Y591S			[40]
28.	Missense			G594E			[40]
29.	Missense			R525Q			[40]
30.	Nonsense			Q234X			[40]
31.	Nonsense			W281X			[40]
32.	Nonsense			Q234X			[40]
33.	Nonsense			Y598X			[40]
34.	Insertion			L138ins140X			[40]
35.	Insertion			1197ins207X			[40]
36.	Deletion			F114delX115			[40]
37.	Missense		164T>C	L11P	PH	+	[41]
38.	Nonsense		169C>T	R13X	PH	+	[41]
39.	Missense		213C>T	R28C	PH	+	[41]
40.	Deletion		472delTTCTCCCC	F114delX115	PH	+	[41]
41.	Missense		502T>C	W124R	PH	–	[41]
42.	Nonsense		503G>A	W124X	PH	+	[41]
43.	Splicing		Intron 6 (-2)A>G		PH	–	[41]
44.	Insertion		546insACAGTGATCT	L138insX140	PH	+	[41]
45.	Deletion		231delCGTGC	T33delX39	PH	+	[41]
46.	Insertion		721insTACATAG	I197insX207	TH	+	[41]
47.	Insertion		696insT	L188insX193	TH	+	[41]
48.	Nonsense		601C>T	Q157X	TH	+	[41]
49.	Nonsense		832C>T	Q234X	SH3	+	[41]
50.	Nonsense		832C>T	Q234X	SH3	+	[41]
51.	Nonsense		895C>T	R255X	SH3	+	[41]
52.	Nonsense		975G>A	W281X	SH2	+	[41]
53.	Missense		1129C>A	H133Y	SH2	+	[41]
54.	Missense		1196T>A	I355N	SH2	+	[41]
55.	Nonsense		1072A>T	K314X	SH2	+	[41]
56.	Deletion		1089delGT	V319delX321	SH2	–	[41]
57.	Nonsense		1690C>T	R520X	TK	–	[41]
58.	Missense		1706G>A	R525Q	TK	+	[41]
59.	Missense		1706G>A	R525Q	TK	+	[41]
60.	Insertion		1735insT	V535insX536	TK	+	[41]
61.	Missense		1736G>A	R544K	TK	–	[41]
62.	Missense		1708G>C	R525P	TK	–	[41]
63.	Splicing		Intron 17 (-2)A>T		TK	+	[41]

(*continued*)

Table 4
(continued).

Pts	Type of mutation	Localization	Nucleotide substitution	Amino acid substitution	Domain	Carrier	References
64.	Splicing		Intron 17 (-2)A>T		TK	+	[41]
65.	Missense		1817G>T	R562L	TK	+	[42]
66.	Deletion		1896delGGA	W588delX588	TK	+	[42]
67.	Missense		1900A>T	I590F	TK	–	[42]
68.	Missense		1904A>C	Y591S	TK	+	[42]
69.	Missense		1913G>A	G594E	TK	–	[42]
70.	Nonsense		2025C>G	Y631X	TK	+	[42]
71.	Nonsense		2025C>G	Y631X	TK	+	[42]
72.	Missense		1822T>C	S564P	TK	+	[42]
73.	Nonsense		2025C>A	Y631X	TK	+	[42]
74.	Missense		1899A>T	E589D	TK	–	[42]

BTK = Bruton tyrosine kinase, NE = not examined, NIL = no mutation detected, PH = Pleckstrin homology, Pts = patients, SH1 = Src homology 1, SH3 = Src homology 3, TH = Tec homology domain, TK = tyrosine kinase domain.

municipality in East China. It might be due to the fact that Shanghai is the center of the district with convenient transportation and advanced medical technology.

Infection is the primary presentation of XLA patients. In our cohort, all but patients 38 and 130 had significant infection before the diagnosis. Similar to other countries, respiratory tract infection is the most common infection among which pneumonia constitutes the largest in our patient group. Otitis media and recurrent diarrhea are also very common, whereas nasosinusitis and cutaneous infection are less frequent. In terms of severe infection, infection of the central nervous system is the most common, whereas sepsis and suppurative arthritis are relatively rare. Examples are osteomyelitis (patient 66) and hepatitis (patient 127), and also tubercular meningitis.

The cohort described here demonstrated higher rates of lower respiratory tract infection (bronchitis/pneumonia, 79.3%), recurrent upper respiratory tract infection, and otitis media, which were different from those from other countries. In Netherlands, 100% of the 15 XLA patients suffered from pneumonia.[29] In USA, 62% of the patients suffered pneumonia and 70% of those suffered from otitis media.[11] In the current study, the incidence of otitis media was 41.5%. Data suggest a higher risk of XLA in patients with pneumonia, recurrent upper respiratory tract infection, otitis media, and chronic nasosinusitis.

On the contrary, the incidence of noninfection arthritis was 18.75% in our patient population, similar to what Wang et al observed.[30] However, such numbers are very different from what was reported from other countries. Of note, the onset of arthritis in XLA patients was 6 years of age in average, with youngest at 1 year of age. This is significantly earlier than that of juvenile idiopathic arthritis (JIA), indicating high risk of septic joint infection in XLA patients.

Cases of poliomyelitis after polio vaccination had been reported in XLA patients.[11,12] In our cohort, 1 patient received the attenuated polio vaccination and presented with flaccid paralysis 2 months later. Due to the lack of viral serotyping, it is not clear if the infection was caused by polio vaccine or natural wild-type polio virus.

Polyarteritis nodosa with cat eye syndrome chromosome region, candidate 1 gene mutation can display low B cells and immunodeficiency. One of our patients was diagnosed with polyarteritis nodosa. This patient was referred to us due to 5 years history of recurrent respiratory tract infection and bilateral intermittent red rash of legs for 6 months. The diagnosis of polyarteritis nodosa was made based on history and physical examination. The laboratory examination showed low B cells and immunodeficiency, but negative rheumatoid factors, antinuclear antibody, and normal ESR. *BTK* gene sequencing confirmed BTK mutation.

The 82 (64.29%) patients showed the recurrent mutations as previously described (BTKbase; http://bioinf.uta.fi/BTKbase). The spectrum of mutations are very similar to a previous cohort study of eastern and central Europe, currently the largest reported group of *BTK* mutation in Europe.[31] It comprised of 122 patients with 53.1% recurrent mutations. Previous studies in Chinese cases of XLA in the literature reported 74 mutations from 74 patients (Table 4).[32–38] When combining our data with published Chinese cases, we found that the collective data are comparable with the large European data and the BTK database (Fig. 3), with missense being most common, followed by frameshift, splicing, and nonsense mutation, whereas the gross deletions being the rarest. In addition, the frequency of nonsense mutation in our group was similar to the European data (16.7% and 17.2%, respectively) that is higher than that in the BTK database (11.53%), whereas previous reported Chinese group had the highest frequency (21.08%). The gross deletion rate was very low in reported Chinese data (1.22%) compared with the European data and BTK database. However, the overall pattern of mutations from our cohort and previously reported Chinese data is consistent with what was found in BTK database.

The most common mutation sites in our study were R520 (1 R520C, 3 R520X) and R525 (1 R52Q, 2 R525X), which are also

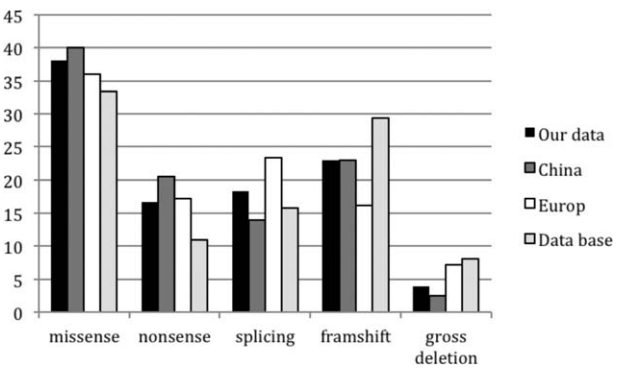

Figure 3. Distribution of BTK mutation types in 4 large cohorts.

the recurrent mutation sites in BTK in arginine-coding CpG dinucleotides. These sites contain the sequence of purine-CpGpyrimidine, which is the single most mutable tetranucleotide.[16,39] Several studies have described mutations at this codon.[40–42] BTK database showed 20 of the mutations resulting in R520X and 17 of them leading to R520. BTK database also lists 4 additional mutations in codon R525 in 23 patients. The defined "hot spot" suggests an important functional element associated with the wild-type arginine in the BTK domain.

The second most common recurrent mutation site in our study was R28 (3 R28H, 1 R28C). It was reported that different amino substitution in this site resulted in varying degree of impairment and dysfunction. The functional mutation in R28C was shown to be less severe than R28H.[43] As reported previously, the effect of mutation of R28C was milder on XID (sex-linked immunodeficiency) mice compared with that on XLA patients.[44] This was consistent with the finding in our study that the patients with R28C mutation had milder clinical features.

A582D was the most frequently identified novel mutation in this study and was found in 3 patients (cousins) from 1 family. The other 2 substitutions of Ala to Asp in BTK database are A508D and A607D. The mutation A508D introduces a charged residue in the hydrophobic core of the domain and, therefore, is likely to alter its conformation. Polar residues are not allowed to reside in a protein core unless the charge can be neutralized. Another compensatory mutation would be required to tolerate the A508D mutation.[45] A582 site was reported to be changed to Val in BTK database. The side chain of Trp-563 is sandwiched between Arg-562 and Ala-582. Mutation of either of the surrounding residues could thus interfere with it sterically.[46]. Serine does not have the bidentate structure of valine. We speculate that A582D may lead to alteration of BTK structure.

Not all patients with the early onset of infections, profound hypogammaglobulinemia, and markedly reduced or absent B cells are found to have BTK mutations. Mutations in pre-B-cell receptor can disrupt B-cell development.[47] In the present study, 15 patients were diagnosed as XLA, but no *BTK* mutation was detected. Conley et al stated that BTK mutation accounts for about 85% of patients with defects in early B-cell development. The remaining patients have defects that are heterogeneous. About one-third have mutations in μ-heavy chain; a small number have defects in λ5, Igα, or B-cell linker. In 5% to 10% of patients, no genetic abnormalities have been identified.[20]

Whether there is relationship between genotype and phenotype in XLA patients remains to be delineated. Some studies support such correlation, whereas others demonstrated otherwise.[15,16,20] In this study, we found that there was no relationship between clinical symptoms and BTK mutations, but both the onset age and the diagnosis age of patients who had severe genotype were earlier than those who had mild genotype. This was similar to the results of the study by Lee et al that patients with less severe missense mutations had higher age of onset than those with severe missense mutations.[25] We reviewed these patients' medical history, and found that the incidence of infection in patients with severe genotypes was higher than those with milder genotypes. We speculated that the doctors were more alert for the patients who often got infection, so the age of diagnosis was earlier.

There is no significant difference between the onset age of XLA in our research and that of other countries.[11,12,14,29] The average diagnosis age in our research is 7.09 years, with 5 patients diagnosed within 1 year, 14 patients (8.05%) within 2 years, and 24 patients (13.79%) within 3 years. The average age of diagnosis of patients without family history is 5.37, with half within 2 years of age in the United States.[11] The average age of diagnosis is 6.2 years, with half diagnosed within 3 years of life in Spain[14] and 6.5 years in Holland.[29] Obviously, the age of diagnosis of XLA patients is rather late in mainland China.[11,14] However, there has been significant improvement on this in XLA patients, with some patients being diagnosed at the stage of primary symptoms. Unfortunately, in the current study, all patients except patients 38 and 130 were not diagnosed at the time of primary symptoms. Since Chinese patients carry the same spectrum of *BTK* mutations as in other countries, the delayed age of diagnosis is probably due to the less awareness and low index of suspicion of community physicians. However, we noticed progress in recognition since many more cases were diagnosed after 2007, and the interval time between symptom onset and age of diagnosis was sharply decreased.

There are still some limitations of this study. First, we did not analyze the transcriptional and translational levels of *BTK* gene of these patients. This study is a retrospective study, so we did not carry out the analysis at the very beginning, and due to many reasons, a part of these patients could not be followed up. Furthermore, the advanced analysis of novel mutations, such as analysis of BTK by flow cytometry, immunofluorescence staining, and/or Western blotting, was not performed, which may weaken the comprehensive understanding of XLA patients.

5. Conclusions

We presented here the data of 174 patients with BTK mutations identified in our center over the past decade, which represents the largest number of XLA patients from China. A total of 127 mutations, including 45 previously unknown sequence variants, were detected. The spectrum of BTK mutations in China is similar to that of other countries and continents in BTK database.

The severe genotypes were associated with younger age of onset and positive family history. Other definite relationships between genotypes and phenotypes were not confirmed in this study. Further investigations need to be done to fully elucidate the genotype–phenotype correlation in XLA patients.

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