ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2006) 24: 57-63

X-Linked Agammaglobulinemia in Northern Thailand

Muthita Trakultivakorn[1] and Hans D. Ochs[2]

SUMMARY X-linked agammaglobulinemia (XLA) is a primary immunodeficiency characterized by a failure to generate immunoglobulins of all isotypes due to the absence of mature B cells and plasma cells, secondary to mutations in the Bruton's tyrosine kinase (*Btk*) gene. We report six patients with XLA, confirmed by mutation analysis, from northern Thailand. The mean age of onset was 2.5 years and the mean age at diagnosis was 7.3 years. All patients had a history of otitis media, pneumonia and arthritis at the time of diagnosis, five patients had developed bronchiectasis and 3 patients septicemia. Other infections reported included sinusitis (5/6), pericarditis (1/6), meningitis (1/6) and pyoderma (1/6). *Haemophilus influenzae, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated on multiple occasions. One patient died of sepsis at the age of 16 years. These observations demonstrate that early diagnosis and treatment can improve prognosis and quality of life.

X-linked agammaglobulinemia (XLA) is considered the prototypic primary humoral immunodeficiency characterized by a profound reduction of all immunoglobulin isotypes, low number of circulatory B cells and absence of plasma cells. The gene responsible for XLA is Bruton's tyrosine kinase (*Btk*).[1] The syndrome was first recognized in 1952 by Bruton.[2] Mutations in the *Btk* gene, located on the long arm of the X-chromosome, result in absence or nonfunctioning Btk protein.[3,4] Btk, a member of the Tec family of protein tyrosine kinases, is expressed in most hematopoietic cells and is of unique important for B-cell development but selectively down-regulated in T cells and plasma cells.[5,6] Absence of Btk or lack of function results in the arrest of B-cell development, absence of circulatory B cells and, as a result, profound hypogammaglobulinemia. Most XLA patients are asymptomatic during the first few months of life because of the protection by maternally derived IgG antibodies. Onset of recurrent infections varies between 4 and 12 months of age.[7] The most common clinical manifestation is bacterial infections and the most prevalent organisms are pyogenic or encapsulated bacteria such as *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus,* and *Pseudomonas* species. The infections are most often localized in the respiratory tract causing otitis media, sinusitis, bronchitis, and pneumonia. Systemic bacterial infections are frequent and include sepsis, meningitis, osteomyelitis, and septic arthritis. Resistance to viral infections is generally

From the [1]Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, [2]Department of Pediatrics, University of Washington School of Medicine, Seattle, USA.
Correspondence: Muthita Trakultivakorn
E-mail: mtrakult@mail.med.cmu.ac.th

intact except for an unusual susceptibility to infections with enteroviruses, such as echovirus, coxsackievirus, and poliovirus,[8] which require neutralizing antibody as an immune mediator during the virus passage through the extracellular space or the blood stream.[9] Early diagnosis and prophylactic infusion of high-dose intravenous immunoglobulin (IVIG), along with adequate treatment of acute infections, markedly improve the long-term prognosis of patients with XLA.[10]

In Thailand, the diagnosis of XLA is usually based on family history, clinical manifestations, panhypogammaglobulinemia, and low numbers of circulating B cells. There have been only few reports on primary immunodeficiency diseases in Thailand, with only one report of two cases of XLA, confirmed by mutation analysis, from Bangkok.[11] Here we report six unrelated boys with XLA from northern Thailand, each with a unique mutation of *Btk*.

PATIENTS AND METHODS

Clinical and laboratory data on six patients were reviewed. All fulfilled the criteria for a definitive diagnosis of XLA[12] and were followed in the Pediatric Allergy/Immunology Clinic, Chiang Mai University, from April 1993 to August 2005. Two patients (numbers 4 and 5) were from Chiang Mai, the rest were referred from three other cities in northern Thailand. The diagnosis of XLA was confirmed by demonstrating absence or reduced amount of Btk protein in monocytes or platelets using flow cytometry,[13] and by mutation analysis.

The age at diagnosis of XLA was defined as the age at which the patient was recognized to have panhypogammaglobulinemia and a very low circulating B-cell number. Mutation analysis was also performed.

RESULTS

The clinical and laboratory data collected prior to diagnosis are shown in Tables 1 and 2. The age of onset of recurrent infections ranged from 1 to 5 years (mean = 2.5 years), and the age at diagnosis was 5 to 11 years (mean = 7.3 years). Only one patient had a positive family history, i.e. the death of a maternal uncle at 2 years of age from infection of unknown etiology. The most common presenting symptom was pneumonia (4 of 6), and all had a history of recurrent otitis media. Five patients had developed bronchiectasis by the time of diagnosis. Arthritis was documented in all patients and was the presenting symptom in 2 patients. In three patients (1, 3 and 4), the arthritis was of caused by bacteria, the others had aseptic chronic recurrent arthritis resembling juvenile rheumatoid arthritis (JRA). Three had developed septicemia due to *Haemophilus influenzae* before diagnosis. Organisms isolated from various locations included *Haemophilus influenzae, Streptococcus pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Virus isolation was not attempted except in patient 2 who had progressive CNS disease but was negative for enteroviruses by PCR. Patient 4 had developed a right peroneal nerve palsy after chronic arthritis and synovitis of the right knee. Patient 6 had suffered from recurrent and severe arthritis of his left hip joint resulting in avascular necrosis of the femoral head. Patient 1 had meningitis suspected but not proven to be of bacterial etiology. Patient 2 had progressive deterioration of his motor and cognitive function but normal CSF profiles and negative PCR for enteroviruses. The CSF was also negative for acid fast bacilli. The electroencephalogram showed no findings suggestive of Creutzfeldt-Jakob Disease (CJD) or Subacute Sclerosing Panencephalitis (SSPE). MRI imaging revealed diffuse cerebral and cerebellar atrophy, with calcification of basal ganglia and thalamus and abnormal periventricular white matter resembling some of the patients reported by Ziegner *et al*.[14] There was no evidence of multifocal leukodystrophy as seen in JC virus infections, and no characteristic abnormality as seen in CJD. He died of sepsis one year after developing CNS disease at the age of 16 years at a hospital in his hometown, no brain necropsy or autopsy was performed. Four of the patients are well without serious acute infections since treatment with intravenous immunoglobulin (IVIG) was initiated at a dose of 300-400 mg/kg every 4-5 weeks. Patient 1 had an episode of pneumonia, two episodes of *H. influenzae* septicemia and recurrent cellulitis during replacement therapy with 200-300 mg/kg IVIG every 4-5 weeks. He has been well after increasing the dose of IVIG to 400 mg/kg every 4-5 weeks. Tonsils were hypoplastic or absent in all patients and all had very low serum immunoglobulin levels and <1% B (CD19/20+) cells. Neutropenia was not observed.

Table 1 Clinical and laboratory data on 6 XLA patients prior to diagnosis

	Patient number					
	1	**2**	**3**	**4**	**5**	**6**
Onset of symptoms (yrs)	5	3	3	1	2	1
Age at diagnosis (yrs)	9	11	5	5	6	8
Family history	None	None	None	None	Suspected	None
First symptom manifested	Pneumonia	Chronic arthritis	Pneumonia	Pneumonia	Chronic arthritis	Pneumonia
Otitis media	Yes	Yes	Yes	Yes	Yes	Yes
Sinusitis	Yes	-	Yes	Yes	Yes	-
Pneumonia	Yes	Yes	Yes	Yes	Yes	Yes
Bronchiectasis	Yes	Yes	Yes	Yes	Yes	-
Progressive degenerating CNS dis-	-	Yes	-	-	-	-
Arthritis	Yes	Yes	Yes	Yes	Yes	Yes
Skin infection	-	-	-	Yes	-	-
Septicemia	-	-	*H. influenzae*	*H. influenzae*	-	*H. influenzae*
Meningitis	Yes	-	-	-	-	-
Pericarditis	Yes	-	-	-	-	-
Organisms encountered						
- *H. influenzae*	+ (Joints, pericardium)	-	+ (Blood, ear, sputum)	+ (Blood, ear, sputum)	+ (Ear)	+ (Blood)
- *S. pneumoniae*	-	+ (Sputum)	+ (Sputum)	+ (Skin abscess)	-	-
- *P. aeruginosa*	+ (Ear)	-	+ (Ear, sputum)	-	+ (Ear)	-
- *S. aureus*	-	-	-	+ (Ear)	-	-
Tonsils	Absent	Hypoplastic	Absent	Absent	Absent	Hypoplastic
Serum IgG (mg/dl)	16	< 180	< 141	< 146	< 141	< 146
IgA (mg/dl)	3.5	< 30	< 23	< 23	< 22	< 23
IgM (mg/dl)	9	< 102	< 17	< 17	< 18	< 18
IgE (IU/ml)	-	-	-	< 17	< 17	< 17

ND = not done, yrs = years.

Btk protein, measured in monocytes or platelets, was markedly reduced or absent in all patients. Mutations affecting genomic DNA of *Btk* were identified in five patients. Patient 6, who had a normal sequence analysis of all exons and exon-intron junctions was found to have a deletion of exons 4 and 5 in cDNA. We suspected but could not proof, a splicing defect in introns 3, 4 or 5. This splicing defect resulted in the absence of Btk protein as determined by flow analysis of patient's platelets and the presence of two peaks, one abnormal and one normal in platelets from his mother confirming the diagnosis of XLA in patient 6.

DISCUSSION

The clinical manifestations of the six XLA patients from northern Thailand were typical and similar to those reported from the US and Europe,[7,10,15] with respiratory tract infections pre-

Table 1 Clinical and laboratory data on 6 XLA patients prior to diagnosis (continued)

	Patient number					
	1	**2**	**3**	**4**	**5**	**6**
CD19/20 (per mm^3) (%)	29 (0.95%)	0 (0%)	37 (0.65%)	65 (1%)	0 (0%)	0 (0%)
CD3 (per mm^3) (%)	2,950 (95%)	4,464 (96%)	5,392 (96%)	6,275 (96%)	4,281 (87%)	9,849 (98%)
CD4 (per mm^3) (%)	1,087 (35%)	1,302 (28%)	2,528 (45%)	2,484 (38%)	1,181 (24%)	2,814 (28%)
CD8 (per mm^3) (%)	1,180 (38%)	1,395 (30%)	2,190 (39%)	3,399 (52%)	2,953 (60%)	7,035 (70%)
CD4/CD8	0.9	0.9	1.2	0.7	0.4	0.4
CD56 (%)	5%	ND	ND	5%	14%	5%
Btk protein expressed in monocytes or platelets	Markedly reduced	Absent	Absent	Absent	Absent	Absent
Mutation analysis	Splice site mutation (intron 4) IVS4 -2a>c 21bp insertion between exons 4, 5	Missense mutation (exon 19) 2075T>C (Leu648Pro)	Missense mutation (exon11) 1171G>C (Ala347Pro)	Nonsense mutation (exon 8) 895C>T (Arg255X)	Missense mutation (exon 18) 2021T>G (Met630Arg)	deletions in exons 4, 5 possible splicing defect in introns 3, 4 or 5
Mother	Carrier	Carrier	Carrier	Carrier	Carrier	Carrier (based on Btk protein expression)
Complications	-	CNS (Negative PCR for enterovirus in CSF)	-	Right peroneal nerve palsy	-	Avascular necrosis of left hip
Note	Alive (21 yrs of age) on monthly IVIG	Died of sepsis at 16 yrs of age	Alive (11 yrs of age) on monthly IVIG	Alive (9 yrs of age) on monthly IVIG	Alive (9 yrs of age) on monthly IVIG	Alive (11 yrs of age) on monthly IVIG

ND = not done, yrs = years.

dominating (Table 2). Otitis media and pneumonia were consistently reported, with pneumonia being the predominant manifestation in most of our patients (4 of 6). The organisms encountered were pyogenic encapsulated bacteria similar to those reported by others.[7,15] Bronchiectasis were presented in five of six patients, possible reflecting the delay of diagnosis. However, chronic lung disease is a complication which can occur in the absence of a history of acute pneumonia.[15] Arthritis, reported in 11-20% of XLA,[7,10] was present in all of the six patients. Three patients had a history of chronic arthritis and were treated as JRA for many years before these symptoms led to the diagnosis of XLA. None of the six patients had neutropenia, which has been observed in up to 25% of XLA patients.[1]

The age of onset of infections in this study was between 1 and 5 (mean 2.5) years. Conley *et al*.,[15] reported that 48.3% of their XLA patients had onset of symptoms between 13 and 40 months, and 31.7% after 40 months of age. The small study from Bangkok also reported a delayed onset of symptoms (6 and 8 years of age).[11] There have been reports of patients with less severe and later onset of disease, often misdiagnosed as having common variable immunodeficiency (CVID), diagnosed as late as 23 and 59 years of age, respectively.[16,17] Usui *et al.*[18] reported a mild case of XLA with onset of recurrent pneumonia at 25 years of age leading to the diagnosis at 27 years. A number of "leaky" phenotypes with less severe disease have been described, in which the affected patients had more than 2% B cells

Table 2 Clinical presentations of XLA patients and organisms encountered in infections prior to diagnosis

	This study (n = 6)	**Lederman *et al.*[7]** (n = 96)	**Hermaszewski *et al.*[10]** (n = 44)	**Vichyanond *et al.*[11]** (n = 2)
Mean age of onset (yrs)	2.5	0.8	0.5	7
Mean age at diagnosis (yrs)	7.3	2.9	ND	9.5
Family History (%)	17	26	ND	0
Presenting symptom	Pneumonia 67%	Respiratory/ GI infections 91%	ND	Pneumonia 100%
Otitis media (%)	100	59	52	100
Sinusitis (%)	67	14	(otitis media and sininusitis)	-
Pneumonia (%)	100	56	32	100
Bronchiectasis (%)	83	46	7	-
Meningitis (%)	17	16	4.5	-
GI infection (%)	0	35	11	-
Pericarditis (%)	17	-	-	-
Arthritis (%)	100	20	11	-
Osteomyelitis (%)	0	3	4.5	50
Skin infection (%)	17	28	14	50
Septicemia (%)	50	10	7	50
Organisms encountered (%)				
- *H. influenzae*	83	9	ND	-
- *S. Pneumoniae*	50	5		-
- *P. aeruginosa*	33	7		100
- *S. aureus*	17	2		-

ND = no data, yrs = years.

and significantly high levels of serum immunoglobulins.[19,20]

Pan-hypogammaglobulinemia and low number of circulating B cells are the most characteristic features of patient with defects in early B-cell development, of which approximately 85% have XLA, and 5-7% have mutations in one of the components of the pre-B cell and B-cell antigen receptor complex.[1,21] In the latter, with mutations affecting autosomal recessive genes, the patients are of either sex but clinically indistinguishable from XLA. They tend to be younger at the age of diagnosis and B cells are absent in their peripheral blood, while most XLA patients have a small number of circulating B cells.[21]

The treatment of XLA includes prophylactic therapy with IVIG and appropriate antibiotics for acute and chronic infections. Our XLA patients were free of serious infections while on 300 - 400 mg/kg IgG replacement therapy every 4-5 weeks. It is of interest that patient 1 developed multiple episodes of serious infections while he was receiving 200-300 mg/kg of IVIG every month but was well after the dose had been increased to 400 mg/kg. This confirms earlier reports suggesting that high-dose IVIG replacement therapy (> 400 mg/kg every 3 weeks) is more effective than low-dose (< 200 mg/kg) in patients with XLA.[22]

Historically, CNS complications in XLA patient are most frequently due to chronic enteroviral infections.[8,23] Patient 2, who developed progressive deterioration of motor and cognitive functions over one year period, was negative for enteroviral infection by PCR. His clinical course resembled that of a subgroup of patients with primary immune deficiencies who presented with chronic progressive lethal CNS disease.[14]

The *Btk* consists of 19 exons and mutations have been found throughout the gene. Molecular analysis in our six patients showed six unique mutations (three missense, one nonsense and two splicing) affecting different sites in the *Btk*. Mutations in *Btk* are highly variable[24] and there is no clear genotype-phenotype correlation in XLA. An international database cataloguing *Btk* mutations, BTKbase, has been established. To date, there have been 512 unique molecular events in 855 patients from 746 families.[25] Identification of the mutations is essential for genetic counseling, detection of carrier female, and for the diagnosis of XLA in patient with an atypical phenotype. Mutation analysis of *Btk* has recently become available in Thailand.

In conclusion, we report six male patients with XLA who presented with typical clinical findings and whose diagnosis was confirmed by mutation analysis. Because the diagnosis was made late and only after many episodes of serious infections, the majority of patients had developed complications by the time of diagnosis. This study should alert pediatricians and physicians taking care of children, to consider the diagnosis of XLA in a male presenting with recurrent sinupulmonary infections or chronic arthritis and absent tonsils. Early diagnosis and proper treatment is crucial for prevention of complications and thus will contribute to the patient's quality of life.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Samreung Rangdeang, Department of Pathology, Faculty of Medicine, Dr. Kittipong Rungruengthanakit, and Rassamee Keawvichit, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, for performing immunophenotyping of the six patients.

REFERENCES

1. Rawlings DJ. X-linked agammaglobulinemia. In: Stiehm ER, Ochs HD, Winkelstein JA, eds. Immunologic Disorders in Infants & Children. 5th edition, Philadelphia, Elsevier Saunders, 2004; pp. 357-69.
2. Bruton OC. Agammaglobulinemia. Pediatrics 1952; 9: 722-7.
3. Vetrie D, Vorechovsky I, Sideras P, *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.
4. Tsukada S, Saffran DC, Rawlings DJ, *et al*. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell 1993; 72: 279-90.
5. de Weers M, Verschuren MCM, Kraakman MEM, *et al*. The Bruton's tyrosine kinase gene is expressed throughout B cell differentiation, from early precursor B cell stages preceding immunoglobulin gene rearrangement up to mature B cell stages. Eur J Immunol 1993; 23: 3109-14.
6. Smith CIE, Baskin B, Humire-Greiff P, *et al*. Expression of Bruton's agammaglobulinemia tyrosine kinase gene, *Btk*, is selectively down-regulated in T lymphocytes and plasma cells. J Immunol 1994; 152: 557-65.
7. Lederman HM, Winkelstein JA. X-linked agammaglobulinemia: an analysis of 96 patients. Medicine 1985; 64: 145-56.
8. McKinney RE Jr, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. Rev Infect Dis 1987; 9: 334-56.
9. Hammon WM, Coriell LL, Stokes J Jr. Evaluation of Red Cross gamma-globulin as a prophylactic agent for poliomyelitis. I. Plan of controlled field tests and results of 1951 pilot study in Utah. JAMA 1952; 150: 739-49.
10. Hermaszewski RA, Webster ADB. Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. Quart J Med 1993; 86: 31-42.
11. Vichyanond P, Visitsunthorn N, Tuchinda M. X-linked agammaglobulinemia in Thailand: report of two new cases and review of a current progress of its immunogenetics. Siriraj Hosp Gaz 1998; 47(Suppl-3) 159-64.
12. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999; 93: 190-7.
13. Futatani T, Watanabe C, Baba Y, Tsukada S, Ochs HD. Bruton's tyrosine kinase is present in normal platelets and its absence identifies patients with X-linked agammaglobulinaemia and carrier females. Br J Haematol 2001; 114: 141-9.
14. Ziegner UH, Kobayashi RH, Cunningham-Rundles C, *et al*. Progressive neurodegeneration in patients with primary immunodeficiency disease on IVIG treatment. Clin Immunol 2002; 102: 19-24.
15. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr 2002; 141: 566-71.
16. Stewart DM, Tian L, Nelson DL. A case of X-linked agammaglobulinemia diagnosed in adulthood. Clin Immunol 2001; 99: 94-9.
17. Morwood K, Bourne H, Philpot R, Gold M, Gillis D, Benson EM. Phenotypic variability: clinical presentation between the 6th year and the 60th year in a family with X-linked agammaglobulinemia. J Allergy Clin Immunol 2004; 113: 783-5.
18. Usui K, Sasahara Y, Tazawa R, *et al*. Recurrent pneumonia with mild hypogammaglobulinemia diagnosed as X-linked agammaglobulinemia in adults. Respir Res 2001; 2: 188-92.
19. Saffran DC, Parolini O, Fitch-Hilgenberg ME, *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.
20. Zhu Q, Zhang M, Rawlings DJ, *et al*. Deletion within the Src homology domain 3 of Bruton's tyrosine kinase resulting in

X-linked agammaglobulinemia (XLA). J Exp Med 1994; 180: 461-70.
21. Conley ME, Broides A, Hernandez-Trujillo V, *et al*. Genetic analysis of patients with defects in early B-cell development. Immunol Rev 2005; 203: 216-34.
22. Liese JG, Wintergerst U, Tympner KD, Belohradsky BH. High- *vs* low-dose immunoglobulin therapy in the long-term treatment of X-linked agammaglobulinemia. AJDC 1992; 146: 335-9.
23. Wilfert CM, Buckley RH, Mohanakumar T, *et al*. Persistent and fatal central-nervous-system ECHOvirus infections in patients with agammaglobulinemia. N Engl J Med 1977; 296: 1485-9.
24. Vihinen M, Kwan SP, Lester T, *et al*. Mutations of the human *Btk* gene coding for Bruton tyrosine kinase in X-linked agammaglobulinemia. Hum Mutat 1999; 13: 280-5.
25. Väliaho J, Smith CIE, Vihinen M. (2004, December 27). Recent results from the analysis of BTKbase [Online]. Available at: http://bioinf.uta.fi/BTKbase/btkresult.html [2005, December 22].