Clinical manifestations and *BTK* gene defect in 4 unrelated Taiwanese families with Bruton's disease

Kuo-Hsi Lee[1], Shyh-Dar Shyur[1,2], Szu-Hung Chu[1], Li-Hsin Huang[1], Yu-Hsuan Kao[1], Wei-Te Lei[1], Chieh-Han Cheng[1], Chia-Yi Lo[1], Chen-Kuan Chen[1] and Ling-Chun Liu[1]

Summary

Background and objective: **X-linked agammaglobulinemia (XLA, also called Bruton's disease) is is an X-linked recessive disorder characterized by recurrent bacterial infections, usually occurring in the first few years of life. Here, we report the results of a *BTK* gene mutation screening study that was performed in Taiwanese families with the *BTK* gene defect to further understand the inheritance patterns of XLA patients in Taiwan and to avoid new cases of XLA within families.**

Materials and methods: In this study, 52 members of 4 unrelated Taiwanese families with the *BTK* gene defect were enrolled. We studied the immunologic reports of 6 symptomatic living male patients with confirmed *BTK* gene defects and correlated the findings with their clinical symptoms. The genomic DNA of the subjects was subjected to direct sequencing mutation analysis.

Results: **We screened 52 members of 4 unrelated Taiwanese families with the *BTK* gene defect for *BTK* gene mutation and found that there were 6 symptomatic living patients with a confirmed defect, 7 symptomatic deceased patients highly suspected to have had the defect and 11 asymptomatic female carriers.**

Conclusions: **This is the first report in a series of the thorough screening for the *BTK* mutation and its carrier status in 4 unrelated Taiwanese families. One pedigree of our study comprises 4 generations. A complete *BTK* gene mutation study for the patient's family members is strongly suggested. *(Asian Pac J Allergy Immunol 2011;29:260-5)***

From the [1]Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan
[2]Taipei Medical University, Taipei, Taiwan
Corresponding author: Shyh-Dar Shyur
E-mail: eji6vu@me.com
Submitted date: 1/5/2011
Accepted date: 30/8/2011

Key words: *X-linked agammaglobulinemia, Bruton's disease, XLA, BTK gene*

Introduction

Bruton's agammaglobulinemia (also called X-linked hypogammaglobulinemia, XLA, Bruton type agammaglobulinemia, Bruton's syndrome, or sex-linked agammaglobulinemia) was the first primary immunodeficiency disease to be described. In 1952, Colonel Ogden Bruton noted the absence of immunoglobulins (Igs) in a boy with a history of pneumonia and other bacterial sino-pulmonary infections[1]. Affected individuals have hypogammaglobulinemia, markedly reduced levels of serum antibodies and markedly reduced levels of B cells[2]. Recurrent otitis is the most common infection prior to diagnosis. Frequent conjunctivitis, sino-pulmonary infections, recurrent diarrhea and skin infections are also observed. Almost 60% of individuals with XLA are diagnosed with immunodeficiency when they develop a severe, life-threatening infection such as pneumonia, empyema, meningitis, sepsis, cellulitis, or septic arthritis. *Streptococcus pneumoniae and Haemophilus influenzae* are the most common organisms found prior to diagnosis, and these microorganisms may continue to cause sinusitis and otitis after diagnosis and the initiation of gammaglobulin therapy. The prognosis for individuals with XLA has improved markedly in the last 25 years as a result of early diagnosis, development of preparations of gammaglobulin that allow normal concentrations of serum IgG to be achieved and the liberal use of antibiotics. We present the results of a *BTK* gene mutation screening study in 52 members of 4 unrelated Taiwanese families with the *BTK* gene defects. This study was conducted to further understand the inheritance pattern of XLA in Taiwanese patients and to avoid new cases of XLA within the patients' families. A complete *BTK* gene mutation study for the patients' family members is strongly suggested. We also analyzed the results of immunologic studies of 6 symptomatic living male patients with confirmed *BTK* gene defects and Bruton's

Table 1. Serum immunoglobulin levels and complement activity

	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (IU/mL)	CH50
Patient 1	7	3	10	7	30.3
Patient 2	137	8	21	18	36.4
Father	1700	277	97	90	40.5
Mother	1300	279	221	37	22.2
Reference	1097-1518	142-277	95-188	10-180	23.5-43.5

Table 2. Levels of lymphocyte subsets in peripheral blood

	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD57 (%)
Patient 1	86.6	38.0	35.8	1.8	2.1
Patient 2	83.0	45.4	31.5	2.1	4.4
Father	55.5	37.2	20.0	15.6	14.8
Mother	76.4	36.9	37.9	7.0	8.5
Reference	67–81	41–55	30–40	8–32	10–19

disease and correlated these findings with the patients' clinical manifestations. Direct sequencing mutation analysis was performed for the genomic DNA of all 52 subjects of our study. We collected information on the clinical manifestations of the disease and the results of immunologic studies of the 6 symptomatic living male patients with confirmed *BTK* gene defects and Bruton's disease and correlated them with the clinical manifestations.

Methods

Informed consent for genetic analysis was obtained from the patient's parents. Total cellular RNA was prepared from peripheral blood leukocytes using the TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. The cDNA was synthesized from RNA via a reverse transcription reaction by using an avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). The BTK cDNA was amplified and then sent for direct sequencing. The mutation nomenclature was based on the Gen-Bank reference sequence (NM_000061.1; GI:4557376), with the A of the ATG translation initiation start being numbered as nucleotide +1.

Family 1

Patient 1 was an 11 year-old boy with growth retardation (body weight < 3rd percentile) who was hospitalized for life-threatening *Pseudomonas* pneumonia with empyema. In the past, the boy had developed recurrent sinusitis, acute otitis media (AOM) with eardrum perforation, pneumonia and meningoencephalitis. Patient 2 was patient 1's younger brother. The younger one (9 years old) had been limping and had right knee swelling for more than 1 year before diagnosis and had been treated for juvenile rheumatoid arthritis (JRA) in many hospitals but had no history of recurrent AOM, sinusitis, or other serious infections. The right knee physical examination revealed the following: range of motion limitation (0^o–120^o) and mild pain on passive range of motion. Immunologic studies revealed a profound decrease in the Ig levels of all isotypes and a marked decrease in the number of B cells (Table 1. and 2.). Both of them had small tonsils and lymph nodes on physical examination. The 2 brothers had the same *BTK* gene defect (c.839+4 A>G (p.E280 fsx 281) mutation in the *BTK* gene), but their clinical manifestations varied widely. After direct sequencing mutation analysis, we found that their mother was a carrier. By tracing their family history, we found that many male members of this family (4 generations) had died in childhood because of overwhelming infection, and we further found that these deceased members had carrier mothers (Figure 1.). We suspected that those who died had the c.839+4 A>G (p.E280 fsx 281) mutation in the *BTK* gene.

Family 2

Patient 3 was an 11-year-old boy with multiple pustules all over his body, particularly on the 4 extremities. He was healthy before the age of 7 years. However, he developed recurrent furunculosis and skin abscesses at the age of 7 years. He was administered oral antibiotics for skin lesions in another hospital, but the lesions were refractory to these drugs. He developed *H. influenzae* meningitis at the age of 9 years and *H. influenzae* pneumonia and sepsis at the age of 11 years. Physical examination revealed paucity of lymphoid tissue. Immunologic studies also showed a profound decrease in Ig levels of all isotypes and a decrease in the number of B cells (Table 3.).

In family 2, we found that the only patient with Bruton's disease had a de novo mutation in his *BTK* gene (c.1630 A>G (p.R544G) de novo mutation in the *BTK* gene)

Family 3

Patient 4 was a 3-month-old male patient who was admitted to our hospital because of drowsiness and grunting. According to his family, he had a frequently spiking fever and recurrent diarrhea with yellowish watery stools and mucus for 3 days prior to hospitalization. Pallor and abdominal distension

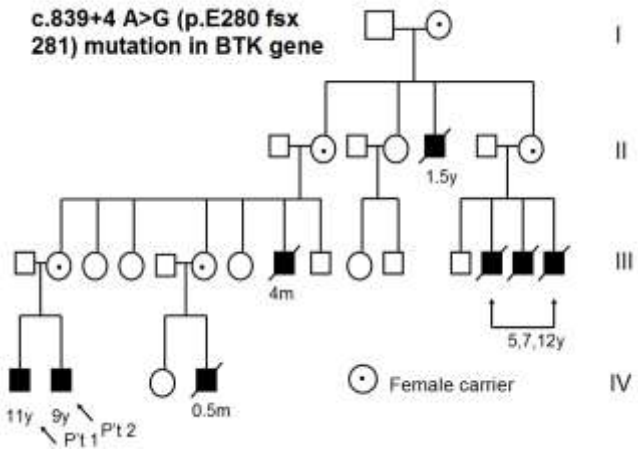

Figure 1. The pedigree of Family 1

Table 3. Serum immunoglobulin levels and complement activity of patient 3 and levels of lymphocyte subsets in the peripheral blood of patient 3

	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (IU/mL)	CH50
Patient 3	35	29	8	42	27.8
Reference	633-1280	48–207	33–202	103-1613	23.5-43.5
	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD57 (%)
Patient 3	70.6	35.5	27.6	0.4	9.8
Reference	55–78	27–53	19–34	10–31	10–19

were noted. We also found 1 bulla of about 1 × 1 cm on his right forearm. During hospitalization, we performed routine examination and bacterial cultures (blood, cerebrospinal fluid, urine, stool, skin wounds). Routine examination revealed small tonsils and lymph nodes. Unfortunately, the patient died within hours of admission. All bacterial cultures were positive for *Pseudomonas aeruginosa*. Immunologic studies revealed a profound decrease in the levels of IgG and IgA and a decrease in the number of B cells in his cord blood (Table 4.).

In family 3 (comprising 4 members), we found only patient with Bruton's disease and he had the c.1106 T>C (p.L369P) mutation in his *BTK* gene. Under direct sequencing mutation analysis, we also found that his mother was a carrier. This patient 4 died because of *Pseudomonas* sepsis.

Family 4

In family 4, patients 5 and 6 were brothers and were aged 2.10 years and 1.9 years, respectively. They denied any major systemic diseases and had a normal growth curve. However, they had recurrent furunculosis and skin abscesses all over their bodies which had been present for 6 months. The 2 brothers had hypotrophic lymphoid tissue. Patient 5 had reduced levels of serum IgA and IgM and markedly reduced levels of B cells. Patient 6 also had similar immunodeficiency. (Table 5.) The 2 brothers had the same *BTK* gene defect, c.271 C>T (p.Q91X) mutation in the *BTK* gene.

Results

In this study, we screened 52 members of 4 unrelated Taiwanese families with *BTK* gene defects for *BTK* gene mutation. Six symptomatic male patients with confirmed *BTK* gene defects and Bruton's disease had symptoms ranging from JRA and skin pyoderma to serious *H. influenzae* and *Pseudomonas* infections and multi-organ lethal *Pseudomonas* infections. Seven symptomatic male patients had died because of associated overwhelming lethal infections and all of them had a carrier mother, except for one who had a de novo mutation. We strongly believe that the patients had *BTK* gene defects and Bruton's disease. Eleven asymptomatic female carriers were confirmed to have a *BTK* gene defect. Twenty-eight asymptomatic family members were found without any *BTK* gene defect. In this study, 4 different *BTK* gene mutations were detected: c.839+4 A>G (p.E280 fsx 281), c.1630 A>G (p.R544G), c.1106 T>C (p.L369P), and c.271 C>T (p.Q91X). In family 1, 2 brothers had the same gene defect (c.839+4 A>G (p.E280 fsx 281) mutation in the *BTK* gene), but they exhibited completely different clinical manifestations. The elder brother had growth retardation, recurrent sinusitis, AOM with eardrum perforation, pneumonia that needed hospitalization (>10 days for each hospital stay), serious *Pseudomonas* infection, and meningoencephalitis. The younger one had only limping and right knee swelling for more than 1 year before diagnosis and he had been treated for JRA in many hospitals. He had no history of recurrent AOM, sinusitis, or other serious infections. In family 2, we found that the only patient with Bruton's disease had a de novo mutation in his *BTK* gene. In family 3, the 3 month-old male patient with the c.1106 T>C (p.L369P) mutation expired because of lethal *Pseudomonas* infection. The brothers from family 4 differed from the brothers in family 1. The brothers from family 4 had the same *BTK* gene defect (c.271 C>T (p.Q91X) mutation in the *BTK* gene and exhibited similar symptoms.

Table 4. Serum immunoglobulin levels and complement activity of patient 4 (cord blood) and levels of lymphocyte subsets in the peripheral blood of patient 4 (cord blood)

	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (IU/mL)	
Patient 4	60	2	9	5	
Reference	176–581	24–89	4.6–46	0.18–3.76	
	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD57 (%)
Patient 4	77.8	64.1	14.7	0.2	15.7
Reference	67–81	38–46	30–40	8–32	5.6–31

Table 5. Serum immunoglobulin levels and complement activity of patients 5 and 6 and levels of lymphocyte subsets in the peripheral blood of patients 5 and 6

	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (IU/mL)	CH50
Patient 5	366	<7	13	<1	66.6
Patient 6	146	<7	7	40	44.4
Reference	345-1213	43-173	14-123	0.31-29.5	23.5-43.5
	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD57 (%)
Patient 5	83.3	34.7	41.3	0.2	12.1
Patient 6	75.5	38	35.8	0.6	22.4
Reference	39–76	23–50	11–33	14–44	10–19

Discussion

XLA or Bruton's disease was first described by Bruton in 1952 as an X-linked recessive disorder characterized by recurrent bacterial infections, usually present in the first few years of life. Bruton's disease is a rare genetic disorder in which the development of B cells is arrested during differentiation. Mutations in the gene coding for a tyrosine kinase (Bruton tyrosine kinase) have been identified as responsible for XLA. XLA is caused by a variety of mutations in the gene encoding Bruton tyrosine kinase (Btk)[3,4]. BTK is a signal-transducing protein expressed in all hematopoietic lineages, except T cells and natural killer (NK) cells. Our study results are consistent with this theory. The *BTK* gene for XLA has been mapped to Xq21.3-Xq22.

Infection was the most common initial clinical presentation (85%), followed by a positive family history (41%) and neutropenia (11%). In our study, 6 symptomatic living male patients with confirmed *BTK* gene defects and Bruton's disease had symptoms ranging from juvenile rheumatoid arthritis and skin pyoderma to recurrent furunculosis, serious pneumonia, and lethal multi-organ infections. Although the average age of diagnosis was earlier in patients with a positive family history (mean, 2.59 years) than in patients with a negative family history (mean, 5.37 years) (p <0.001), only 34.5% of patients with a positive family history were diagnosed at the time of their birth before clinical symptoms developed, that is, only 34.5% of patients with a positive family history were diagnosed on the basis of family history alone.

Typically, affected males develop recurrent bacterial infections in their first 2 years of life and are diagnosed with immunodeficiency before 5 years of age. In fact, pulmonary and gastrointestinal infections are still the major clinical problems, whereas episodes of sepsis, encephalitis, or meningoencephalitis are rare. Respiratory infections are still the major cause of morbidity and mortality in patients with Bruton's disease. Although the incidence of sepsis and meningoencephalitis are rare, these complications tend to be associated with high morbidity and mortality, like in patients 1, 2, and 4. The most common infecting organisms are *H. influenzae* and *S. pneumoniae*.

A clinical diagnosis of XLA is considered in individuals with the following: (1) recurrent bacterial infections before the age of 5 years, (2) severe lethal bacterial infection, (3) paucity of lymphoid tissue (small tonsils and lymph nodes on physical examination) and (4) family history of immunodeficiency.

DNA sequencing techniques are performed for the early diagnoses before lethal infections occur. Although missense mutations affecting non-conserved residues in the amino acid sequence for each domain and mutations in non-invariant splicing positions are less severe mutations[5], the genotype-phenotype correlation is not well-characterized[6]. An obvious feature of XLA is recurrent bacterial infection because of humoral immunodeficiency. However, according to several studies, 30–50% of all XLA patients have a positive family history, with the remaining patients developing sporadic mutations[7,8]. A history of septic arthritis can be another diagnostic clue for XLA. If physicians suspect this disorder, they should thoroughly study the family history and order an immune workup. Specific blood tests and findings can also help confirm

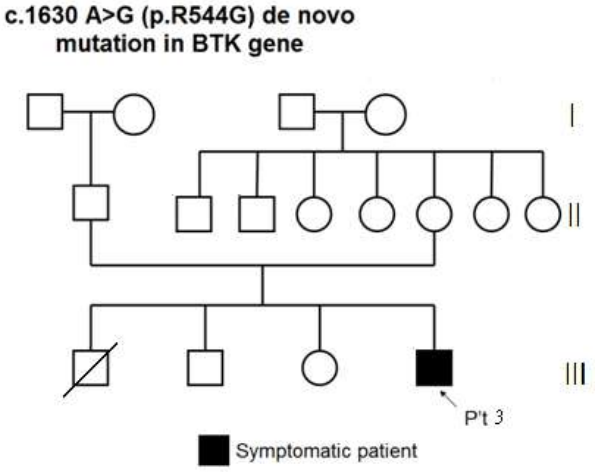

Figure 2. The pedigree of Family 2

the diagnosis of XLA. The serum IgG concentration is typically less than 200 mg/dL with other Igs being markedly reduced or absent and markedly reduced levels of B cells (CD 19^+ cells) in the peripheral circulation (<1%). In our study, all 6 symptomatic patients had markedly decreased serum IgG levels[9]. Normal NK cell activity was noted. XLA patients had low or undetectable antibody titers to ubiquitous antigens (e.g., isohemagglutinins, antistreptolysin, and anti-*Escherichia coli*) and to previously administered vaccine antigens. Individuals with XLA fail to make antibodies to vaccine antigens such as tetanus, *H. influenzae*, *S. pneumoniae*, or diphtheria[10]. BTK protein testing and molecular genetic testing can also be used to confirm the diagnosis of XLA.

About 90% of males who are presumed to have XLA based on the early onset of infections, severe hypogammaglobulinemia and markedly reduced number of B cells have detectable mutations in the *BTK* gene. The majority of females with an XLA-like phenotype and males with an XLA phenotype who do not have identifiable *BTK* mutations are likely to have defects including μ heavy chain deficiency, IgA deficiency, λ5 deficiency, and B-cell linker protein (BLNK) deficiency[11,12].

Prevention of bacterial infections in XLA patients is very important. Administration of intravenous gammaglobulin (400–600 $mg \cdot kg^{-1} \cdot month^{-1}$) can prevent recurrent bacterial infections[13]. Slow subcutaneous immunoglobulin (SCIG) infusions or more rapid SCIG administrations are additional options that could improve patient compliance, decrease side effects, limit fluctuations in IgG levels, and result in lower cost[14].

Before antibiotics and Ig therapy, few XLA patients survived past infancy and early childhood[15]. Early diagnosis, regular Ig treatment and prompt use of antibiotics have increased their longevity dramatically and now an increasing number of XLA patients become adults. Two follow-up studies in genetically defined XLA patients indicate that infectious and noninfectious morbidity remains high[16,17]. The mortality rates in XLA are also unclear but can be as high as 30% over approximately 10 years, even with current comprehensive care.

This is the first series of in which thorough screening for *BTK* mutations and carrier status in 4 unrelated Taiwanese families has been carried out. One pedigree in our study included 4 generations There was wide variability in the clinical presentation even within the same family and with the same *BTK* gene defect. Bruton's disease with a de novo mutation in the *BTK* gene, c.1630 A>G (p.R544G), was found in one of the families that we examined. In this study, 7 symptomatic male patients had died because of associated overwhelming lethal infections. Finally, to avoid new cases within the patients' families, a complete *BTK* gene mutation study of patients' family members is strongly suggested.

References

1. Bruton OC. Agammaglobulinemia. Pediatrics. 1952;9:722-8
2. Conley ME, Rohrer J, Minegishi Y. X-linked Agammaglobulinemia. Clin Rev Allergy Immunol. 2000;19:183-204.
3. Parolini O, Hejtmancik JF, Allen RC, Belmont JW, Lassiter GL, Henry MJ, et al. Linkage analysis and physical mapping near the gene for X-linked agammaglobulinemia at Xq22. Genomics. 1993;15:342–9.
4. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993;72:279–90.
5. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr. 2002;141:566–71.
6. Jo EK, Song CH, Park JK, Lee JH, Kim DS. Protein and genetic analysis of Bruton's tyrosine kinase (Btk) in three Korean x-linked agammaglobulinemia (XLA) families. J Korean Pediatr Soc. 2002;45:44–54.
7. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine (Baltimore). 2006;85:193–202.
8. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr. 2002;141:566–71.

9. Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol.. 2009;27:199-227
10. Farrar JE, Rohrer J, Conley ME. Neutropenia in X-linked agammaglobulinemia. Clin Immunol Immunopathol. 1996;81:271–6.
11. Hoffman T, Winchester R, Schulkind M, Frias JL, Ayoub EM, Good RA. Hypoimmunoglobulinemia with normal T cell function in female siblings. Clin Immunol Immunopathol. 1977;7:364–71
12. Conley ME, Sweinberg SK. Females with a disorder phenotypically identical to X-linked agammaglobulinemia. J Clin Immunol. 1992;12:139–43
13. Eijkhout HW et al. The effect of two different dosages of intravenous immunoglobulin on the incidence of recurrent infections in patients with primary hypogammaglobulinemia. A randomized, double-blind, multicenter crossover trial. Annals of Internal Medicine. 2001; 135**:**165-74
14. Gardulf A, Andersen V, Bjorkander J, et al. Subcutaneous immunoglobulin replacement in patients with primary antibody deficiencies: safety and costs. Lancet. 1995;345:365–9
15. Hermaszewski RA, Webster AD. Primary hypogamma globulinaemia: a survey of clinical manifestations and complications. Q J Med. 1993;86:31–42
16. Plebani A, Soresina A, Rondelli R, Amato GM, Azzari C, Cardinale F, et al. Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. Clin Immunol 2002;104:221-30
17. Van der Hilst JH, Smits BW, van der Meer JM. Hypogammaglobulinemia: cumulative experience in 49 patients in tertiary care institution. Neth J Med. 2002; 60:140-7.