

Phenotypic variability: Clinical presentation between the 6th year and the 60th year in a family with X-linked agammaglobulinemia

To the Editor:

X-linked agammaglobulinemia (XLA) was first described by Bruton in 1952 as an X-linked recessive disorder characterized by recurrent bacterial infections, usually presenting in the first few years of life. Immunologic testing typically demonstrates a profound decrease in Ig levels of all isotypes and a marked decrease in the number of B cells. The gene for XLA has been identified and mapped to the Xq21.3-q22. It was known before the discovery of the gene that there was wide variability in the clinical presentation, even within the members of the one family who were likely to be carrying the same gene.¹

In this report we present a family with Bruton's XLA in whom the index case presented with recurrent bacterial infections at the age of 6 years. At that time the maternal grandfather was being treated with replacement Ig for common variable immunodeficiency after presenting in his 60th year with recurrent pneumococcal infection and normal gamma globulin levels that subsequently decreased. The 6-year-old boy was found to have a mutation in exon 10 of the BTK gene. The same mutation was found in the maternal grandfather and in a 1-year-old asymptomatic brother.

This case describes the oldest patient in the literature to present with immunodeficiency associated with a BTK gene mutation and highlights the heterogeneity of presentation and phenotype that can occur within the same family.

We describe a family of patients with XLA with phenotypic variability.

The index patient presented at age 6 years with complicated pneumococcal pneumonia. This required prolonged admission to hospital, intravenous antibiotics, thoracostomy, and drainage of an empyema. He had a history of recurrent chest and sinus infections, none of which required hospitalization. Investigations showed panhypogammaglobulinemia (Table I). Lymphocyte studies showed absence of CD19⁺ cells. Other immune function studies were normal. DNA sequencing of his BTK gene showed a point mutation at gene position 994 showing a substitution of C to T. This results in a change from arginine to tryptophan at amino acid residue 288 (994C > T [R228W]), thus confirming the diagnosis of XLA.

He was commenced on intravenous Ig (Intragam P, CSL) replacement treatment. He has not had recurrence of clinical chest infection but has required surgery for chronic sinus disease.

His 1-year-old asymptomatic brother was investigated and found to be profoundly panhypogammaglobulinemic

(Table I), and he was also commenced on intravenous Ig (Intragam P, CSL) replacement therapy. Genetic testing in the brother showed the same genetic mutation in the BTK gene as found in the index case.

The maternal grandfather was diagnosed with common variable immunodeficiency at the age of 59 years after a long history of recurrent infections. His history before the diagnosis included 16 episodes of pneumonia to the age of 21 years, with childhood bronchiectasis and a lobectomy at age 9 years. He had frequent episodes of pneumonia throughout his adult life. He had an episode of spontaneous bacterial peritonitis at age 48 years; this was in association with heavy alcohol intake and possible alcoholic liver disease. He had an episode of pneumococcal diskitis at age 56 years. He had Igs tested with this episode (Table I).

At age 58 years he had further complicated pulmonary infections, which resulted in a pneumonectomy. His Ig levels were repeated and showed panhypogammaglobulinemia as outlined in Table I. His total lymphocyte count was 0.5 (normal, 1 to 4), and subsequent lymphocyte studies showed absence of CD19⁺ cells.

He was commenced on intravenous Ig replacement (Intragam P, CSL) and has remained relatively well since. Molecular studies of his BTK gene were undertaken after the diagnosis of XLA in his grandson, and these showed that he carried the same BTK gene mutation as his 2 grandsons.

Genomic DNA was obtained from whole blood. All 19 exons of the BTK gene, including the exon-intron boundaries, were individually amplified. Primer sequences were based on Conely et al.² PCR was carried out in a 50 µL volume containing 2.5 µL of DNA, 37.5 pmol of each primer (Sigma Aldridge, Sydney, Australia), 0.25 mmol/L deoxynucleotide triphosphate (Astral Scientific, Sydney, Australia), 1× PCR buffer (Applied Biosystems, Sydney, Australia), 2.5 mmol/L MgCl₂ (Applied Biosystems), and 1 U of AmpliTaq Gold Polymerase (Applied Biosystems).

PCR products were purified by polyethylene glycol precipitation before cycle sequencing kit (Applied Biosystems) with an automated ABI373 DNA sequencer. Detected mutations were confirmed by sequencing in the opposite direction.

In this report we describe a family spanning 3 generations who were confirmed to have a mutation in the BTK gene. The diagnosis of common variable immunodeficiency in the grandfather was made at the age of 58 years, although he had been symptomatic for many years. Previously a diagnosis of selective IgA deficiency had been made, because the IgG and IgM levels were normal. His 6-year-old grandson was diagnosed with XLA more than 1 year later when he presented with recurrent infections. This resulted in increased awareness

TABLE I. Immunoglobulin levels

	Brother 1, age 6 y	Brother 2, age 1 y	Grandfather, age 56 y	Grandfather, age 58 y
Total IgG	1.01 (>8.6 g/L)	1.08 (>2.08 g/L)	10.2 (7-14 g/L)	4.29 (7-14 g/L)
IgG1	0.51 (>4.2 g/L)	1.37 (>1.84 g/L)	Not tested	3.2 (>4.2 g/L)
IgG2	4.2 (>1.4 g/L)	0.43 (>0.35 g/L)	Not tested	0.77 (>1.4 g/L)
IgG3	0.09 (>0.63 g/L)	0.05 (>0.08 g/L)	Not tested	0.63 (>0.28 g/L)
IgG4	0.03 (>0.4 g/L)	0.052 (>0.007 g/L)	Not tested	0.11 (>0.08 g/L)
IgA	<0.07 (>0.6 g/L)	<0.007 (>0.17 g/L)	<0.07 (>0.7 g/L)	<0.07 (>0.7 g/L)
IgM	0.24 (>0.42 g/L)	0.07 (>0.2 g/L)	0.52 (>0.3 g/L)	0.14 (>0.5 g/L)

Immunoglobulins and subclasses measured by rate nephelometry (Beckman Coulter Image Immunochemistry System, Beckman Coulter, Sydney, Australia). Numbers in parentheses indicate lower limit of reference ranges.

and early investigation of his 1-year-old brother, who also had markedly low IgG. The increased awareness also prompted further investigation of the grandfather.

The mutation identified in the family in exon 10 resulted in a nucleotide change from C to T at nucleotide position 994 of the BTK gene. This resulted in an arginine being substituted for a tryptophan at amino acid residue 288 (994C>T (R228W)). This mutation has previously been associated with a large Dutch XLA pedigree whose affected members exhibit heterogeneity in their clinical and immunologic phenotype.³ By x-ray crystallography and modeling of the Src/SH2 domain, it has been suggested that this domain forms a socket, which can bind specific phosphorylated tyrosine residues. The mutation R228W can be predicted to severely disrupt phosphotyrosine binding to the SH2 domain, disrupting the functional activity of BTK. The late presentation of the grandfather might imply that binding of BTK to Src through the SH2 domain might not be necessary for full function of the protein.

Phenotypic variation within a 3-generation family has been previously described.⁴ They described a 51-year-old man with recurrent sinusitis and sporadic pneumonia who was confirmed to have a mutation resulting in a premature stop codon in BTK. The index patient was also initially diagnosed with isolated IgA deficiency, but when he was reinvestigated years later, he was found to be panhypoglobulinemic. On review of family members there was marked phenotypic variation within affected members confirmed to have the same mutation. Despite the establishment of an XLA mutation registry BTKbase (<http://www.uta.fi/mut/bioinfo/BTKbase>) in 1994,⁵ there have been no clear genotype-phenotype correlations established.

Other factors such as infection exposures have been postulated as possible reasons for phenotypic variation. However, family members are likely to be exposed to similar infections, and older family members are less likely to have received frequent antibiotics in their youth, making this explanation less plausible.

Other possible explanations for the phenotypic variability are interacting genetic factors including mannose-binding lectin variant alleles. A previous study has shown that the severity of lung disease in patients with cystic fibrosis can be associated with heterogeneity of the

mannose-binding lectin,⁶ and several studies have looked at the risk of invasive pneumococcal disease and MBL genotype and have shown an increased susceptibility in patients who are MBL homozygotes.⁷ Summerfield et al⁸ have also reported a patient aged 56 years who presented with cryptosporidial diarrhea and was found to have MBL deficiency and isolated IgA deficiency. Factors such as this might be playing a role in the phenotypic variability in this and other families with XLA.

This grandfather showed evidence of delayed onset to measurable hypogammaglobulinemia; however, he had a clear life-long history of infections that might be associated with Ig deficiency. His diagnosis was delayed because of lack of measurable gamma globulin deficiency. It raises the question about whether earlier testing for B-cell numbers or mutations in the BTK gene might have altered his treatment or subsequent life course.

This patient also highlights the value of measuring Ig subclasses and specific antibody responses in patients with a suggestive history of immunodeficiency. Selective IgA deficiency might be associated with IgG2 subclass deficiency and greater risk of infection, despite normal total IgG levels. Patients with an IgG2 subclass deficiency might be unable to produce antibody to polysaccharide antigens such as pneumococcus. This ability can be tested by immunization with the currently available pneumococcal vaccinations, with levels of pneumococcal-specific antibody measured before and after vaccination. Antibody-specific responses can also be measured by using protein antigens such as tetanus toxoid and *Haemophilus influenzae*.⁹ These additional measurements can clearly be of benefit in patients with a clinical history of recurrent infections in whom the total Ig levels might be normal.

In summary, we present a set of cases reinforcing the absence of genotype phenotype correlation in patients with identical mutations in the BTK gene and highlight that late presentation of the disease might occur. XLA is not restricted to presentation in childhood, and patients with a characteristic clinical history and immunologic findings should be screened for this disease.

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Occupational asthma and IgE sensitization to 7-aminocephalosporanic acid

To the Editor:

Cephalosporins cause occupational asthma, and it has been suggested that the pathogenesis is mediated by IgE.^{1,2} 7-ACA (aminocephalosporanic acid) is a major intermediate metabolite in the synthesis of cephalosporin. There has been one published report of occupational asthma caused by 7-ACA, in which a nonimmunologic mechanism was suggested.³ Ours is the first investigation to detect serum IgE specific to 7-ACA-HSA (human serum albumin) conjugate in an exposed worker with occupational asthma.

This report describes the clinical findings in two asthmatic workers in a pharmaceutical plant who were exposed to 7-ACA powder used to synthesize ceftriaxone. To evaluate the IgE response, skin prick tests (SPTs) were performed in 5 exposed workers. To confirm bronchial sensitization in the two symptomatic workers, a methacholine bronchial challenge and specific bronchial provocation tests (BPTs) with 7-ACA and ceftriaxone were done. 7-ACA-HSA and ceftriaxone-HSA conjugates were prepared in our laboratory according to a method described previously.⁴ For IgE-ELISA, sera was incubated in 7-ACA-HSA conjugate or sham HSA coated well, and anti-human IgE and second antibody were added. Twenty nonatopic, healthy subjects with no history of exposure, who had negative SPTs to ACA and ceftriaxone, were enrolled as control subjects. Final absorbance value was determined by subtraction from that of sham HSA coated well. The positive cutoff value for specific IgE was determined by using the mean $\pm$ 3 SD of the absorbance values (patient LB). For the ELISA inhibition test, serum with high levels of specific IgE was preincubated with the free forms of 7-ACA and ceftriaxone, as well as with 7-ACA-HSA and ceftriaxone-HSA conjugates, and then subjected to IgE ELISA.

Table I summarizes the clinical features of the five exposed workers. None of the asymptomatic workers had positive responses to 7-ACA or ceftriaxone on SPT. However, two asthmatic subjects with work-related respiratory symptoms with or without rhinitis were found. Both patients had airway hyperresponsiveness to methacholine. Patient LB had a strong positive response to 7-ACA on SPT and had immediate bronchoconstriction after inhaling 7-ACA-powder solution during the specific BPT. Furthermore, high serum-specific IgE binding to 7-ACA-HSA conjugate was noted on ELISA (Fig 1, A). Patient NS had a negative response to 7-ACA on SPT but developed significant bronchoconstriction on 7-ACA BPT. Serum-specific IgE antibody was not detected. None of the workers had positive responses to ceftriaxone on specific BPT. When the IgE-ELISA inhibition test was performed by using the serum from patient LB, significant inhibition was noted in a dose-dependent manner with the serial addition of 7-ACA-HSA conjugate, whereas there was partial inhibition with free 7-ACA (Fig 1, B). Minimal inhibition was noted with free ceftriaxone, sham HSA, or ceftriaxone-HSA conjugate.

The pathogenesis of occupational asthma induced by low-molecular-weight drugs requires further study. These drugs may act as haptens and combine with a protein to elicit an immunologic response. In this study, we can suggest two different pathogenic mechanisms leading to occupational asthma caused by 7-ACA. In patient LB, an IgE-mediated mechanism was the major mechanism, as he showed positive responses on SPT and BPT to 7-ACA and had high serum levels of IgE specific to 7-ACA-HSA conjugate. By contrast, although patient NS clearly had work-related asthma symptoms and a positive response to 7-ACA on BPT, serum-specific IgE was undetectable,