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# X linked agammaglobulinaemia with a 'leaky' phenotype

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**Abstract**

**Typical X linked agammaglobulinaemia (XLA) is characterised by absence of immunoglobulin production and lack of mature B cells. The gene responsible for XLA has recently been identified, and codes for a B cell tyrosine kinase, BTK. A family affected by a B cell immunodeficiency, which is less severe than classical XLA, is described but they had a pedigree suggestive of X linked inheritance. Demonstration of a mutation in the *BTK* gene confirms that this is a mild form of XLA.**

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X linked agammaglobulinaemia (XLA) is a disorder of B cell maturation characterised by absence of circulating mature B cells, lack of detectable immunoglobulin of all classes, and of specific antibody production. [1] Affected boys present with severe bacterial infections after passive maternal immunity has declined, but usually before the age of 2 years, and require lifelong treatment with immunoglobulin replacement to prevent potentially fatal infections. The gene responsible for XLA was recently identified as that coding for a tyrosine kinase, BTK, [2] and mutations in this gene have now been demonstrated in a large number of families with typical XLA phenotypes. [3] The function of the *BTK* gene is not yet known, but, consistent with the phenotype, it has been shown to be essential for normal development of mature B cells by demonstration of apparent unilateral X chromosome inactivation in B cells of obligate female carriers of XLA. [4] BTK is also expressed in a number of other cell series, including monocytes and neutrophils, and is probably involved in intracellular signalling pathways.

*Table 1 Investigations in three cases studied*

| | 1 | 2 | 3 |
|---|---|---|---|
| Haematology | | | |
| Leucocytes ($\times 10^9$/l) | 3.0 | 8.3 | 7.2 |
| Neutrophils ($\times 10^9$/l) | 0.69* | 3.32 | 0.86* |
| Lymphocytes ($\times 10^9$/l) | 1.77 | 4.23 | 5.68 |
| Blood group | O Rh + | O Rh + | ND |
| Isohaemagglutinins | | | |
| Anti-A | 1:4* | 0* | ND |
| Anti-B | 0* | 0* | ND |
| Immunology | | | |
| IgG (g/l) | 8.0 9 | 3.84 | 4.00 |
| IgA (g/l) | 0.46 | 0.16* | < 0.03* |
| IgM (g/l) | 0.25* | 0.22* | 0.07* |
| $IgG_1$ (mg/100 ml) | 488 | 204* | ND |
| $IgG_2$ (mg/100 ml) | < 3* | < 3* | ND |
| $IgG_3$ (mg/100 ml) | 30* | < 2* | ND |
| $IgG_4$ (mg/100 ml) | 12 | < 2* | ND |
| Lymphocyte subpopulations (%) | | | |
| CD3 | 85 | 86 | 92 |
| CD4 | 44 | 41 | 67 |
| CD8 | 44 | 48 | 26 |
| CD19 | 1* | 0* | 2* |
| Vaccine responses | | | |
| Diphtheria | Positive | Negative* | ND |
| Tetanus | Positive | Positive | Positive |
| Pneumococcus | Negative* | Negative* | ND |
| Chromosomes | Normal | Normal | Normal |

* Low.
ND = not done.

## Case reports

We describe three brothers who were all affected by an immunodeficiency characterised by low B cell numbers and hypogammaglobulinaemia, but who had normal T cell numbers and function. Their immunodeficiency was less severe than typical XLA, but the pedigree was highly suggestive of X linked inheritance. There was no previous family history. The case histories follow and the results of immunological investigations are shown in table 1.

Case 1 presented at 2 years with pneumococcal pneumonia and empyema requiring thoracotomy, and he had a history of recurrent chest infections and severe otitis media. He developed pneumococcal meningitis at 5 years, at which time the diagnosis of hypogammaglobulinaemia was made. Since then he has been maintained on prophylactic penicillin only. He recently received a course of immunoglobulin treatment during a chest infection. He has received all his routine immunisations, as well as Pneumovax.

Case 2 also presented at the age of 2 years, with a cervical abscess, followed several months later by an episode of pneumococcal meningitis. At 3 years he developed pneumococcal pericarditis requiring pericardiectomy. This occurred concurrently with his elder brother's pneumococcal meningitis, and as a result both boys were investigated, and found to have hypogammaglobulinaemia. He also receives prophylactic penicillin and remains well. He has received the same immunisations as his elder brother.

Case 3 was screened at the age of 8 weeks in view of the history of immunodeficiency in his older brothers.

None of the boys has so far received regular immunoglobulin replacement treatment.

Investigations (table 1) revealed variable degrees of hypogammaglobulinaemia in the three brothers. In case 1, total IgG and IgA concentrations were within the normal range, but IgG subclass 2 was undetectable. All three had very low (absent in one) B cell (CD19 positive) numbers. Responses to vaccine antigens were variable, but notably positive to tetanus in all three and to diphtheria in case 1. Iso-

haemagglutinin values were low in the two elder boys. Cases 1 and 3 were moderately neutropenic at presentation, a finding not uncommon in XLA as well as other immunodeficiencies. None of the boys has a phenotype typical of XLA.

In view of the strong possibility of an X linked disorder, and the immunological findings suggestive of a mild form of XLA, further investigations were performed to elucidate the underlying defect in this family.

**X chromosome inactivation analysis**

At an early stage in the development of all normal female cells, one X chromosome becomes inactivated (lyonisation). In female carriers of XLA, B cell precursors which retain as active the X chromosome carrying the abnormal *BTK* gene fail to mature. This results in an apparently 'non-random' or 'unilateral' pattern of X chromosome inactivation in mature B cells, which contrasts with the random pattern seen in other haemopoietic cell series and other tissues, where the *BTK* gene is not essential for normal development. [4]

Purified B cells from the mother of the three affected boys were shown to demonstrate unilateral X inactivation using a method dependent on the different methylation states of active and inactive X chromosomes. [5] The X inactivation pattern in maternal whole blood was random. This difference was strongly suggestive that she was a carrier of an X linked immunodeficiency affecting B cell development, the most likely explanation being a modified form of XLA.

**Mutation analysis**

In order to test this hypothesis, *BTK* mutation analysis was performed in the affected boys, together with a large number of other boys with 'classical' XLA phenotypes. Various mutations were identified throughout the gene. [3] The analysis was performed on cDNA, which was prepared from mRNA purified from peripheral blood. The gene was divided into seven overlapping fragments, each of which was amplified using the polymerase chain reaction, and screened for the presence of mutations using the technique of single strand conformation polymorphism analysis. [6] Any fragment found to contain an alteration was sequenced. Analysis of cDNA prepared from the three affected boys in the family described revealed a single nucleotide alteration (C to A) at position 1952. This resulted in a non-polar to polar amino acid substitution (alanine to aspartic acid) in the kinase domain near the C terminal end of the BTK protein.

**Discussion**

Boys affected by X linked agammaglobulinaemia present with severe bacterial infections after passive maternal immunity has declined. Typically they have absent mature B cells in peripheral blood and undetectable serum concentrations of all immunoglobulin isotypes. Two of the brothers in this family presented with severe pneumococcal infections, but their immunodeficiency is less severe than would be expected in typical XLA, with respect to both age of presentation and lack of requirement for immunoglobulin replacement. All three boys have detectable immunoglobulin concentrations, and some specific antibody production. However, the occurrence of three affected male siblings strongly suggests X linked inheritance, and the phenotype bears a closer resemblance to XLA than to other described X linked immunodeficiencies. Demonstration of non-random X chromosome inactivation in purified B cells from the mother provides further evidence of an X linked disorder affecting the B cell lineage. Identification of a mutation in the *BTK* gene confirms the diagnosis of a 'forme fruste' of XLA. This mutation results in a relatively conservative amino acid substitution, which presumably disrupts, but does not completely abolish, the kinase activity of the protein, allowing retention of some enzyme activity, and generating the mild clinical phenotype seen in this family. This is in contrast to mutations found in most typical XLA patients, where more profound alterations in amino acid structure result in complete loss of function.

Significant numbers of families exist in which there are sporadic male cases of hypogammaglobulinaemia with low but not absent B cell numbers. Usually there is no clear diagnosis in these boys. After the identification of the gene responsible for XLA (and also that for X linked hypogammmaglobulinaemia with hyper-IgM), and the recognition that mild forms of XLA exist, it is likely that the underlying defects will now be definable in at least some of these families. This should also allow accurate prenatal diagnosis and carrier detection in individuals at risk, as well as contributing to further elucidation of the nature of the functional defect in XLA.


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