SHORT REPORTS

A new restriction fragment length polymorphism at the DXS101 locus allows carrier detection in a family with X linked agammaglobulinaemia

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Abstract

The gene responsible for X linked agammaglobulinaemia (XLA) lies in Xq22 and has recently been identified as *atk*. DXS101 is a polymorphic locus which is closely linked to the disease locus. In this report we describe the identification, by pulsed field gel electrophoresis, of a new polymorphism at the DXS101 locus with a predicted heterozygosity of 4.9%. Despite this low value, we show how this polymorphism has been important in carrier status determination in a family with XLA where assessment was not possible by other means.

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X linked agammaglobulinaemia (XLA) is a rare genetic immunodeficiency disease characterised by a low level or absence of circulating B cells and thus a lack of immunoglobulins of all subclasses. The gene responsible, *atk*, has recently been isolated and found to encode a new non-receptor tyrosine kinase expressed mainly in B lineage cells.¹ This gene was isolated by a positional cloning approach which relied on the precise localisation of the XLA locus in Xq22. The order of loci was determined as: *cen*-DXS3-DXS366-DXS442-XLA-(DXS178, DXS265)-DXS101-DXS94-DXS17-tel.¹⁻³ The DXS101 polymorphic locus was identified as a distal flanking marker for the XLA locus on the basis of a recombination event in a family affected by this disorder² and is estimated to lie about 600 to 700 kb distal to the *atk* gene.¹ There are additional copies of DXS101 interspersed throughout Xq22.⁴ We have recently isolated single copy probes for two of the DXS101 sequences, one of which maps near to the polymorphic locus and the second of which lies near the DXS54 and PLP loci.⁵ DXS54 and PLP lie between DXS178 and DXS94, within 2 Mb distal to DXS178.⁶

In an attempt to identify patients who may have deletions in the region of the XLA gene we investigated patient DNA by pulsed field gel electrophoresis (PFGE) analysis and probes which map to this region. We found that one of the fragments recognised by the DXS101 probe cX52.5 was altered in size in two of these patients. Similar analysis of a panel of 26 normal female controls, showed

two subjects heterozygous for this fragment, confirming that it was a new, rare, restriction fragment length polymorphism (RFLP), and not a deletion or other alteration associated with the XLA gene. Application of the single copy probes for DXS101⁵ showed that the RFLP lay in the vicinity of the PLP gene locus. Use of this RFLP has allowed carrier status determination in a female in a family with XLA which had previously not been possible since DNA from one key family member was unavailable.

Materials and methods

Very high molecular weight DNA was prepared in agarose plugs from blood leucocytes collected from XLA patients, normal controls, and members of family LO.⁷ Diagnosis of XLA was based on patients having severely decreased serum immunoglobulin levels and circulating B cells.

DNA in agarose plugs was restricted with *Eag*I (New England Biolabs) using a large excess of enzyme, according to the manufacturer's recommendations, and separated by PFGE on 1% agarose gels, electrophoresed at 170 V, with a switch time of 70 seconds for 40 to 44 hours at 8°C, using the LKB Pulsaphor system with CHEF electrode array.⁷ These gels were blotted onto Hybond N+ membranes (Amersham Int, UK) and the membranes hybridised with either probe B550⁵ which recognises the copy of the DXS101 sequence associated with the new polymorphism, or probe cX52.5 which recognises all copies of DXS101.⁴ Genomic DNA was investigated by Southern blot analysis and hybridisation with single copy probes for the DXS3, DXS366, DXS178, and DXS17 loci.⁸ All probes were labelled to a high specific activity with [³²P]-dCTP using the random priming technique.

Results and discussions

Patient and control DNA samples, digested with various rare cutting restriction enzymes, were analysed by PFGE and hybridisation. The DXS101 probe cX52.5 identified *Eag*I fragments of 250 kb, 350 kb, 400 kb, and 780 kb

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Figure 1 (A) DNA from two unrelated XLA patients (BC and DLO) together with DNA from unaffected males (m) digested with *EagI* and hybridised with cX52.5. (B) DNA from four normal females (f) digested with *EagI* and hybridised with cX52.5. The 810 kb fragment can be seen in one of the females along with the more common 780 kb fragment. LM indicates the zone of limiting mobility of the DNA. Chromosomes from *S cerevisiae* strain YP148 were used as size markers.

Figure 2 DNA from members of family LO digested with *EagI* and hybridised with probe B550.

in control DNA (fig 1A). Analysis of the patient DNA samples identified two subjects in whom the 780 kb fragment was apparently increased in size to 810 kb (fig 1A). Probe B550, which maps close to the PLP gene locus,⁵ also recognised the altered size fragment in these patients (results not shown).

To investigate whether this apparent size alteration could be because of a rare RFLP, DNA from a panel of 26 normal females and 27 normal males was digested and separated in the same way. Membranes were hybridised with probe cX52.5. Of the 27 normal males, none showed the larger sized fragment, but of the 26 normal females, two showed the larger fragment in addition to the 780 kb species (fig 1B and results not shown). That this altered fragment could arise as a result of methylation of the inactive X chromosome in these females was considered unlikely as the altered fragments were only observed in a total of two out of 26 normal females and two unrelated affected males. It seemed more likely that we were observing a new RFLP with two alleles, 780 kb and 810 kb. Frequencies for the 780 kb and 810 kb alleles were calculated as 0.975 and 0.025, respectively, giving a heterozygosity for the locus of 4.9%. This very low value would normally lead to a probe being dismissed as not useful for genetic linkage analysis, as the great majority of subjects would be uninformative and the chromosome with which the disease is travelling could not be identified.

Although the low level of heterozygosity of this RFLP will limit its usefulness in the majority of XLA families, we have identified informative females in two out of 20 XLA families investigated so far and we have been able to use this probe for carrier status determination in one of these families. One of the patients (DLO) who possessed the 810 kb allele had a maternal half sister (CM) for whom it had previously been impossible to ascertain carrier status as DNA from her father was not available. Since both she and her mother were heterozygous with all the probes tested, DXS3, DXS366, DXS178, and DXS17 (results not shown), it was not possible to determine which X chromosome she had inherited from her father. Her mother (KLO) was an obligate carrier as she had an affected brother in addition to her affected son. To give a carrier risk to the half sister, CM, her mother would have to be informative (heterozygous) with a closely linked probe, while she herself would have to be uninformative (homozygous) with the same probe. Since her affected half brother had the rare 810 kb allele with probe B550, it was likely that his mother would be heterozygous, as the frequency of the 810 kb allele made homozygosity unlikely. In addition, if CM had inherited an 810 kb allele this would probably have come from her mother as its low frequency in the population makes it unlikely that her father would also possess this allele.

DNA from the mother and half sister was analysed as previously with probe B550 (fig 2). The mother, KLO, proved to be heterozygous

as expected. The daughter, CM, was found to be homozygous for the 780 kb allele. She had therefore inherited the 780 kb allele from her mother, the opposite to that inherited by her affected half brother. She could now, therefore, be given a carrier risk assessment of approximately 5% based on the frequency of recombination between XLA and DXS94 and DXS17. The cumulative two point lod score between XLA and DXS94 is 11.26 at $\theta_{\max} = 0.05$ and between XLA and DXS17 is 13.04 at $\theta_{\max} = 0.05$.² In this instance the low frequency of the 810 kb allele would have allowed us to give a probable diagnosis even had CM proved to be heterozygous as this could have been based upon the probability of her father also having the 810 kb allele (0.025) in combination with the probability of recombination (0.05). This would have been substantially different from the 50% probability of carrier status that is the case for all daughters of known carriers of X linked diseases before any analysis is carried out.

This probe also identifies another member of the pedigree, MKE, as a probable carrier as she is also heterozygous at this locus (fig 2), although she has had no affected boys. One of her daughters, EKE, does not appear to have inherited the affected chromosome and her grandson, AKE, who does not have XLA, has the 780 kb allele as might be expected. Furthermore, MKE has five additional daughters for whom carrier assessment using this probe may be informative.

Although the gene that is causative of XLA in the majority of families has been identified, it is not yet possible to use it for carrier assessment in the majority of cases. No polymorphisms associated with the gene have as yet been identified and the majority of families affected by XLA investigated so far appear to have mutations which do not result in gross

changes in the gene (unpublished observations). Those mutations that have been detected so far appear to be unique to families which means that mutation analysis will have to be performed for each carrier on a family basis. The family described here has no obvious detectable alteration in the *atk* gene (our unpublished observations) and so it is not possible at present to determine carrier status in this family definitively. This study has shown that use of such rare RFLPs may facilitate diagnosis of carrier status in a minority of families.

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