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Detection of translocation breakpoints by pulsed field gel analysis: practical considerations

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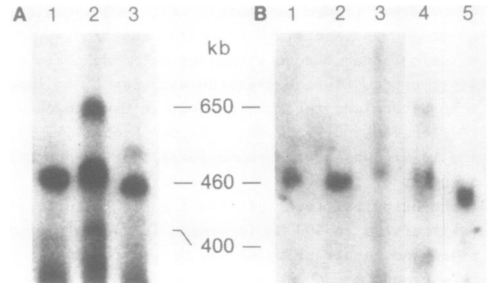
Analysis of chromosomal translocation breakpoints by pulsed field gel electrophoresis has the potential for bridging the gap between identification of tightly linked markers and initial molecular mapping of human disease gene(s) of interest. We are screening Xq24-q26 probes by field inversion gel electrophoresis (1) to identify a marker that detects an altered restriction fragment containing the translocation breakpoint in a female patient with the X-linked Lowe Oculocerebrorenal Syndrome and a de novo X,3 translocation (t(X;3)(q25;q27)) which most likely disrupts the disease locus at Xq25 (2,3). Several controls are necessary to determine whether an altered fragment does, in fact, contain the breakpoint or is due to rare polymorphisms and/or methylation differences. In particular, we have found that methylation differences arising during cell culture can be a misleading source of altered fragments.

One marker, St 1 (DXS86, Xq26) detects altered BssH II fragments of 400 kb and 650 kb in the translocation patient's lymphoblast line in addition to the normal 460 kb fragment present in the patient and five control lymphoblast lines. However, results from follow up experiments reveal that the most likely explanation for the altered fragments is differences in methylation of the BssH II sites in the patient's lymphoblast line rather than identification of the translocation breakpoint. First, the altered BssH II fragments are not detected in blood samples from the patient's parents and therefore are not due to rare restriction site variants (fig.A, lane 1, father; 2, patient's lymphoblast line; 3, mother). Next, we attempted to confirm the result in a different tissue and found that the altered fragments are not present in the patient's fibroblasts (not shown). Third, we obtained a new blood sample from the patient to test uncultured cells directly. Only the normal 460 kb fragment is present is present in the patient's blood, fig.B, lane 2 (lane 1, normal male; 3, normal female; 4, patient's lymphoblast line). Finally, an altered fragment containing the breakpoint would also be expected to be present in a somatic cell hybrid made from the patient's lymphoblasts and retaining the derivative chromosome 3 (including Xq25-qter) and this was not found (fig.B, lane 5).



Our studies point out the need for controls to confirm that an altered restriction fragment detected in a translocation patient does contain the breakpoint. In some cases, identification of the breakpoint is supported by the presence of altered fragments detected with several restriction enzymes, eg. Fountain et al.(4) report altered fragments detected with three enzymes in both parent and hybrid cell lines. In contrast, two different translocation breakpoints described by Compton et al. (5) were each detected with one probe and one enzyme combination. As the authors point out, differences due to polymorphism and/or methylation have not been excluded in their study. Reports of variability of methylation patterns in cultured fibroblasts (6) and lymphoblasts (7) combined with methylation-sensitivity of rare-cutting restriction enzymes makes confirmation of altered fragments in more than one tissue, somatic cell hybrids, and/or uncultured cells important controls for identification of a translocation breakpoint.

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