Elucidation of structural abnormalities of the X chromosome using fluorescence in situ hybridisation with a Y chromosome painting probe

R T Howell, Rachel Millener, Susan Thorne, Jill O'Loughlin, J Brassey, A McDermott

South Western
Regional Cytogenetics
Centre, Southmead
Hospital, Bristol BS10
5NB, UK
R T Howell
R Millener
S Thorne
J O'Loughlin
J Brassey
A McDermott

Correspondence to
Dr Howell.

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Abstract
Particular regions of the X and Y chromosomes share DNA sequence homology to the extent that cross hybridisation occurs. Thus, chromosome painting with a whole Y chromosome probe consistently results in fluorescence on specific regions of the X chromosome as well as the complete Y chromosome. This phenomenon has been exploited to elucidate the structure of unusual X chromosome rearrangements, without Y involvement, in two females.

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Identical DNA sequences are found in the X and Y chromosomes within the pseudoautosomal region located at the tip of the short arm of each sex chromosome.¹ There are also sequences which share greater than 95% homology outside this region, mapping to the proximal part of the long arm of the X and to the short arm and proximal segment of the long arm of the Y.^{2,3}

When undertaking fluorescence in situ hybridisation with a painting probe constructed from flow sorted Y chromosomes it is usual to observe hybridisation to those parts of the X chromosome sharing homology with Y sequences,⁴ the regions displaying a positive fluorescent signal being the distal part of the short arm, corresponding to the pseudoautosomal region, and a portion of the long arm at band Xq21. This phenomenon has been exploited in two cases to confirm the structure of unusual X chromosome rearrangements, by making use of a commercially available Y chromosome painting probe (Cambio, Cambridge), biotinylated after its construction from flow sorted Y chromosomes.⁵

Chromosome preparations from standard whole blood cultures were analysed by trypsin G banding using Leishman's stain. Replication banding was achieved by Hoechst 33258 staining, followed by UV photolysis and Leishman's stain, in preparations from cultures in which a methotrexate block was released by 5 bromodeoxyuridine for the final six hours of culture.

For fluorescence in situ hybridisation studies, slides were subjected to RNase treatment followed by a serial dehydration in ethanol. The chromosome preparations were denatured in 70% formamide/2 × SSC at 70°C for two minutes before hybridisation, and the probes were denatured for 10 minutes at 70°C and preannealed for two hours to allow the competitor DNA (whole human DNA) to suppress repeated sequences. Hybridisation took place at 37°C overnight (17 hours). A single stringency wash was performed in 50% formamide/1 × SSC at 43°C for 10 minutes. The probe was visualised by the two layer avidin-fluorescein isothiocyanate (FITC) detection system (avidin-FITC, biotin-antiavidin, and avidin-FITC).⁶ Slides were mounted in antifade solution (AF1, Citifluor) containing DAPI (4',6-diamidino-2-phenylindole, 0.8 µg/ml) and propidium iodide (0.4 µg/ml) and analysed under a Leitz Diaplan epifluorescence micro-

Figure 1 X chromosomes from case 1, G bands and ideogram. The deleted X is to the right. The arrows on the ideogram define the extent of the deletion.

Figure 2 Case 1: fluorescence in situ hybridisation with the Y chromosome paint showing hybridisation to the tip of the short arm of the normal and deleted X chromosomes, and also to Xq21 in both.

scope fitted with a 50 W mercury lamp and a Leitz I2/3 filter.

Case reports

CASE 1

Case 1 was a 3 year old girl referred for chromosome studies because of developmental delay, hypotonia, short stature, and dysmorphic features including epicanthic folds, widely spaced nipples, and webbed neck. G banded analysis showed loss of part of the short arm of the X chromosome, considered to be either an interstitial deletion or possibly an unbalanced translocation product (fig 1). In situ hybridisation with the Y chromosome paint confirmed the abnormality to be an interstitial deletion, a positive fluorescent signal being found on the tip of the short arm of

the deleted chromosome as well as the tip of the normal X short arm (fig 2). Use of an X chromosome paint, not illustrated, showed hybridisation to the whole of the abnormal chromosome, providing further evidence that no autosomal material was involved, and the karyotype was interpreted as 46,X,del(X)(p11.3p22.2). The deletion was subsequently identified in the mother and grandmother of the patient, neither of whom displayed any significant abnormal clinical features. Replication banding showed the abnormal X to be late replicating in all cells of the child, the mother, and the grandmother.

Herva *et al*⁷ reported an apparently identical deletion to this family in two generations of phenotypically normal females ascertained through a single mentally retarded girl, the proband.

CASE 2

Case 2 was a 31 year old woman with primary amenorrhoea who had originally been reported by a laboratory in Asia as having a 45,X karyotype. As she had normal development and no stigmata of Turner's syndrome apart from ovarian agenesis, a sample was sent for reinterpretation. She was found to have a mosaic karyotype with approximately 75% of cells having a 45,X complement while the remainder contained 46 chromosomes including an abnormal X, apparently a duplication deficient structure with short arms at both ends. C banding showed the abnormal chromosome not to be dicentric and it was considered to have the structure Xpter→q21.2::p11.3→pter (fig 3).

This conclusion was supported by in situ hybridisation using the Y chromosome paint (fig 4), the presence of a fluorescent signal at each end of the abnormal chromosome confirming the interpretation of G banded analysis. The long arm breakpoint, although within Xq21, could be localised proximal to the region of cross hybridisation owing to the absence of fluorescence in this region. The abnormal X chromosome was shown to be universally late replicating.

Although the duplication deficient X chromosome described here is apparently unique, similar X chromosome rearrangements with other breakpoints have been reported previously, for example, monocentric in a girl with mental retardation and dysmorphic features,⁸ and dicentric in a female with stigmata of Turner's syndrome.⁹

Discussion

In situ hybridisation painting kits are now widely available for use in the routine diagnostic laboratory, and the straightforward application of a commercially available Y paint in rapidly elucidating X chromosome abnormalities is aptly demonstrated here. However, it is clear that caution should be used in the interpretation of results of Y painting in cases where unidentified sex chromosome material may possibly be of X or Y origin.

Figure 3 X chromosomes from case 2, G bands and ideogram of the abnormal chromosome. The duplication deficient X is to the right.

Figure 4 Case 2: fluorescence in situ hybridisation with the Y chromosome paint showing hybridisation to both ends of the duplication deficient chromosome. Note the absence of fluorescence in the proximal part of the long arm of the abnormal chromosome.

Phenotypic variation, ranging from normal to Turner's syndrome, in females with deletion of the short arm of the X chromosome is well recognised.¹⁰ The occurrence of clinical abnormality only in the probands of the families in case 1 and that cited by Herva *et al*,⁷ two instances with an apparently identical interstitial short arm deletion, is remarkable, and

similarly suggests variability in the phenotypic effect of X chromosome short arm deletions. Jauch *et al*,⁴ when using a Y chromosome paint, suggested the cross hybridising region on the X long arm to be Xq13. However, in case 2, the absence of fluorescence on the proximal part of the abnormal arm of the duplication deficient chromosome permitted localisation of the cross hybridising region to Xq21.3, distal to the breakpoint at Xq21.2. Cell lines are not currently available from these patients.

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