A Restriction-Fragment-Length Difference Detected by the Anonymous Probe DXS199 Exhibits Non-Mendelian Inheritance

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Summary

Anonymous DNA probes were isolated from an X chromosome–enriched flow-sorted library. One of these probes, DXS199, identified a restriction-fragment difference that failed to show Mendelian segregation. All normal females were found to have two AvaII fragments of 6.5 kb and 6.0 kb, whereas all normal males had only the 6.5-kb fragment. DNA from a 49,XXXXY male was found to have both 6.0- and 6.5-kb AvaII fragments, in the same 3:1 ratio as seen in the inactive:active number of X chromosomes. This variant, which reflects a structural difference between active and inactive X chromosomes, is likely to be due to a methylation site on the active X chromosome.

Introduction

Differences between individuals in the structure of their DNA can be conveniently detected by hybridizing Southern (1975) blots with appropriate DNA probes to reveal restriction-fragment variation. These differences in DNA structure, known as RFLPs (Botstein et al. 1980), are being sought in order to construct linkage maps (White et al. 1985) and to provide markers for inherited disease (Goodfellow et al. 1985). RFLPs invariably have shown codominant inheritance, as is expected for markers that detect structural features of DNA, although rare dominant or recessive inheritance was anticipated (Botstein et al. 1980). We now report a restriction-fragment-length difference (RFLD), detected by the anonymous probe DXS199 located on the short arm of the X chromosome, that does not follow classical Mendelian inheritance. This variant is correlated with the presence of an inactive X chromosome and consequently provides a molecular marker for the latter.

Material and Methods

DNA Probe

The anonymous probe DXS199, a single-copy 3.3-kb HindIII fragment, was isolated from a flow-sorted X-chromosome library (Kunkel et al. 1982) and localized to the distal short arm of the X chromosome, Xp11-Xpter (Starr and Wood 1987). This probe detects AvaII fragments at 6.5 and 6.0 kb.

Human DNA Samples

Cultured lymphoblasts obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research (Camden, NJ) were used for DNA preparation. Members of the Utah pedigrees (White et al. 1985) were used for both polymorphism screening and segregation analysis. DNA also was prepared from GM3384 lymphoblasts derived from a 49,XXXXY male.

Hybridization

AvaII restriction fragments were separated by electrophoresis on a horizontal 0.5% agarose gel and transferred to Nytran® (S & S). The Xp-specific probe DXS199 was ^{32}P-labeled using random priming (Feinberg and Vogelstein 1984). Prehybridization for ≥2 h at 62 C in 6 × SSC, 5 × Denhardt's, 100 μg sheared salmon sperm DNA/ml, 0.1% SDS was

Table I

Phenotypes Observed in AvaII Digests Probed with DXS199

Fragment Size (kb)	Male	Female
6.5	10	0
6.5/6.0	...	21
6.0	0	0

followed by overnight hybridization in the same solution containing ^{32}P-labeled DNA probe. The filter was washed in 0.1 × SSC, 0.1% SDS for 15 min at room temperature and twice at 62 C for 30 min. The filter was autoradiographed for 2 days using Kodak XRP-1 X-ray film with DuPont Cronex Lightening Plus® intensifying screens.

Results

Typing AvaII digests of unrelated individuals with DXS199 revealed two peculiarities. No individual was found who possessed the 6.0-kb fragment alone, and the restriction-fragment pattern was correlated with the sex of the individual (table 1). All males had a single fragment at 6.5 kb, whereas all females had fragments at 6.5 and 6.0 kb. These data show that DXS199 does not detect a two-allele system that is in Hardy-Weinberg equilibrium ($\chi^2 = 29.2$; $P < .01$).

We then examined the segregation of the AvaII variant within families (fig. 1). Four families with a total of 16 male and 13 female offspring were typed. Again we obtained an unanticipated result, since all
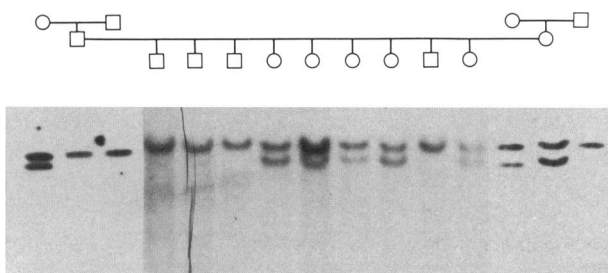
Figure 1 AvaII digests of Utah pedigree K-1331 probed with DXS199. The lymphoblasts used for DNA preparation were, from left to right: GM7340, GM7057, GM7007, GM6992, GM7030, GM6983, GM6988, GM6999, GM7005, GM7059, GM7033, GM7023, GM7050, GM6990, and GM7016. The greater intensity of the upper 6.5-kb band in GM6999 and GM7005 was not reproducible.
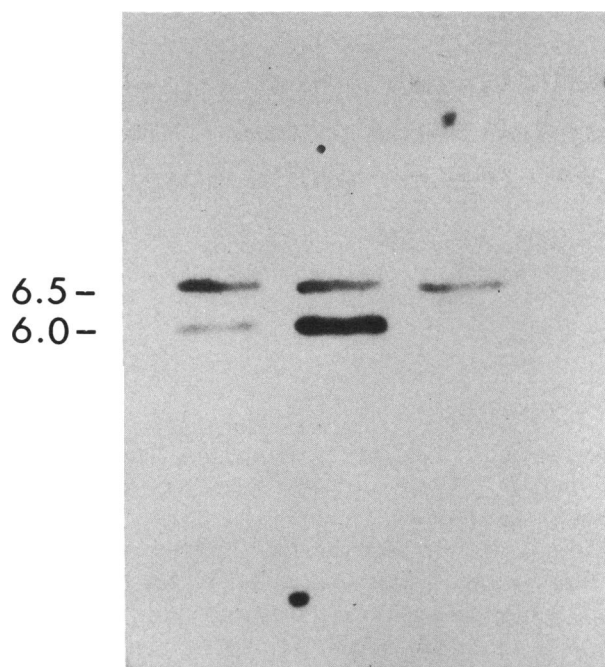
Figure 2 AvaII digests of genomic DNA hybridized with the probe DXS199. The lymphoblasts used for DNA preparation were, from left to right: GM7041, a normal 46,XX female; GM3384, a 49,XXXXY male; and GM7046, a normal 46,XY male.

females had bands at 6.5 and 6.0 kb whereas all males had a single 6.5-kb band. It thus appeared as if mothers invariably transmitted the 6.5-kb fragment to their sons and the 6.0-kb fragment to their daughters. This inheritance pattern was clearly non-Mendelian.

Variation that is not inherited might result from the secondary postzygotic structural alteration of chromosomal DNA that occurs as part of the X-inactivation mechanism in normal females (Lyon 1961). If the 6.0-kb fragment were produced by restriction digestion of DNA from inactive X chromosomes, then the intensity of this fragment would correspond to the number of inactive X chromosomes. This 6.0-kb fragment would also be present in males with multiple X chromosomes. Both of these predictions were tested (fig. 2) when DNA from a 49,XXXXY male was examined. The intensities of the 6.0- and 6.5-kb fragments in this male reflect the 3:1 inactive:active X-chromosome ratio. Thus the RFLD detected by DXS199 has the properties expected of a variant that arises owing to a structural feature of inactive X chromosomes.

Discussion

RFLPs detected by anonymous DNA probes derived from the human X chromosome have been found to exhibit a number of inheritance patterns. In addition to the expected X-linked pattern, some fragments exhibit Y-linked inheritance (Page et al. 1982). These fragments, which are derived from the Y chromosome, are detected by DNA probes from regions of the X chromosome that share homology with the Y. In addition, probes derived from the telomeric region of the short arm (Xp), which undergoes pairing and recombination with the Y chromosome, show either pseudoautosomal inheritance (Cook et al. 1985) or partial sex-linked inheritance (Rouyer et al. 1986).

The variant RFLD described here shows none of these inheritance patterns. It appears to be a consequence of a secondary somatic structural modification associated with the differential activity of X chromosomes. The difference between inactive and active chromosomes, which produces 6.0- and 6.5-kb AvaII fragments, respectively, mimics the gain of a site on the inactive chromosome or the loss of a site on the active chromosome. The gaining of a site on the inactive chromosome would require some form of structural alteration, which would need to be reversed prior to meiosis. There is, however, precedence for the loss or masking of sites by DNA methylation, and it has been proposed that the inactive X is inactivated by methylation (Gartler and Riggs 1983). AvaII is known to be sensitive to methylation, with the sequence $GG(A/T)C^mC$ being protected against digestion (Sutcliffe and Church 1978). Differential methylation between inactive and active X chromosomes has been described for a number of X-linked genes (Toniolo et al. 1984; Wolf et al. 1984; Yen et al. 1984; Keith et al. 1986). In these instances restriction sites on the inactive chromosome are masked by methylation. Contrary to these observations, our finding of the apparent loss of an AvaII site on the active X chromosome indicates that, at this particular site, the active X is methylated whereas the inactive X is not. At least one HpaII site with these same contrary methylation properties has been reported within the housekeeping HGPRT gene (Wolf et al. 1984; Vogelstein et al. 1985).

We have not observed other RFLDs when using DXS199 with any of 18 additional restriction enzymes, including HpaII and MspI. Whether the structural modification that we have detected represents a feature of the inactivation process or a feature of a transcribed element is unknown. An understanding of the molecular nature of this difference between active and inactive human X chromosomes must await cloning of the region involved.

Acknowledgments

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