

Paternal inheritance of translocation chromosomes in a t(X;21) patient with X linked muscular dystrophy

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SUMMARY A number of DNA probes from the short arm of the X chromosome have been used to study the inheritance of the translocation chromosomes in a girl with an X;autosome translocation and muscular dystrophy. The two translocation chromosomes were found to be derived from the father's single normal X chromosome, ruling out maternal inheritance of a pre-existent mutation and enhancing the concept that the de novo translocation is responsible for the dystrophic phenotype.

A neuromuscular disorder resembling Duchenne or Becker muscular dystrophy has been described in a number of females, each with an X;autosome translocation involving an exchange point in Xp21 at or near the site of the Duchenne locus. These females show preferential inactivation of the remaining normal X chromosome. A widely held view is that in the cells of these subjects the translocation has in some way disrupted the activity of the Duchenne or Becker gene on one X chromosome and non-random inactivation of the normal X chromosome has silenced the normal gene, thus causing the dystrophic phenotype.¹⁻⁷

This hypothesis is supported by the fact that, in all cases reported, the translocation and the neuromuscular disease have both appeared de novo in the affected female. In each case, however, the possibility has remained that the Duchenne mutation was inherited from a carrier mother independent of the translocation event. Demonstration that the translocation chromosomes are derived from a normal paternal chromosome would rule out this possibility and would further strengthen the argument that the disease in the affected female is a direct result of the translocation.

One of these patients is an affected female with the translocation t(X;21)(p21;p12). Although her mother was considered at one time to be a probable

carrier of the Duchenne mutation,⁸ closer examination failed to confirm her carrier status.⁷ Examination of the karyotypes for chromosome heteromorphisms failed to reveal the parental origin of the translocation chromosomes.⁷

We now report definitive evidence from a study of DNA restriction fragment length polymorphisms (RFLPs) that the patient's translocation chromosomes are of paternal origin.

Methods and results

To examine the inheritance of the patient's X chromosomes we used several DNA probes that reveal polymorphisms in the short arm of the X chromosome. Southern blot analysis (figure) on DNA from the patient showed that she is heterozygous for five of the markers (table). Her father, of course, has only one allele for each marker on his single X chromosome, allowing assignment of her paternal and maternal alleles at each locus.

To determine whether the paternal or maternal alleles are carried on the translocated chromosomes, DNA was prepared from a pair of somatic cell hybrids (patient fibroblasts fused to mouse A9 cells), each carrying only one of the two translocation chromosomes, der(X) or der(21).¹⁴ Southern blot analysis of these hybrids (figure and table) showed that for each marker it is the paternal allele that is carried on one or the other of the translocation chromosomes. We conclude that the transloca-

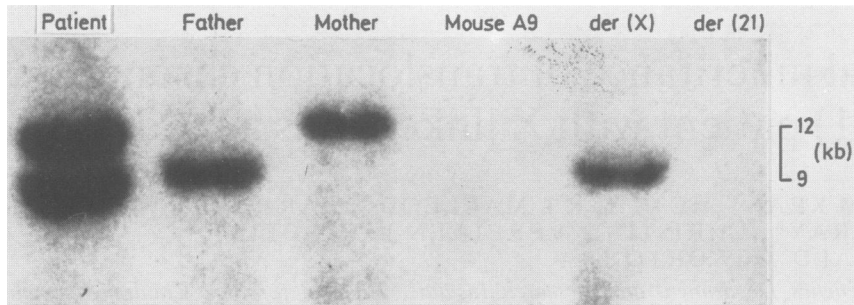


FIGURE Autoradiogram of lymphoblast DNA hybridised with the L1-28 probe. DNA was digested with *TaqI*, separated on 0.6% agarose gel, blotted to Zetabind, and hybridised at 42°C in 50% formamide with the L1-28 probe. The lanes marked der(X) and der(21) contain DNA from somatic cell hybrids, each carrying one of these translocation derived chromosomes and no normal X chromosome.

TABLE Summary of informative polymorphic marker data*.

Marker	Patient	Father	Mother	der(X)	der(21)
782	D1/D2	D2	D1/D1	-	D2
99-6	F1/F2	F2	F1/F2	-	F2
OTC	G3/G2	G2	G3/G2	G2	-
L1-28	A1/A2	A2	A1/A1	A2	-
58-1	L1/L2	L1	L1/L2	L1	-

*The five informative markers are designated by their common names. These, together with the allele designations, follow the convention set out by Bakker *et al.*⁹ The DNA probes were kindly provided by Drs P Pearson (754 and L1-28), L Kunkel (99-6 and 58-1), and A Horwich (OTC).¹⁰⁻¹³

tion originated in the X chromosome inherited from her father, ruling out the possibility that the translocation chromosome carries a pre-existing mutation for muscular dystrophy from a carrier mother.

The only caveat in this type of experiment, the possibility of non-paternity, is not an issue in this case. Analysis of red cell antigen and serum protein polymorphisms in the patient and her parents, coupled with the DNA polymorphism data reported here, results in a probability of non-paternity of only 0.02%. Furthermore, for two of the markers (782 and L1-28), the patient's mother is homozygous for the allele not found on the translocation chromosome, constituting absolute proof that the translocated chromosomes are not of maternal origin.

Discussion

Paternal inheritance of the translocation chromosome strengthens the argument that the muscular disease in this patient is a direct result of the chromosomal rearrangement, perhaps by disruption of the gene itself. This result is particularly interesting in light of recent detailed cytogenetic analysis¹⁵

revealing that the chromosomal exchange points in female patients may occur at opposite ends of band Xp21, separated by several hundred to a few thousand kilobases of DNA. If each of these translocations disrupts the activity of the Duchenne/Becker gene in band Xp21, then the affected locus must contain a very large gene, a multigene complex, or a single gene with cis acting regulatory elements separated from the gene by several hundred kb of DNA. Alternatively, at least some of the translocations may act by altering the chromatin structure in such a way as to suppress gene expression at a considerable distance from the site of the translocation. The recent isolation by molecular cloning of DNA sequences from the Duchenne/Becker locus,^{16,17} including sequences from the t(X;21) translocation junction described here,¹⁷ should allow the molecular characterisation of this region and a distinction among these various models.

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