Detection of germinal mosaicism in a DMD family

SARAH BULLOCK, CLIVE FELIX, SAMIRA ISKANDER-GABRA* and VAL DAVISON

West Midlands Regional Genetic Laboratory, Birmingham Women's Health Care NHS Trust or Clinical Genetics Unit, Birmingham Maternity Hospital, Birmingham B15 2TG, U.K.

Female carriers of Duchenne or Becker muscular dystrophy are usually asymptomatic and the identification of dystrophin gene deletions is confounded by the presence of a normal X chromosome. Various approaches to the problem have been described, including deletion analysis of small amounts of dystrophin mRNA transcribed in blood lymphocytes by reverse transcription and amplification using exonic primers [1]. Females carrying a deletion give products of both normal and reduced size which are identifiable by gel electrophoresis. A simpler strategy uses highly polymorphic markers within commonly deleted ('hotspot') regions of the dystrophin gene [2].

If enough informative family members are available, heterozygosity or hemizygosity of one or more of these loci can be determined and carrier status ascertained. We used the latter technique combined with fluorescence in situ hybridisation studies employing an intragenic cosmid. Cosmid probes specific for commonly deleted areas of the gene are proving to be very useful tools [3].

The pedigree of the family studied is shown in Figure 1. Initially, individual II.2 and her daughter (II.2) requested advice regarding their carrier status. The son of II.2 (boy II.3) had died of DMD and no DNA was available from this isolated case. Subsequently, one of II.2's sisters (II.4) also wanted to know her carrier status. Sisters II.2 and IV.4 both had relatively high creatine kinase levels whereas a further sister, II.6, lay within the normal range. Individual II.2 had not had her creatine kinase assayed.

Linkage analysis was performed on DNA extracted from whole blood using the intragenic (CA)n repeat polymorphisms STR 50, STR 49, STR 45 and STR 44 and the extragenic flanking markers 5C and 5G. Fluorescence in situ hybridisation was carried out on fixed lymphocyte cultures using an X centromeric probe, DX 27, and a cosmid probe, cPT1, specific for exon 45 within the dystrophin gene at Xp21.

As shown in Figure 1, molecular analyses of 9 family members from 3 generations using short tandem repeat markers indicated that 2 of the sisters (II.2 nd II.4) carry a deletion of the locus recognised by STR 45 from their maternally derived X chromosomes. Their mother is, however, heterozygous for this marker. Further, their unaffected brother (II.5) has inherited the same maternal X chromosome but does not carry the STR 45 deletion. The daughter of II.2 (II.3) has inherited her mother's deletion-bearing X chromosome. In situ hybridisation studies of individual II.4 revealed cosmid signal on only one X chromosome in all cells examined, indicating her other X to have a deletion within the dystrophin gene encompassing exon 45. Her mother, however, showed cPT1 signal on both X chromosomes and therefore the region recognised by this probe did not appear to be deleted.

The combined molecular linkage and in situ hybridisation studies described above have enabled us to positively identify an individual (I.1) who is a germinal mosaic for the mutation of the dystrophin gene seen in this family. Whilst molecular analysis gave an initial strong indication of the somatic deletion present in females II.2, II.4 and II.5, in situ hybridisation provided confirmation of this and also of the fact that female I.1 does not carry the deletion in her somatic cells. Because the mutation is a detectable deletion we are now able to offer reliable prenatal diagnosis for any subsequent at risk pregnancy in this family.