Medical genetics: advances in brief

Rapid direct diagnosis of deletion carriers of Duchenne and Becker muscular dystrophies

Fassati, A, Tedeschi S, Bordonni A, *et al*. *Lancet* 1994;343:302-3.

The detection of females who carry Duchenne or Becker muscular dystrophy (DMD/BMD) can still be problematical. Rapid carrier diagnosis may be necessary in the event of pregnancy in a woman at risk, before results of restriction fragment length polymorphisms (RFLPs) become available. Pulsed field gel electrophoresis can provide accurate results where there is a deletion or duplication, but can be time consuming. Fassati *et al* describe a new method that will allow rapid results to be obtained in women at risk of being carriers of deletions in the DMD/BMD gene. Amplification of two exons in the DMD/BMD gene is carried out by means of the polymerase chain reaction (PCR); one of the amplified exons is deleted in the affected male in the pedigree, the other is used as a control. A limited quantity of *Taq* polymerase is used so that the two primer pairs compete for amplification. In carrier women there is reduced efficiency of amplification of the deleted exon, which can be detected after agarose gel electrophoresis and ethidium bromide staining. The ratio between control and target exon bands for each woman is calculated, after taking the average of six measures. Carrier diagnosis is made if the difference between control and proband mean values was 50% or more and statistically significant. The authors have used the technique in 13 pedigrees and 34 at risk females; 13 obligate carriers on pedigree analysis were correctly diagnosed by this competitive PCR technique and seven further women with a risk of more than 99% on RFLP analysis also gave positive results with the PCR assay. An equivocal result in one female was shown to be negative when other deleted exons were used in the assay, in agreement with a less than 1% risk from restriction fragment length polymorphisms. This technique, although not applicable to all families, promises to be a useful addition to the methods available for carrier diagnosis in DMD.

ANGELA BARNICOAT

The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation

Migeon BR, Luo S, Jani M, Jeppesen P. *Am J Med Genet* 1994;55:497-504.

Most females with a straightforward 45,XO karyotype (Turner's syndrome) have a fairly benign condition, and females with one normal and one structurally abnormal X chromosome (for example, an iso(X) chromosome, an X deletion, or a large ring X chromosome) are usually similar clinically, presumably because the abnormal X chromosomes are usually inactivated in all cells, leaving the normal X to be the functional one. Occasionally, however, females with significant mental retardation and severe con-

genital abnormalities are found to have a form of Turner's syndrome with a tiny ring X chromosome in some cells (45,X/46,X,r(X)), that is, they are mosaic. Studies of these severely affected females show that the XIST locus (residing in the region of the putative X inactivation centre on Xq13.2) on their tiny ring X chromosome is either not present or not expressed. XIST transcription correlates well with X chromosome inactivation, and so non-expression of the locus, even when it is present, suggests that these tiny chromosomes are transcriptionally active. In this report, the transcriptional activity of ring X chromosomes lacking XIST expression from three females with a severe phenotype was studied. An antibody specific for the acetylated isoforms of histone H4 marking transcribed chromatin domains labelled the tiny ring X chromosomes at a level consistent with significant activity. Genes which are normally silent on an inactive X were also shown to be expressed. Overall, there is now convincing evidence that ring chromosomes associated with severe phenotypes are unable to undergo X chromosome inactivation, and that the severe phenotype is the result of functional disomy resulting from a lack of dosage compensation for genes present within the ring chromosome. This information will be particularly important during genetic counselling after a small ring X chromosome is found coincidentally at amniocentesis.

FRANCES FLINTER

A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations

Highsmith WE, Burch LH, Zhou Z, *et al*. *N Engl J Med* 1994;331:974-80.

Until recently, the diagnosis of cystic fibrosis has rested on sweat chloride findings. However, it has been known for some time that there is a group of patients who have chronic lung disease similar to that seen in cystic fibrosis but who have normal sweat chloride values. This paper describes an evaluation of a group of such patients. By direct sequencing of PCR amplified mRNA transcripts, an identical point mutation in intron 19 of CFTR was found in 13 of 23 patients. The mutation, found in patients from different ethnic groups and associated with different extended haplotypes, leads to the creation of a partially active splice site in intron 19 and to the insertion into most CFTR transcripts of a new 84 base pair "exon", containing an in frame stop codon, between exons 19 and 20. Normally spliced transcripts were also detected at a level approximately 8% of that found in normal subjects. While sweat ductal potential differences were normal in eight tested patients with the intron 19 mutation and pulmonary disease, sweat acinar epithelial chloride secretory function was abnormal as were the bioelectric properties of nasal epithelium. The group of patients with this mutation had lung disease that was similar to or milder than that expected for their age and, while at least one patient had ob-

structive azoospermia, a note added in proof indicated that a number of males with this mutation have fathered children. There are a number of implications arising from this molecularly delineated mild CF phenotype. First, consideration of the diagnosis of cystic fibrosis must extend to patients with suppurative pulmonary disease and normal sweat chloride values. Second, the finding of low levels of normal CFTR mRNA patients with this mutation may serve as a guide for therapy and, finally, screening for the mutation should prove diagnostically useful in this group of patients.

DAVID RAVINE

Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13

Wooster R, Neuhausen SL, Mangion J, *et al*. *Science* 1994;265:2088-90.

A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1

Miki Y, Swensen J, Shattuck-Eidens D, *et al*. *Science* 1994;266:66-71.

BRCA1 mutations in primary breast ovarian carcinomas

Futreal PA, Liu Q, Shattuck-Eidens D, *et al*. *Science* 1994;266:120-2.

A breast cancer susceptibility locus was linked to the long arm of chromosome 17 four years ago by Marie-Claire King and colleagues. Now Miki and 44 co-authors have isolated a gene in which mutations cosegregate with early onset breast cancer, or breast and ovarian cancer in five families. Single nucleotide substitutions, a deletion, and an insertion were found. The gene has 22 exons, encodes a protein of 1863 amino acids, and includes a probable zinc finger domain. Expression is found in breast, ovary, thymus, and testis with alternative splicing seen in breast and ovary. Although these mutations are associated particularly with breast cancer in women in their 20s and 30s, occasional women with the mutation remain unaffected at well above retirement age. The large size of the gene and the alternative splice sites may make the search for mutations difficult, but the identification of this gene does make it possible to distinguish persons within families with a high risk from those who have not inherited predisposing mutations. In a related paper Futreal and 26 co-authors contrast the high degree of loss of heterozygosity (LOH) for the BRCA1 containing region of 17q in sporadic breast and ovarian tumours with the low number of BRCA1 mutations so far identified within them. In their series, 3/32 breast and 1/12 ovarian carcinomas were found to contain single BRCA1 nucleotide changes. All four mutations were present in the germline and were associated with early onset cancers. It seems likely, therefore, that within the limitation of current techniques, the majority of sporadic breast and ovarian cancers are not associated with mutations of BRCA1. A week before publication of the positional cloning of BRCA1, Wooster and 30 co-authors reported linkage between a second heritable early onset breast cancer