
The detection of females who carry Duchenne or Becker muscular dystrophy (DMD/BMD) can still be problematic. 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 complicated. 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 this 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, calculated with 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.

FRANCES FLINTER


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 5% of that of wild type. While sweat results are 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 congenital abnormalities are found to have a form of Turner's syndrome with a tiny ring X chromosome and one normal X chromosome, 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 inactive. 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 transcriptional chromatin domains labelled the 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.

ANIELA BARNICCOAT


Most females with a straightforward 45,XO karyotype (Turner’s syndrome) have a fairly normal phenotype, and females with one normal and one structurally abnormal X chromosome (for example, an is(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
susceptibility locus and polymorphisms centred on D13S260. The putative BRCA2 gene at this locus is distinct from the retinoblastoma (RB1) gene although LOH for chromosome 13 in tumour tissue frequently involves both loci. As with BRCA1, BRCA2 haplotypes cosegregate with early onset breast cancer; by contrast with BRCA1, BRCA2 is less often associated with ovarian cancer, but does confer a small increase in the risk of breast cancer in males. These two genes are likely to account for the majority of families with increased susceptibility to early onset breast and ovarian cancer; a third gene or genes may nevertheless await discovery. Determining the function of these genes should provide significant clues to the underlying molecular events in breast and ovarian cancer.

In conclusion, it is clear that further three families linked completely to the same LQT locus are the majority of families with breast and ovarian cancer, but does confer a small increase in the risk of breast cancer in males. These two genes are likely to account for the majority of families with increased susceptibility to early onset breast and ovarian cancer; a third gene or genes may nevertheless await discovery. Determining the function of these genes should provide significant clues to the underlying molecular events in breast and ovarian cancer.

JOHN C K BARBER

Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity

Prolongation of the QT interval and other repolarisation abnormalities have been associated with a syndrome of episodic cardiac arrhythmia and stroke in a large Utah family. This localised an LQT gene to chromosome 11p15.5. A letter from a French group in the same issue of the journal now confirms that HRAS is excluded from the region containing the LQT gene and that LQT is more centromeric in 11p15.5 than previously thought.

In this current paper Jiang et al describe a genetic linkage analysis localising two new autosomal dominant LQT loci, nine families with first being linked to markers on chromosome 7q35-36 (LQT2) and three families of three loci indicating that the human genome contains at least a fourth LQT locus. Two possible candidate genes for LQT2 have been postulated; a chloride channel and a muscarinic receptor have been mapped to the corresponding region. The gene encoding an L type calcium channel alpha 1 subunit has been mapped to chromosome 3p21-23 and must be a candidate for LQT3. The relative importance of the four or more possible loci is not yet known. The phenotypes of patients with the different forms of LQT are described as being surprisingly similar. The authors postulate that the repolarisation abnormalities underlying different forms of LQT are the same. LQT genes, they feel, may encode elements of a common physiological mechanism of arrhythmia and perhaps by characterisation of this mechanism efforts towards prediction, prevention, and treatment of cardiac arrhythmias may be advanced. In news and views in the same issue of the journal Kerry Schwartz asks when patients will benefit from gene testing. Fong et al. It is suggested that early identification of genotypically affected asymptomatic persons would enable a detailed clinical workup and appropriate therapy. Thus early disease detection might be considered. The natural history and prognosis of such healthy carriers is still largely unknown and a precise phenotype-genotype analysis should be done before systematic therapeutic interventions are applied.

J GRAY

Myophosphorylase deficiency: an unusually severe form with myoglobinuria

Myophosphorylase deficiency (McArdle's disease) is a metabolic myopathy commonly presenting with muscle cramps and fatigue in childhood. The cramps are precipitated by strenuous exercise and relieved by rest. CK levels following strenuous exercise are greatly raised. The differential diagnosis in childhood includes the various forms of glycogen storage disease, Fabry's disease, the muscular dystrophies, and other metabolic myopathies such as phosphofructokinase or lactate dehydrogenase deficiency. Evaluation of these disorders often includes muscle biopsy. After the second or third decade diagnosis of myophosphorylase deficiency is facilitated because 50% patients experience myoglobinuria owing to muscle necrosis following prolonged or strenuous exercise. The authors of this article report an unusual case of myoglobinuria in an affected 8 year old boy. In the past the classic test for diagnosis of myophosphorylase deficiency was the "forearm ischaemic exercise test" which involved measurement of preischaemic and post-ischaemic exercise venous lactate levels. This test is non-specific as metabolic blocks anywhere along the glycogenolytic and glycolytic pathway impair lactate production. Recently several mutations in the myophosphorylase gene have been identified in patients with McArdle's disease with the commonest mutation being present in 75% patients with 50% being homozygous for this mutation. The reported patient with early onset of myoglobinuria and another patient with a fatal infantile myopathic variant of myophosphorylase deficiency were both homozygous for this common mutation although the authors stated that there was no consistent genotype-phenotype variation. The paper concludes that DNA analysis is now an alternative to muscle biopsy for the diagnosis of McArdle's disease and makes biopsy unnecessary in 90% of patients. It is interesting that although the paper goes into detail about the common mutation, which is a G to T transition at codon 49 in exon 1 of the myophosphorylase gene with change of an encoded arginine to a stop codon, the authors omit to mention the chromosomal location (11q13) of the gene itself.

JILL CLAYTON-SMITH

Mutation analysis in patients with possible but apparently sporadic Huntington's disease

Genetic counselling of families where there is an isolated case of possible Huntington's disease can be difficult. Davis et al present 44 cases of possible sporadic Huntington's disease studied using two polymerase chain reaction (PCR) methods. The first amplifies both the critical CAG repeat and the poly-morphic flanking CCG repeat (seven to 12 copies), the second method measures the CAG repeat directly (normal range eight to 33 copies). The cases were divided into probable or doubtful Huntington's disease. The family history was noted as negative (both parents alive or died over 65 years without suggestive features) or suspicious (for example, early death or adoption). Thirty of the 44 cases were confirmed as being affected with Huntington's disease. Thirteen cases gave a borderline result with the first PCR method but were shown to be unequivocally positive when CAG repeat length was measured. Twenty-five of the 28 patients with probable Huntington's disease had positive results (10 with negative family histories). Five of the 16 patients with doubtful disease were shown to have the typical expansion (two with negative family histories). In two of the patients with confirmed diagnoses and negative family histories the parents were still alive. In one, non-paternity was shown and in the other an allele in the intermediate range (35 repeats) had expanded when inherited from the father. This study provides useful confirmation that a majority of patients with typical clinical features but no family history do have Huntington's disease. The possibility that those with negative results may have Huntington's disease in the same gene is considered. The authors suggest that although new mutations do occur (as in one of their cases), non-paternity or mild disease which has been overlooked may be more common causes for the negative family history. Where there is doubt about the clinical diagnosis a much lower proportion of cases are shown to have Huntington's disease. Other potential causes for chorea were present in some of their patients. A proportion of the positive results in this study had repeat numbers in the borderline range and this illustrates the importance of measuring CAG repeat length directly in cases where there is doubt about the diagnosis. This study will aid in the elucidation of the apparently sporadic cases of Huntington's disease but, as the authors point out, the use of diagnostic DNA technology must be done with informed consent and not confused with predictive testing.

ANGELA BARNICOAT

Advances in brief