

## Medical genetics: advances in brief

### The identification of point mutations in Duchenne muscular dystrophy patients by using reverse-transcription PCR and the protein truncation test

Gardner RJ, Bobrow M, Roberts RG. *Am J Hum Genet* 1995;57:311-20.

More than 60% of boys with Duchenne muscular dystrophy (DMD) have a gross deletion in the dystrophin gene, which causes a frame-shift and can be detected with 98% efficacy by a multiplex PCR system. (Milder phenotypes, for example, Becker muscular dystrophy, are, in most cases, caused by in frame exon deletions.) In an additional  $\approx 6\%$  of cases of DMD and BMD, the disease is caused by a gross duplication. More than 70 small mutations causing DMD have also been identified, and most result in premature termination of translation; however, most of these mutations (nonsense mutations, frameshifting splice mutations, and small rearrangements) emerged from projects aimed at screening restricted parts of the gene. Despite the strong evolutionary conservation of the dystrophin primary structure, very few (<2%) of DMD cases appear to be associated with missense mutations. The preponderance of chain terminating mutations may be explained by the proposition that the remaining >98% of mutations act solely by reducing transcript levels, DMD resulting usually only from a virtual absence of dystrophin protein and not from the production of an altered protein. In order to exploit this unusual mutational spectrum where most mutations lead to a disruption of the open reading frame (ORF), the authors assessed the sensitivity of the protein truncation test (PTT) as a mutation screening test for DMD. Illegitimate transcription of the dystrophin gene can be amplified from peripheral blood lymphocytes in 10 sections by reverse transcription and nested PCR. Twenty-two patients with clinical DMD and no detectable deletion on multiplex PCR were studied. Translation terminating mutations were detected in 17/22 patients screened after the whole coding region had been screened. Small structural rearrangements and a splice site mutation were detected by sizing the RT-PCR products, whereas point mutations were determined by the PTT. Of the five "unsolved" cases, a misdiagnosis is unlikely in three, as dystrophin is known to be absent on muscle biopsy, but these data are not available on the other two. It is known, however, that RT-PCR and PTT cannot be used to screen for all large duplications, which account for  $\approx 6\%$  of DMD mutations. RT-PCR/PTT can be used effectively to screen for unknown translation terminating mutations in the dystrophin gene, and the method may be improved further by using muscle RNA extracted from frozen muscle biopsy samples, since the higher levels of dystrophin mRNA would make the RT-PCR (the most challenging step technically) more robust. The clinical implications for families requiring accurate carrier testing and prenatal diagnosis are obvious.

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### A familial Alzheimer's disease locus on chromosome 1

Levy-Lehad E, Wijsman EM, Nemens E, et al. *Science* 1995;269:970-3.

### Candidate gene for the chromosome 1 familial Alzheimer's disease locus

Levy-Lehad E, Wasco W, Poorkaj P, et al. *Science* 1995;269:973-7.

Mutations in the amyloid precursor protein gene on chromosome 21 were the first to be associated with a small number of families who had autosomal dominant Alzheimer's disease (AD) with onset before the age of 65. By contrast, as many as 80% of such families may result from mutations in the AD3 gene on chromosome 14. Now a third gene with intriguing similarities to the second has been identified in an isolated group of German ancestry who have remained culturally and genetically distinct despite living in the Volga region of Russia for over 200 years. In the first paper, the authors established strong linkage between AD in these families and the D1S479 locus on the long arm of chromosome 1. In the second paper, the somewhat unlikely approach of using a sequence with homology to the AD3 gene as a probe for the new locus proved successful when the sequence, obtained from an expressed sequence tagged (EST) library, was found to map to the same region as the D1S479 locus with which linkage had already been established. The new gene was obtained by screening cDNA libraries and sequencing showed a 67% homology between the new gene and the original AD3 candidate gene. A single mutation was found to cosegregate with the disease in seven of nine Volga German families but not in the other two, who the authors believe are phenocopies. This mutation was not found in any of 48 sporadic patients from an AD clinic. Two transcripts are found in a number of tissues, but only one of the two in brain. The proteins of both the AD3 and new gene have no less than seven transmembrane domains and the mutations identified in both genes cluster near the internal or external membrane boundaries leading the authors to speculate that they interfere with the insertion or anchoring of these proteins. Best indications to date suggest that the mutations lead to a build up of amyloid precursor proteins but this work should allow the construction of cellular and animal models with which to unravel the pathogenesis of AD and, in the longer term, to design possible strategies for its prevention.

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### Genetic homogeneity between childhood-onset and adult-onset autosomal recessive spinal muscular atrophy

Brahe C, Servidei S, Zappata S, Ricci E, Tonali P, Neri G. *Lancet* 1995;346:741-2.

Recent advances in the understanding of the molecular pathology of spinal muscular atrophy (SMA) still leave some unanswered questions. The clinical classification of SMA has been problematical in the past, as the different types are defined by age of onset, speed of progression, and age at death which can all be very variable, even within a family. The demonstration of linkage to 5q13 in different types of childhood onset SMA has supported the belief that the separate clinical types may be allelic. Inheritance of adult onset spinal muscular atrophy is variable. Some families clearly show dominant inheritance and some recessive, while a proportion are undefined or appear to be sporadic cases. Clinical examination does not help in predicting the genetics in any particular pedigree. Dominant pedigrees have shown no linkage to 5q. Brahe and colleagues studied six subjects from four families with adult onset SMA. Pedigree information suggested recessive inheritance in

three of the families and the fourth case was sporadic. Single strand conformation polymorphism analysis (SSCP) was used to show deletions of exons 7 and 8 of the survival motor neurone gene (SMN) in all six patients. One patient also showed a partial deletion of the neuronal apoptosis inhibitor gene (NAIP). Deletions in SMN are seen in the vast majority of infantile SMA patients and deletions of NAIP in a small proportion (45% in Werdnig-Hoffmann, 18% in other childhood onset SMA). The authors conclude that the molecular pathology of recessive adult onset SMA is similar to that of childhood onset SMA. Interestingly the clinical presentations of half of the six patients studied were atypical, in that they showed raised creatine kinase levels and calf hypertrophy and the muscle biopsy in one patient was more suggestive of myopathy than spinal muscular atrophy. As the genetic changes causing SMA become more clearly defined, diagnosis may be by DNA studies and genetic advice will become possible for some without a family history of the condition.

ANGELA BARNICOAT

### Minute supernumerary ring chromosome 22 associated with cat eye syndrome: further delineation of the critical region

Mears AJ, El-Shanti H, Murray JC, McDermid HE, Patil SR. *Am J Hum Genet* 1995;57:667-73.

Cat eye syndrome (CES) is a very variable condition in which patients may have ocular coloboma, anal atresia, preauricular skin tags and pits, congenital heart disease, urogenital abnormalities, and mental retardation in association with characteristic dysmorphic features. Typically the karyotype shows a supernumerary bisatellited marker chromosome derived from an inverted duplication of the short arm and proximal long arm of chromosome 22 (inv dup 22pter-22q11.2), resulting in tetrasomy for this region. Previously a study of the size of the marker chromosomes in 10 patients suggested that the critical region (smallest common duplication required to cause the phenotype) spanned a 3.1 Mb region from the centromere to D22S181. In this study, three generations of a family were found to carry a minute supernumerary ring chromosome derived from 22q11.2. The proband, who had the typical phenotype, had a smaller ring chromosome than other typical CES chromosomes and this therefore redefines the distal limit of the CES critical region. His father and paternal grandfather, who were clinically unaffected, were found to have a variable sized, supernumerary ring chromosome which, on further analysis (including estimations of dosage), proved to represent three (not four) copies of the loci D22S9, D22S43, and ATP6E. In previous case reports, three copies of the critical region have been associated with an abnormal phenotype, however, so a simple threshold model is not sufficient to explain the phenotypic variability of CES. It may be that the presence of four rather than three copies of the CES critical region may increase a person's susceptibility to express the phenotype, but other factors (which are currently unidentified) may also play an important role.

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