BIS6

THE DUCHENNE MUSCULAR DYSTROPHY GENE: A GIANT GENE WITH MULTIPLE PRODUCTS

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The DMD gene is the largest known gene to date (~2500 Kbp). The product of the gene in muscle is a 427 kDa protein, dystrophin. Dystrophin is also expressed in the nervous system, where it is regulated by three promoters. Smaller products of the gene are Dp71, Dp116, Dp140 and Dp260. The main DMD gene product in non-muscle cells is a 70.8 kDa protein, Dp71, consisting of the C-terminal region of dystrophin. Dp71 is the main DMD gene product in the brain, and is the first product of the gene expressed during mouse development. Experiments with transgenic *mdx* mice that express in the muscle Dp71 instead of dystrophin, showed that Dp71 restored the level of dystrophin associated proteins, but did not alleviate muscle damage. It is, therefore, suggested that the function of Dp71 is different from that of dystrophin. Gene inactivation techniques are being used to investigate the biological function of Dp71. The possible involvement of Dp71 in embryogenesis and in brain function will be discussed.

BIS7

Analysis of muscular dystrophies gene mutations in Southern Italy

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The muscular dystrophies are genetically heterogeneous. Duchenne and Becker muscular dystrophies, X- linked recessive disorders characterized by variable degrees of progressive muscle wasting, respiratory impairment and cardiomyopathy leading to premature death, originate from mutations in the enormous DMD/BMD gene, with the majority of cases showing gross gene rearrangements. The major muscular transcript, composed of 79 exons, encodes for dystrophin, a subsarcolemmal protein of 427 kD.

Autosomal inheritance is present in a significant percentage of muscular dystrophy cases. In most of them the disease arises from mutations in the sarcoglycan complex, comprising three distinct transmembrane proteins, alfa -sarcoglycan (50 kD DAG, adhalin), beta - sarcoglycan (43 kD DAG, A3b) and gamma - sarcoglycan (35 kD DAG) - strictly anchored to dystrophin.

The analysis of mutations of dystrophin and DAGproteins genes in 274 DMD, 113 BMD and 47 DAG patients afferent to the "Servizio di Cardiomiologia" of the second Naples University are reported. Multiplex PCR - using primers for 72 dystrophin exons - has identified 245 deletions (64.4%), 16 duplications (4.2%) and 19 small mutations (4.9%); correlations with clinical phenotypes are shown.

A direct diagnosis of DMD/BMD carrier status was made in 332 females by quantitative multiplex PCR; 139 mutated carriers, 132 not carriers and 61 not mutated females with mutated progeny have been diagnosed.

BIS8

DETECTION OF DUCHENNE MUSCULAR DYSTROPHY GENE PRODUCTS IN AMNIOTIC FLUID AND CHORIONIC VILLUS SAMPLING CELLS - POSSIBLE APPLICATION FOR PRENATAL DIAGNOSIS

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Duchenne muscular dystrophy (DMD) is one of the most frequent x-linked lethal diseases, affecting one in 3,500 male births. we have examined the expression of several DMD gene products in amniotic fluid (AF) and chronionic villus sampling (CVS) cells. By using a very sensitive procedure of immunoprecipitation followed by Western blot analysis, we could detect variable ammounts of dystrophin in cultures of most CVS and AF samples from normal fetuses. PCR analysis demonstrated the presence of the muscle type dystrophin mRNA in all AF cell cultures tested. The brain type dystrophin mRNA was also detected in some of these cultures. These DMD gene transcripts are of fetal origin and are produced by most or all clonable AF cells. We are attempting to amplify the signal by increasing the sensitivity of the assay method and the culture conditions. A test for prenatal diagnosis of DMD, based on the expression of dystrophin in AF or CVS cells, will be very helpful in DMD cases with detectable deletion and with no sufficient genetic information. Such a test could be potentially used to also detect the new mutations in the DMD gene, which comprise ca. 30% of all cases

BP1

A NOVEL NON SENSE MUTATION IN EXON 72 OF THE DYSTROPHIN GENE PRODUCING EXON SKIPPING IN A BMD PATIENT

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The cases of Duchenne/ Becker muscular dystrophy not due to gross rearrangments are supposed to be small mutations/point mutations in the dystrophin gene. In this paper we describe a novel non sense mutation (C-T nt 10512) in exon 72 of the dystrophin gene in a patient with clinical features indicative of BMD. On muscle biopsy all fibres were continuously labelled with all antibodies with the exception of DYS2 . Three multiplex PCR from genomic DNA showed no deletions. RNA obtained from muscle biopsy was reverse transcribed and the cDNA region spanning exons 68-79 was amplified. The normal RT-PCR product and was replaced by two transcripts , the major one lacking exons 68-76 (out of frame) and a minor one resulting from skipping of exons 68-78 (in frame).DNA sequencing of the missing exons revealed a C-A transversion at position 10512 in exon 72 which led to the substitution of the serine codon TCA with a Stop codon TAA. This mutation should theoretically result in the absence of protein production or in the production of a shortened not-functional protein. However the mutation leads to the production of a minor transcript with skipping of exons 68-78 which restores the Open Reading Frame and is most likely the reason for the mild Becker phenotype. Further confirmation that an in frame transcript was produced derives from the observation that a weak but definite immunostaining was detected with an antibody that is raised to an epitope located 3' to the mutation