INTRODUCTION

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic disorders due to genetic alterations on Xp21, corresponding respectively to absence/reduction-alterations of the protein dystrophin. Dystrophin is located at subarcolemmal level, consists of four domains (C-terminal, N-terminal, Rod domain, Actin binding domain) and is linked to a number of different proteins. The complete function of dystrophin and of the dystrophin-related proteins is still unknown. It is involved in maintaining the sarcolemmal integrity, but it probably also plays some functional role (1).

Even though we know that this protein is responsible for these diseases, we do not know the true pathogenetic mechanism underlying them.

The clinical picture of DMD is quite well known: onset in the first years of life with symptoms and signs related to limb girdle weakness (impairment getting up from the floor, climbing the stairs and jumping, waddling gait). The disease involves all muscles except the extraocular and, usually, the bulbar ones. The disease is slowly progressive: children are wheelchair-bound by the age of 12. After this it involves the respiratory muscles and heart; nowadays, respiratory failure is generally the cause of the death in the third decade of life (1).
Becker muscular dystrophy is a milder form of the disease in which motor symptoms are present but life span is usually normal, although early death can occur as the result of possible severe heart involvement (dilating cardiomyopathy). Furthermore, in the last few years the spectrum of clinical features in people with reduced/altered dystrophin has become greater, these features now ranging from isolated cardiomyopathy to effort-related cramps and myalgias, and isolated myoglobinuria (1).

Having said that, the clinical picture – especially in DMD – is characterized by other features, such as: mild-to moderate cognitive impairment, learning disabilities (in about one third of patients) (2-4); sometimes failure to thrive in the first months/years of life and overweight state/obesity in the following years (5). Even though these are surely not the main clinical problems associated with DMD and BMD, they are peculiar aspects that not only raise questions about the possible functions of dystrophin, but can also have clinical repercussions on the patient’s life.

Immunohistochemistry studies on muscle samples from DMD/BMD patients have shown abnormal staining of neuronal nitric oxide synthase (nNOS), a finding that could be an important clue to the pathogenetic mechanism (6).

Thus, in the last few years our center has focused on these peculiar clinical aspects of these disorders, aspects such as I.Q., and metabolic findings, and we recently performed immunocytochemistry staining of nNOS in samples from BMD patients, in an attempt to establish a correlation with genetic alterations. Here we summarize our studies.

CNS INVOLVEMENT IN DUCHENNE AND BECKER MUSCULAR DYSTROPHIES: KNOWN ASPECTS

As mentioned earlier, it is well known that one third of DMD patients have mild-moderate mental impairment; a frequent finding – often the only early sign justifying referral – is speech delay. Furthermore, learning disabilities, not necessarily correlated with mental impairment, could be related to some visuo-motor impairment. ERG b-wave is absent in DMD, and of reduced amplitude in BMD. No visual alterations are reported in DMD/BMD patients, but it is possible that some subclinical alterations are involved in the learning disabilities of these children. Dystrophin is present in different areas of the central nervous system (CNS), such as Purkinje cells, Schwann cells and glial cells (isoforms Dp140 and Dp 71) where its function is unknown. Another isoform, S-promoter dependent and called Dp116, is found mainly in the peripheral nerve, even though neither clinical nor electrophysiological alterations attributable to peripheral nerve involvement have been described to date (2-8) (See Fig. 1).

We also know that CNS involvement is stable over time, and no major CNS abnormalities are usually reported in these patients.

No clear correlation has been found so far between the above neurological aspects and genetic alterations in DMD, even though it is accepted that distal deletions are related to cognitive impairment (8-11). We read with interest, among others, the report by H. Chen, M. Matsuo et al. (12), which reports contiguous deletion of the S-promoter/first S exon and of the downstream exon 56 in three out of 24 Japanese patients affected by dystrophinopathy and severe mental retardation (I.Q. below 70), and looked for the same deletions in a group of our DMD/BMD patients.

BODY COMPOSITION AND RESTING ENERGY EXPENDITURE IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS

Clinical background

Muscle mass is part of the metabolically active component of the human body; DMD causes...
a progressive wasting of skeletal muscle, which is replaced by adipose and fibrous tissue; energy expenditure may be affected by reduction in muscle mass. We know that a few cases have been referred in the first years of life for failure to thrive, that 50% of dystrophic children are obese by the age of 13 years and that a similar percentage is undernourished by the age of 18 years (5,13-14).

NEURONAL NITRIC OXIDE SYNTHASE IN SKELETAL MUSCLE OF BECKER MUSCULAR DYSTROPHY PATIENTS

**Background**

Neuronal nitric oxide synthase is usually complexed with dystrophin in skeletal muscle. This is not the case in DMD/BMD in which, unlike other kinds of muscular dystrophy, anomalous nNOS distribution is found. A possible role for oxidative stress has been suggested in the pathogenesis of dystrophinopathies. (15,6) (Fig. 2, see over).

THE MATERIALS AND METHODS OF OUR WORKS

**Study one**

We studied a group of 31 BMD and DMD patients (19 BMD and 12 DMD), ranging from 2 years 2 months to 22 years of age, at different stages of the disease.

We used WISC-R scale for subjects older than 7 years, the Griffiths Scale for younger patient and the Leiter Scale for our one foreign language patient.
Study two

This study was carried out in 9 DMD patients aged between 6 and 12 years. Energy expenditure (resting energy expenditure, REE) was estimated using: indirect calorimetry, and a daily food and activity diary. Body composition was studied by magnetic resonance imaging (MRI), using the 0.5 Tesla system (Philips Gyroscan). Images were obtained with a T1 weighted spin-echo sequence (repetition time TR/echo time TE 300 ms/10 ms, two averages). Slice thickness was 10 mm, slice interval 1 mm and plane resolution 1.8 x 1.8 mm. Seven sets of slices, each spanning 8.8 cm, were taken from ankles to shoulders.

Study three

This study was carried out in more than 20 muscle samples from BMD patients, all with known deletion in the dystrophin gene. Slices of 8 micron were cut using a cryostat and immunocytochemistry was carried out with immunofluorescence to identify nNOS (and a series of other proteins, work still in progress). This was done after checking for sarcolemmal integrity by means of Spectrin determination. A correlation was sought between these data and deletions in the dystrophin gene.

RESULTS

Study one

In our patients we did not find any deletion in dystrophin S-promoter; only 1 patient had I.Q.<70, but, in accordance with previous studies the mean I.Q. in our DMD patients was below average intelligence and, in the BMD group, also below the mean I.Q. (Fig. 3).

In most of the DMD patients we found a disharmonic profile (defined individually for each scale used) with significant differences emerging between Verbal and Performance Scale scores.

We also confirmed the previous report of in-
volvement of the second part of the gene in intellectually impaired patients.

Study two

We here report the clinical data of 9 patients at different stages of the disease: body composition was as expected in DMD patients; the lean body mass and especially the metabolically active muscular tissue was severely reduced in comparison with normal subjects (Fig. 4, see over). REE/kg body weight was 38.1 kcal/kg body weight, in line with normal subjects; REE/lean body mass, on the other hand, was 58.6 kcal/kg FFM, higher than in normal subjects (Fig. 1). This is probably due to the reduced amount of lean body mass in DMD patients (66% versus 84%) as a consequence of muscular wasting (19% versus 37%) (Fig. 5, see over).

As a further result, in magnetic resonance imaging, we now have a simple, non-invasive tool to study lean body mass in neuromuscular disorders.

Study three

Neuronal nitric oxide synthase failed to show its sarcolemmal distribution in most of the BMD samples with a deletion in 48-50 exons of dystrophin gene.

Sarcolemmal distribution appeared to be preserved in samples from patients with deletions in different exons of the gene. We did not find any relation with clinical features.

This work is still in progress (Figs. 6 and 7, see over).

FUTURE EVOLUTION OF OUR STUDIES

Study one

With regard to CNS involvement in dystrophinopathies, our next project is to focus on speech delay, in order to detect at risk children through pre-school evaluation of language skills and to try pre-school training as a means of pre-
serving reading ability, which can so positively influence the quality of life of children affected by this very severe disease.

Study two

More than just a cosmetic problem, obesity in DMD can worsen respiratory problems and surely constitutes a serious difficulty for parents who have to manage these children on a daily basis (washing, dressing and so on). It is now clear that obesity in DMD is not due to altered metabolic processes related to muscle changes typical of the disease, but instead to an incorrect balance between food intake and energy expenditure (the latter being reduced due to muscle weakness). Correct dietary advice could improve this aspect of these subjects’ lives.

The imaging tool we have now to study body composition in neuromuscular disorders can be used with the aim of detecting possible differences in various disorders.

Study three

As mentioned before, our work on BMD muscles is still in progress. The results given here are very preliminary and partial. Furthermore, we are going to study parameters of oxidative stress in the muscle of DMD patients, in an attempt to discover possible correlations with clinical and genetic findings.

Fig. 4 - Body composition in patients affected by Duchenne muscular dystrophy and in controls.
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REFERENCES