Interrelationship between gene, its product and phenotype in Duchenne and Becker muscular dystrophy

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Abstract. DNA analysis was carried out in 113 patients of 103 families. In 58 families (55%) deletions were found using different cDNA probes. The attempt of studying the correlation between mental retardation in patients and the exon deletions was made. Dystrophin was evaluated in 80 patients including 12 affected females. One girl had chromosomal translocation X;22 and was a true DMD case. An unusual pedigree typical of X-linked transmission with affected subjects showing clinical features of DMD but with normally expressed dystrophin is presented. Owing to DNA and dystrophin analysis the correct diagnosis in some doubtful cases of muscular dystrophies could be established and some unusual pedigrees detected.

Key words: DMD/BMD - DNA and dystrophin analysis, X-autosomal translocation, mental deficiency
INTRODUCTION

The recent progress in molecular genetics makes possible the new approach to diagnosis of muscular dystrophies. In particular the progress is evident in the case of Duchenne and Becker muscular dystrophy (DMD and BMD).

The gene has been mapped at Xp 21 (Davies et al. 1983, Boyd and Buckle 1986) and cloned in 1987 (Koenig et al., 1987); many probes including cDNA probes were developed.

The gene of DMD/BMD is the largest one known in man. Its characteristic feature are deletions which constitute most (65%) of the mutations. The product of the gene - dystrophin - has been detected and characterized by Hoffman et al. (1987).

The aim of this paper is to summarize our data on interrelationship between phenotype, results of DNA analysis and dystrophin expression in DMD and BMD.

METHODS

Patients population consisted of 113 affected males aged 2-57 years and 12 females aged 4 to 30. Of 113 males 57 were diagnosed between 2nd and 7th and 40 between 8th and 10th year of life, 12 females were diagnosed between 4th and 30th year of life.

In all patients electrophysiological test (electromyography, conduction velocity measurement), biochemical tests such as determination of enzymes activity (namely that of CK) were performed. In 31 affected males IQ was tested using Wechsler scale.

DNA analysis was carried out in 113 patients from 103 families. Dystrophin was studied in 80 patients - 68 males and 12 females. The correlation of tests will be discussed only in respect of this group.

Blood samples taken from patients were treated with lysis buffer, centrifuged and the pellet of white blood cells was treated with 10% SDS and proteinase K (Maniatis et al. 1982). Then the samples were incubated overnight at 37°C. DNA was extracted using phenol/chloroform (2-3 times), precipitated by means of sodium acetate and ethanol, washed, dried and redissolved in TE buffer. Recently phenol free method was introduced. 6M Na Cl was added instead of phenol.

Then the DNA was digested with the appropriate restriction enzymes, and DNA fragments were separated on 0.7-1.5% agarose gel electrophoresis. The gels were stained with ethidium bromide and DNA was fixed by baking the filters. The DNA fragments were transferred from the gel to hybridization membrane - Hybond C (Southern 1975). The hybridization was performed using 32P labelled cDNA probes. Autoradiography was carried out in -70°C. The following cDNA probes obtained from K. Davies and Andrew Read were used (in brackets we give the applied restriction enzymes): cf 27E (Hind III), cf 27F (Hind III), cf 23a (PstI), cf 56a (PstI), Ca 1a (PstI), cf 77, (Hind III). Polymerase chain reaction was also used for detection of deletions (Hentemann M. et al. 1990).

Muscle samples for dystrophin detection were obtained from m. quadriceps femoris. Specimens from 4 children free of neuromuscular diseases were used as normal controls (Fig. 3). Immunohistochemical studies were performed using antibodies raised against dystrophin (a generous gift from Dr. E. Hoffman). Thick (4 µm) sections of muscle samples from normal controls and patients were placed on coverslips and incubated with primary antibody for 2 1/2 h and followed by an appropriate biotinylated secondary antibody for 30 min. To detect antibodies streptavidin Texas R conjugate 1/500 was added for 15 min. In the affected girls also cytogenetic examination was performed.

RESULTS

Deletions were found in 62 out of 113 affected males, i.e. in 55%. Distribution of deletions in DMD and BMD patients is shown in Fig. 1. The level of mental development as related to position and size of deletions and expression of dystrophin is presented in Fig. 2. Verification of initial clinical diagnosis based on dystrophin detection and DNA analysis is shown in Table I (males) and Table II (females). In one severely affected girl we found an X:
Fig. 1. Distribution of deletions in 62 DMD/BMD patients; -, dystrophin absent; +/-, dystrophin abnormal (reduced); n, normal IQ; yes, IQ<70; (yes), borderline of mental retardation.
autosomal translocation with breaking point within the gene of dystrophin. Dystrophin analysis confirmed the diagnosis of DMD (see below, Fig. 4). Four girls showed a pattern of dystrophin described in manifesting carriers (Hoffman 1992). The remaining seven cases were dystrophin positive, i.e. DMD and BMD (Fig. 5) could be excluded; they belong probably to the group of limb-girdle or other recessive autosomal type of muscle dystrophy.

**TABLE I**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Absent</th>
<th>Reduced</th>
<th>Mosaic Pattern</th>
<th>Normal</th>
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</thead>
<tbody>
<tr>
<td>DMD</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manifesting carrier</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-GD</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*a case of translocation X; 22 with breaking point in Xp 21.2
Fig. 3. Immunocytochemical labelling of dystrophin using polyclonal antibody 60 kD. Control muscle showing clear labelling at the periphery of all muscle fibres. x 448

Fig. 4. Immunocytochemical labelling of dystrophin using polyclonal antibody 60 kD. DMD muscle showing total absence of labelling. x 860

Fig. 5. Immunocytochemical labelling of dystrophin using polyclonal antibody 60 kD. BMD muscle showing very fine labelling muscle fibres. x 448
The molecular approach permitted us to find some unusual pedigrees; they will be described elsewhere. Here we present only one family with the most obvious X-linked inheritance and phenotype characteristic of DMD. Surprisingly, however, the expression of dystrophin in the affected subjects was normal and DNA analysis did not reveal any deletions.

**DISCUSSION**

Our material confirms the importance of molecular approach to the diagnosis of muscular dystrophies. DNA analysis and dystrophin detection enable us to confirm or exclude the diagnosis of DMD or BMD. This is very important for the patients and their families. We are also able to find among apparently typical pedigrees some families which might represent a new type of X-linked dystrophy. This subject requires, however, collecting larger number of atypical pedigrees and application of more sophisticated analysis. Due to availability of dystrophin many of "mysterious" cases of females with muscular dystrophy are explained. Among our female patients there is a girl with X; 22 translocation, entirely negative for dystrophin (97% of dystrophin negative fibers in immunofluorescence and in Western blotting). This is an example of true DMD in a female. The other females with reduced amount of dystrophin or its mosaic pattern are manifesting carriers (Table 11). The group of affected females recently described in detail by Hoffman et al (1992), could be sorted out owing to the possibility of detecting dystrophinopathies. Finally we managed to exclude X-linked dystrophy in seven girls who in spite of their phenotypic resemblance to DMD or BMD turned out to be cases of limb-girdle dystrophy (or other autosomal recessive type of muscular dystrophy).

The introduction of molecular methods changed very much our understanding of BMD. This benign variant of the disease which was considered to be ten times less frequent than DMD turned out to be quite frequent. It appeared also that BMD has a very broad phenotypical spectrum - from almost asymptomatic cases to the severe ones, suggesting clinically the diagnosis of DMD. In the presented material all deletions in BMD except one are in frame and located within exons 45-49 (Fig. 1), whereas in DMD the localization of the deletions is variable. Our material, however, is not large enough for drawing definite conclusions regarding the differences in distribution of the deletions in DMD and BMD. The level of mental development in our material does not seem to correlate with size or position of deletions (Fig. 2). Mental brain deficiency occurred both in cases of DMD and BMD.

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**REFERENCES**


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