DNA deletions in mild and severe Becker muscular dystrophy

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Summary. The DNA of 33 patients diagnosed as suffering from Becker muscular dystrophy (BMD) has been probed with cloned DNA sequences from Xp21, known to reveal DNA deletions in patients suffering from the more severe Duchenne muscular dystrophy (DMD). Two BMD cases showed clear deletions. A third case gave aberrant band sizes, which further analysis showed to be caused by a small deletion. This suggests that deletions in DXS164 occur approximately as frequently in BMD as they do in DMD. Of the two cases showing large deletions, one is at the severe end of the Becker clinical spectrum, whilst the other is a classical Becker-type dystrophy. The fact that loci defined by probes commonly deleted in classical DMD patients are also deleted in BMD patients of varying severity is strong additional evidence that these disorders are allelic, and further justifies the use of probes with defined linkage relationships to DMD also being used for counselling in BMD families.

Introduction

Duchenne and Becker muscular dystrophy are X-linked recessive disorders. BMD occurs at a frequency of about 1 in 30,000 male births, an order of magnitude less common than DMD. The clinical pattern of muscle involvement is similar in both disorders, but in the case of BMD the development of muscle weakness starts later, and is less pronounced than in DMD. Although there are classical cases of both DMD and BMD, a continuous range of severity exists between the two. BMD can be differentiated arbitrarily from DMD by maintenance of ambulation beyond the age of 16 years (Emery and Skinner 1976; Dubowitz 1986). BMD and DMD could, therefore, be considered to be milder and more severe allelic forms of the same disease.

Cytogenetic studies and meiotic linkage analysis do not contradict this. Thirteen girls manifesting DMD and with balanced X-autosome translocations involving Xp21 have been described (reviewed by Boyd and Buckle 1986). Two of these cases (Nielsen et al. 1983; Verellen-Dunouville et al. 1984) are clinically mild, falling within the spectrum of Becker rather than Duchenne muscular dystrophy. Thus it appears that chromosomal breaks in Xp21 can give rise to symptoms consistent with the more severe DMD, or the milder BMD.

The assignment of DMD to Xp21 has been confirmed by formal linkage studies utilising restriction fragment length polymorphisms (RFLPs) revealed by probes known to be localised to this chromosomal region (Davies et al. 1983, 1985; Brown et al. 1985; Dorkins et al. 1985; Hofker et al. 1985; Wilcox et al. 1985; Walker et al. 1986). Linkage of BMD to DNA probes known to be localised around Xp21 has also been demonstrated (Kingston et al. 1984; Brown et al. 1985; Dorkins et al. 1985; Roncuzzi et al. 1985; Walker et al. 1986).

The cytogenetic and linkage data thus suggest that the two disorders may be allelic. However, they are by no means conclusive. In the case of the translocations, the limits of resolution of cytogenetic techniques do not allow direct analysis of specific gene sites, while the small sample sizes available for meiotic linkage analysis give results with wide confidence limits, which could mask the presence of two linked, yet quite widely separated loci.

The pERT 87 probes used in this study have shown regions of DXS164 to be deleted in about 7% of patients with DMD (Kunkel et al. 1986). This initial report suggested, however, that deletion in BMD may be less common (2 out of 145 cases). The pXJ1.1 probe also reveals DMD deletions at about the same frequency (Ray et al. 1985). These probes are therefore presumed to be derived from, or very close to, the locus for DMD. In this paper, we describe the screening of DNA from 33 BMD patients with the probes pERT87.1, pERT87.8, pERT87.15, and pXJ1.1. The 33 cases represent 27 different kindreds, and therefore, possibly, 27 independent mutations.

Materials and methods

DNA analysis

DNA was isolated from whole blood leucocytes in EDTA (Kunkel et al. 1977). Three to ten µg DNA was digested with the appropriate restriction enzyme, before separation by electrophoresis on horizontal 0.8% (w/v) agarose gels. The DNA was then transferred to Zetaprobe filters by Southern blotting (Southern 1975). For routine deletion screening, PstI digested DNA was used.

Cloned specific probe sequences were recovered from low-melting point agarose, and were routinely labelled with 32P-dCTP to a high specific activity, using a hexanucleotide-primed reaction (Feinberg and Vogelstein 1983). Unincorporated nucleotides were removed by separation in G50 columns.

Filters were initially probed with 754 and pERT87.8 simultaneously. Probe 754 was used on all blots as a control.
for the technical adequacy of individual DNA preparations for hybridisation. The same filters were subsequently hybridised with the other probes.

Prehybridisation was carried out in 3 x SSC (0.45 M NaCl, 0.045 M sodium citrate), 0.1% sodium dodecyl sulphate (SDS), 2 x Denhardt's solution (0.04% (w/v), each of bovine serum albumin, Ficoll 400, and polyvinylpyrrolidone 40), and 100 μg/ml denatured salmon sperm DNA at 65°C. Hybridisation was performed in the same solution with addition of the denatured radiolabelled probe. Filters were washed for 30 min each in 2 x SSC, 0.1% SDS; 1 x SSC, 0.1% SDS, and 0.2 x SSC, 0.1% SDS at 65°C.

Autoradiography was at −70°C with DuPont intensifying screens, exposing for 1-6 days. For reprobing, filters were stripped in 0.4 M NaOH for 1 h at 42°C, followed by a 30 min wash in 0.2 M Tris-C1 (pH 7.5), 0.5% SDS, and 0.1 x SSC.

Probe origins

The original pERT87 clone (DXS164) was derived from a library highly enriched for human DNA from Xp21 (Kunkel et al. 1985), utilizing genomic DNA from a male patient who had a microscopically visible deletion around Xp21, and who also suffered from three X-linked disorders (DMD, chronic granulomatous disease, and retinitis pigmentosa) (Francke et al. 1985). Three unique sequence subclones covering 50 kilobases (kb) from DXS164 (pERT87.1, pERT87.8, and pERT87.15), which had previously been shown to detect deletions in DXS164 of DMD patients, were used in this study (Kunkel et al. 1986).

pXJ1.1 was derived from the DNA of a female patient affected with relatively mild muscular dystrophy, who was found to have an X-autosome translocation, t(X;21)(p21;p12) (Verellen-Dumoulin et al. 1984). The autosomal breakpoint is within a region of ribosomal RNA genes (Worton et al. 1984). Using rRNA sequences as probes, the region spanning the breakpoint was cloned, and a unique sequence from the X-chromosomal portion was isolated, and shown to reveal deletions in some DMD patients (Ray et al. 1985).

754 (DXS84) was selected from a flow-sorted X-chromosome library (Hofker et al. 1985). It detects a Pst1 polymorphism and has been localised to Xp21−11.3. The locus defined by this probe has a recombination fraction of 0.15 at a maximal lod score of 15.84 with the D/BMD locus (Walker et al. 1986).

99.6 (DXS41) was also isolated from a flow-sorted X-chromosome library (Aldridge et al. 1984). The locus defined by this probe maps distal to the D/BMD locus with a recombination fraction of 0.15 at a maximal lod score of 11.642 (Walker et al. 1986).

Clinical details

The patients described were ascertained as part of a systematic survey of a large muscular dystrophy clinic (Hodgson et al. 1987). Of 189 patients ascertained, 33 individuals from 27 kindreds had been diagnosed as BMD. Ten of these patients were still ambulant after 16 years of age. The remainder were diagnosed as BMD because of the late onset and relatively mild clinical features. Clinical diagnoses were established independently of, and prior to, the DNA tests.

Case A

This patient is an isolated case of dystrophy in his family, having a normal maternal uncle and one normal brother (Hodgson et al. 1986). He sat at 10 months, stood at 17 months, and walked at 21 months. His speech development was also late, his first sentence being at 3 years of age. He always had a clumsy gait, and was never able to run. He first managed to walk upstairs at 5 years of age. He began to walk on his toes at 8 years, and his calves were noted to be enlarged at that time. Following this, he experienced increasing difficulty in locomotion until 16 years 2 months, when he went into a wheelchair. An IQ test in childhood gave an overall score of 103. His creatine phosphokinase (CPK) was 2,450 iu/l (N = < 120) at 14 years old. Nerve conduction was normal. EMG suggested a myopathy, and his muscle biopsy was myopathic, but it is not possible to distinguish between DMD and BMD by this means. His mother had a normal CPK and muscle biopsy. He is now 21 years of age, and his condition remains stable. He is mobile in his wheelchair, and has a good sedentary job. His spine is straight, but he has some contractures at the elbows, hips, knees, and ankles.

Case B

This patient is an isolated case of dystrophy in his family, having two normal brothers. He also has a daughter. He was well until the age of 19 years, when he noticed easy exhaustion and muscle cramps in his legs. Weakness gradually developed in the legs, and subsequently also in the arms. An EMG and muscle biopsy performed when he was 26 years of age were compatible with a dystrophic process, and he was diagnosed as suffering from Becker muscular dystrophy.

The patient's muscle power deteriorated gradually over the years, until he was forced to retire from his job as an electrician in his late 30s. At the age of 38 years he lost the ability to walk, although he could still stand with support.

On examination at 37 years of age, he was noted to have bulky calves, but otherwise he was generally wasted, with limb weakness more marked in proximal muscles. There was a suggestion of facial weakness, and definite weakness of the neck flexors and trapezi. Tendon reflexes were absent, abdominal reflexes present, and plantar reflexes were flexor. Sensation was normal.

On re-examination at 42 years of age, he was unable to stand, and had a kyphoscoliosis. He was of normal intelligence and had normal speech. The slight weakness of his facial and neck muscles and of his limbs had progressed. CPK was elevated at 324 iu/l (N = < 80).

Case C

This boy is an only child, and there is no known family history of muscular dystrophy. However, information is scarce, because his mother lost contact with her family when she was 16 years old.

The patient had slightly delayed motor milestones: he crawled at 7 months, sat at 1 year, and stood and walked at 18 months. He spoke his first sentences at 3 years of age. His school reported that he was a bit slow in physical activities, such as running, when he was 5 years old.

On examination at 9 years of age, he was noted to have prominent, firm calves, tight Achilles tendons, and a mild