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A normal male with an inherited deletion of one exon within the DMD gene

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Summary. We describe two brothers with identical inherited deletions of one single exon within the middle of the DMD gene; one brother has Becker muscular dystrophy diagnosed at 11 years of age, whereas the older brother is normal at 18. These results have implications for genetic counselling and prenatal diagnosis in families with Becker muscular dystrophy.

Introduction

The genetic defect underlying Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) have been elucidated in detail at the molecular level (Kœnig et al. 1987; Forrest et al. 1987; Cross et al. 1987; Darras et al. 1988; Burghes et al. 1987). Deletions within the DMD locus have been detected in the majority of affected boys, and such mutations are thought to be responsible for abnormal expression of the gene product, dystrophin, in DMD/BMD muscle (Hoffman et al. 1987a). Similarly, female gene carriers of the defective gene also show deletions but in hemizygous form (Bonilla et al. 1988b). Gene deletions are detected at the molecular level by Southern blotting and hybridisation with cDNA probes isolated from the DMD gene (Kœnig et al. 1987; Forrest et al. 1987; Cross et al. 1987; Darras et al. 1988; Burghes et al. 1987). The corresponding absence of dystrophin or abnormal dystrophin expression in muscle tissue may be detected at the mRNA level by Northern blotting (Burghes et al. 1987), or at the protein level with antibodies directed against dystrophin (Hoffman et al. 1987a, b; Bonilla et al. 1988a; Arahata et al. 1988). However, we have found an 18-year-old male with an inherited deletion of one exon, but with an apparently normal expression of dystrophin in skeletal muscle and with no clinical signs of muscular dystrophy.

Materials and methods

The family shown in Fig. 1 was referred for genetic counselling since the youngest boy was diagnosed as having BMD at 11 years of age and his two older half-sister required definition of their carrier status. DNA samples from the different family members were digested with *Hin*dIII, Southern blotted and hybridised with the six cDNA probes cDMD 1–2a, cDMD 2b–3, cDMD 4–5a, cDMD 5b–7, cDMD 8, cDMD 9–14, covering the DMD gene (Kœnig et al. 1987), according to standard procedures (Bergerheim et al. 1989). To exclude mislabelling, new blood samples of the two brothers were obtained and the Southern blots were repeated.

For immunocytochemical labelling of dystrophin, unfixed frozen muscle sections were labelled with a specific monoclonal primary antibody and a rhodamine-conjugated second antibody as described (Nicholson et al. 1989a, b).

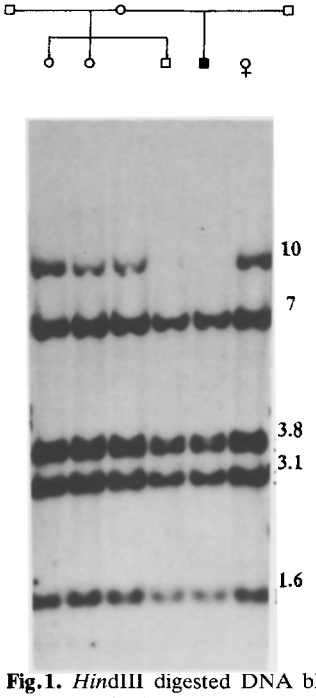

Fig. 1. *Hin*dIII digested DNA blotted and hybridised to the probe cDMD8. The lanes in the autoradiogram (*below*) correspond to the individuals indicated in the pedigree, and *numbers to the right* indicate the sizes of the *Hin*dIII restriction fragments in kilobase pairs. The *lane to the far right* displays DNA from an unrelated normal female

Results and discussion

As shown in the autoradiogram (Fig. 1), one exon-containing restriction fragment was delected in the affected boy. This deletion, detected with the probe cDMD8, affected a 10kb *Hin-*

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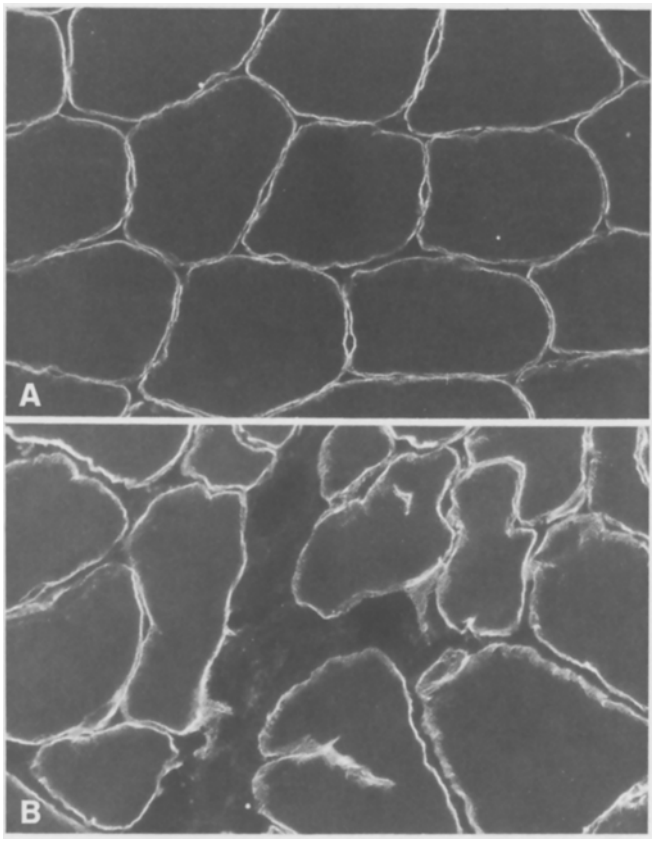

Fig. 2A, B. Immunocytochemical labelling of dystrophin in muscle sections. **A** Biopsy from the unaffected older brother. **B** Biopsy from the brother with BMD

dIII fragment; no additional deleltions could be detected with cDNA probes spanning the whole DMD gene. The deletion is located approximately 6900 bases from the 5′ end of the transcript; on the gene level, this corresponds to about 100 kb telomeric to the J-Bir locus (Kœnig et al. 1987; Darras et al. 1988). A clustering of deletions in this region has been reported in DMD/BMD (Kœnig et al. 1987).

Strikingly, as shown in Fig. 1, three additional members of this family, the older half-brother, the mother, and the younger half-sister were found to have the same deletion. The brother, five years older than the affected boy, has normal muscular function and strength. Aged 18, he recently registered for military service with normal muscular performance for his age, has an athletic body and practises many sports, including weightlifting. The hemizygous deletions of the 10 kb*Hin*dIII fragment in two female family members are evident from the reduced (hemizygous) signal intensities of this band, whereas the other restriction fragments in this autoradiogram show normal signal intensities.

Histopathological examination of muscle biopsies (m. quadriceps fem.; vast. lat.) from the two brothers showed morphological changes consistent with X-linked muscular dystrophy in the affected boy and muscle with normal characteristics in the older brother. The appearance of immunocytochemical labelling, using a monoclonal antibody to dystrophin, was markedly different in the two biopsies. As shown in Fig. 2, muscle from the older brother showed normal uniform dystrophin labelling at the periphery of the fibres, whereas the labelling of muscle from the affected boy was more diffuse and frequently appeared to extend further into the cytoplasm. It is

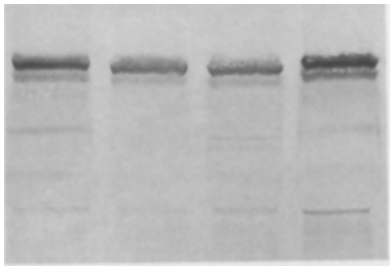

Fig. 3. Western blots of muscle the biopsies probed with dystrophin antibodies. The lanes represent *from the left*: a control; the unaffected older brother; the affected brother with BMD; and a control. The bands at the *top* show the full length dystrophin

not clear whether this abnormal appearance resulted from artifactual deformation of the muscle fibres, or from a more fundamental abnormality of the way dystrophin is associated with the cell surface. The intensity of labelling was similar in both brothers indicating that the deletion in both brothers is 'in frame', and thus does not interrupt the translation of the dystrophin mRNA. Western blots of muscle biopsies probed with the same monoclonal antibodies revealed dystrophin of identical size in both brothers, but their full length molecules were slightly smaller than normal dystrophin (Fig. 3).

It seems, therefore, that some additional factor(s) precipitated the disease expression in the younger boy. One possibility is that any alternative splicing (Feener et al. 1989) or post-translational processing required for the correct attachment and localisation of the dystrophin is defective or incomplete. Another is that the protein to which dystrophin binds at the muscle cell surface (Cambell and Kahl 1989) is defective. It is also possible that the still unaffected brother may develop symptoms later in life. Late onset of muscular dystrophy was reported in a mildly affected case with a deletion within the DMD locus, although his affected relatives presented in adolescence with classical BMD (Forrest et al. 1987). In a second family, an unaffected male had an intron deletion within the DMD gene, and his affected brother possessed the same deletion plus an additional deletion of exons in a more distal part of the gene (Koh et al. 1987; Bartlett et al. 1989).

The clinical implications of our findings are significant. A small deletion within the DMD gene may not necessarily lead to expression of clinical symptoms; this imposes problems in the genetic counselling in relatives of boys with mild forms of Xp21 linked muscular dystrophy. In the family shown in Fig. 1, prenatal diagnosis was not performed in the younger sister, who carries the deletion and who was pregnant at the time that these results were obtained.

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