Dystrophin analysis in the diagnosis of muscular dystrophy

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SUMMARY We present a family in which the differential diagnosis between X linked Duchenne muscular dystrophy and autosomal recessive Duchenne-like muscular dystrophy was resolved in favour of the latter by analysis of dystrophin, which is the protein product of the Duchenne muscular dystrophy locus.

X linked Duchenne muscular dystrophy is the commonest of the neuromuscular diseases of childhood, occurring in approximately 1 in 3300 live male births.¹ In contrast, autosomal recessive Duchenne-like muscular dystrophy is rare.^{2,3} We describe a family where the differential diagnosis lay between these two conditions, and was resolved by analysis of dystrophin, which is the protein product of the Duchenne muscular dystrophy locus.⁴

Case reports

The family was referred for genetic counselling because the eldest daughter (DS), now aged 13 years, had a diagnosis of muscular dystrophy. The parents were healthy and unrelated; there was no family history of muscle disease. They also had a son of 2 years (JS) and a normal daughter aged 10 years.

CASE 1

DS toe walked from the age of 10 months, suffered noticeable difficulty walking at age 5 years, and by the next year had trouble climbing stairs. Muscle strength continued to deteriorate slowly and she was able to walk but unable to rise from the lying to standing position unaided at 12 years. On examination by one of us (HEH) there was proximal muscle weakness and wasting affecting both limb girdles and hypertrophy of both calves. Serum creatine kinase activity at age 9 years was 1500 IU/l and a muscle biopsy specimen taken at the same age showed a necrotising myopathy compatible with muscular dystrophy but without a focal pattern of distribution of the necrotic muscle fibres. Electrocardiography was normal at 13 years. Her karyotype was normal 46XX by high resolution banding.

CASE 2

JS, now aged 2 years, was late in walking at 18 months. He was slightly awkward when running and climbing stairs, and grasped furniture in order to rise from the floor. On examination there was slight weakness of latissimus dorsi, and mild enlargement of both calves, but muscle strength was otherwise normal. His serum creatine kinase activity at 21 months was 4526 IU/l. Electrocardiography was normal. A muscle biopsy specimen showed extensive areas of normal muscle fibres, but with small groups of fibres showing some atrophy and considerable basophilia suggesting focal regeneration. There were evenly scattered hyaline fibres and no excess of fat or connective tissue or disruption of muscle architecture. The histochemical profile was normal. These appearances were considered to be more in favour of autosomal recessive muscular dystrophy than Duchenne muscular dystrophy because of the focal distribution and lack of excess connective tissue.

INVESTIGATIONS

Moser and Emery suggested that manifesting carriers of Duchenne muscular dystrophy are as common as patients with 'limb-girdle dystrophy', at least in adults.⁵ Because of her calf hypertrophy we were concerned that DS might be a manifesting carrier of Duchenne muscular dystrophy. Furthermore her brother had clinical features consistent with Duchenne muscular dystrophy and there was some evidence that he might be more severely affected. Autosomal recessive Duchenne-like muscular dystrophy is rare and some possible cases have been rediagnosed as X linked Duchenne muscular dystrophy.^{3,6} Accordingly, we screened DNA from JS with dystrophin cDNA probes cDNA

Fig 1 *TaqI* digest of DNA probed with cDNA 8. There is a polymorphism with two alleles as arrowed. Allele 1 is 6.5 kb and allele 2 is 5.6 kb in length. The mother of DS and JS is heterozygous. DS has inherited allele 2 and JS allele 1. This would be expected in 5% of families with Duchenne muscular dystrophy where the site of mutation is unknown.

1-3 and cDNA 6-8, which detect molecular deletions in at least 60% of boys with Duchenne muscular dystrophy.^{7,8} No such deletion was found. Several polymorphisms have been described with these probes.^{7,8} However, we only found the mother of JS to be heterozygous (and thus informative) with the cDNA 8/*TaqI* polymorphism: JS and DS had inherited opposite alleles (fig 1), which would be

Fig 2 Western blot analysis of dystrophin in control (C) and patient (JS) muscle samples. Binding of dystrophin monoclonal antibody was visualised with horseradish-peroxidase labelled second antibody exposed to diaminobenzidine. Relative molecular mass (Mr) measurements are approximate.

expected in only 5% of families with Duchenne muscular dystrophy.

METHODS

Dystrophin analysis was performed by homogenising small samples of muscle in sodium dodecyl sulphate polyacrylamide gel treatment buffer and separating the extracted proteins on a 4-8% gradient slabgel.⁹ After electrophoretic transfer¹⁰ of the proteins to nitrocellulose paper (western blotting) the samples were probed with a specific monoclonal antibody to dystrophin.¹¹ The antibody produces a characteristic pattern of bands from muscle of normal control individuals and the result from the muscle sample from JS was indistinguishable from these controls (fig 2).

Discussion

This family highlights the difficulty in distinguishing between the rare manifesting carrier of the relatively common Duchenne muscular dystrophy gene, and the rare autosomal recessive Duchenne-like muscular dystrophy. Although the latter form of muscular dystrophy is well documented in North Africa and Arabia,^{12,13} it is very uncommon among Europeans.^{2,3,14} The diagnosis could not be resolved by traditional methods. Although muscle histology favoured autosomal recessive dystrophy, this interpretation is not absolute, and Duchenne muscular dystrophy remained a possible diagnosis on clinical grounds. DNA analysis with dystrophin

cDNA probes did not support but could not exclude Duchenne muscular dystrophy in this family. Dystrophin analysis showed no abnormality in the affected boy, however, whereas abnormalities are found in at least 97% of boys with Duchenne muscular dystrophy.¹⁵ Therefore we conclude that this family is a genuine example of autosomal recessive Duchenne-like muscular dystrophy and counselled them accordingly. It is possible that the one Duchenne muscular dystrophy boy without dystrophin abnormality in the study of Hoffman *et al* may also have this diagnosis.¹⁵

This family represents one of the few proved European families reported with autosomal recessive Duchenne-like muscular dystrophy and to our knowledge there has been no previous report of dystrophin analysis being used to resolve this problem.

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