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LESSON OF THE MONTH

Sporadic lower limb hypertrophy and exercise induced myalgia in a woman with dystrophin gene deletion

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Abstract

A 25 year old woman, without family history of muscular dystrophy, had had an isolated lower limb hypertrophy since infancy and later experienced exercise-induced myalgia. Genomic DNA analysis showed a deletion of exons 45 to 52 of the dystrophin gene. Uncommon phenotypes of dystrophinopathies and consequences in genetic counselling in women are emphasised.

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Recent advances in molecular genetics have focused interest on previously unrecognised phenotypic expressions of dystrophinopathies.[1] These conditions are suspected in males with a family history suggestive of an X linked disorder. Because of the high rate (30%) of spontaneous dystrophin gene mutations, however, immunocytochemistry for detection of dystrophin on muscle fibres and DNA analysis on muscle or blood samples are also important in the evaluation of isolated or sporadic muscle dystrophies. Analysis of DNA is also useful to assess symptomatic female carriers of Duchenne and Becker muscular dystrophies (DMD, BMD), which are recognised with increasing frequency.

We report the case of a young woman, without family history, who had dystrophin gene deletion manifest by bilateral hypertrophy of her calves and thighs, and myalgia on exertion.

Case report

A 25 year old woman was referred by her occupational medical control for pronounced bilateral lower limb hypertrophy. She had no children. Her parents and her six brothers and sisters were asymptomatic. Hypertrophy of calves and thighs was first noticed by her parents in her infancy. During childhood, she developed a progressive toe walking gait. Achilles tenotomy was performed at the age of 16 with a good result on gait. Cramps and painful sensations induced by exercise developed as she grew older. In adulthood, she experienced discomfort in her lower limbs during prolonged walks, running, or climbing stairs. In recent years, some exercise induced myalgia developed in the upper limbs, but, as a whole, worsening of muscular symptoms was minimal.

Examination showed global and symmetric pseudohypertrophy of her lower limbs, predominating in her calves and thighs (fig 1). Upper limbs were normal. The muscles in the lower limbs were rubbery and percussion elicited no pain. Testing of muscle strength and tendon reflexes was normal. Other physical examination was normal.

Serum creatine kinase concentration was 680 (normal range 40–225) U/l. Electro-

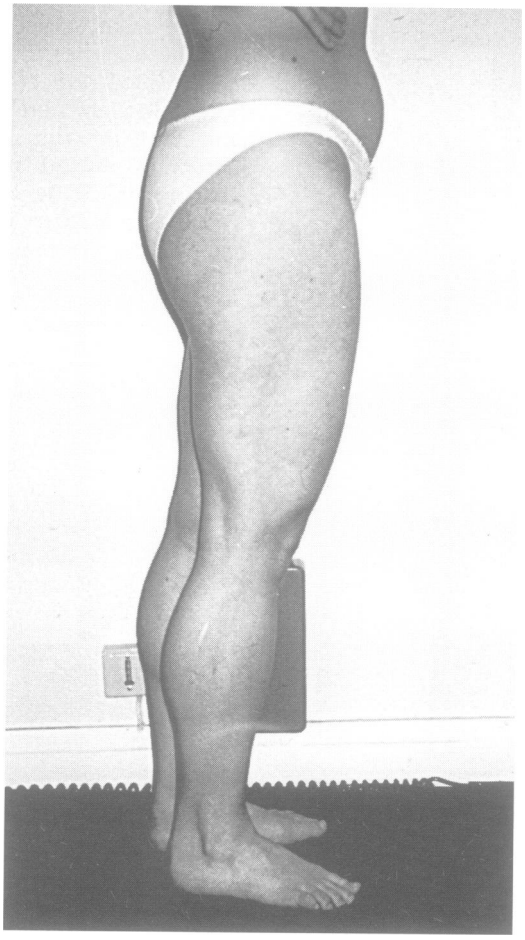

Figure 1 Bilateral hypertrophy of calves and thighs.

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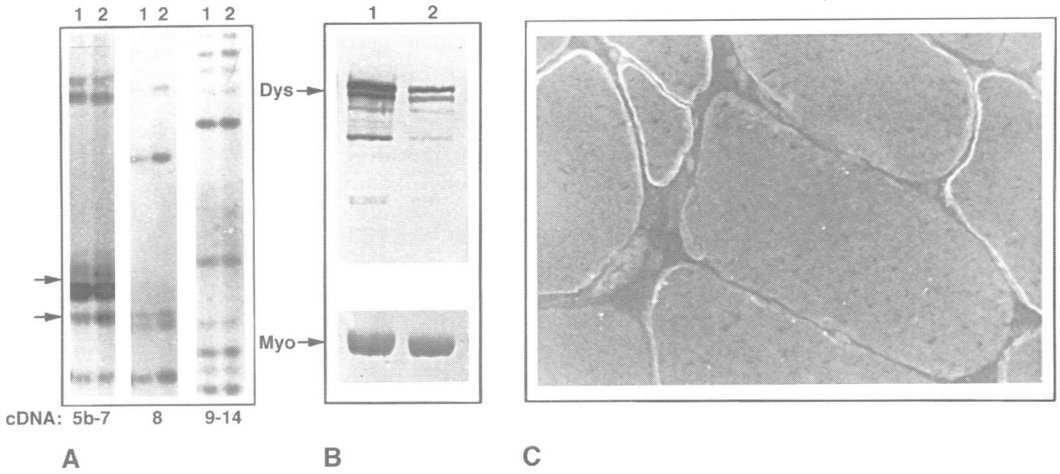

Figure 2 DNA and dystrophin analysis. (A) Southern blot performed with three dystrophin cDNA probes after Hind III digestion. cDNA probe 5b-7 (corresponding to exons 30 to 46): 2 bands (black arrows) containing exons 45 and 46 show half intensity of labelling in the patient (lane 1) compared with a female control DNA (lane 2). The other bands, corresponding to non-deleted exons, are similarly expressed in the patient and the control. cDNA probe 8 (corresponding to the deleted exons 47 to 52): all bands show half intensity of labelling in the patient (lane 1) compared with the control (lane 2). cDNA probe 9–14 (corresponding to the non-deleted exons 53 to 64): all bands show a similar intensity of labelling in the patient (lane 1) and the control (lane 2). (B) Western blot analysis with antibody dys-1. The doublet of dystrophin is normal sized for the patient (lane 2) compared with the control (lane 1) but in reduced amount (20% estimated by densitometry). Dys = dystrophin; Myo = myosin. (C) Immunofluorescence study with dystrophin antibody dys-2 showing a dystrophic myopathological pattern with a mixture of positive and completely negative fibres

myography, ECG, and echocardiography were normal; CT of the lower limbs was in keeping with a fatty degeneration of muscles. The karyotype was normal (46, XX).

A biopsy of quadriceps femoris muscle showed abnormalities consistent with a muscular dystrophy, including considerable variability in fibre size with numerous hypertrophic fibres, splitting, central nuclei, necrotic fibres, small groups of regenerating fibres, endomysial fibrosis, and mild fatty degeneration. An immunofluorescence study of dystrophin was performed with monoclonal antibodies against the rod domain (dys 1), the C-terminal (dys 2), and the N-terminal (dys 3) of dystrophin (Novocastra, Newcastle-upon-Tyne, UK). It showed a mixture of positive fibres with continuous sarcolemmal staining, and completely negative fibres that accounted for 30% of muscle fibres (fig 2). Negative fibres were found with all three antidystrophin monoclonal antibodies. The quantity of dystrophin was estimated at 20% of the normal with western blot analysis using the monoclonal antibody dys 1.

Genomic DNA was analysed by hybridisation with 6(^{32}P)-dCTP labelled cDNA probes encompassing the dystrophin gene, after digestion by restriction enzymes Hind III and Southern blotting. The gene dosage showed a deletion ("simple dose" intensity of labelling) of exons 45 to 52 (fig 2).

Discussion

This woman was evaluated for sporadic bilateral lower limb hypertrophy without weakness, and exercise induced myalgias. She had mildly increased creatine kinase concentrations, and muscle biopsy showed dystrophic changes with absent dystrophin expression in 30% of muscle fibres. Karyotype was normal. Analysis of DNA showed a deletion of exons 45 to 52 of the dystrophin gene on one X chromosome.

Dystrophinopathies are due to mutations in the dystrophin gene on the short arm of the X chromosome at p21,[2] and, therefore, are virtually confined to males. In about 70% of cases of BMD/DMD, mutations of the dystrophin gene result in detectable deletions and duplications.[3-5] Deletion of exons 45 to 52 of the dystrophin gene found in our patient disrupts the transductional reading frame, and gives a shortened and non-functional dystrophin product. This deletion is found on the DNA of male patients with DMD in whom no dystrophin is detected in muscle tissue.[6]

A range of 45–70% of definite female carriers of DMD have increases in serum creatine kinase concentrations,[7 8] and about 10% have clinical symptoms.[9] The proportion of manifesting BMD female carriers is probably much lower.[10 11] So called "manifesting" or "symptomatic" DMD carriers were first recognised in the early 1960s.[12-14] It was the presence of unilateral calf hypertrophy and associated cramps on exercise that prompted Dubowitz in 1963 to report the first muscle biopsy of a known carrier of DMD, and to show overt pathological changes in the muscle.[12] Manifesting female carriers with previously X linked history for DMD in males usually present with proximal limb weakness (80%), and much less often with muscle symptoms without weakness, including calf hypertrophy, myalgia, or cardiac failure.[15] Unlike asymptomatic carriers of DMD, manifesting DMD carriers do tend to show a mosaic pattern on dystrophin immunocytochemistry in their muscle.[16] A lack of correlation between dystrophin expression and clinical weakness, however, has been found in manifesting DMD carriers.[16]

Some reports have described DMD in women with chromosomal abnormalities such as Turner (X0) or Turner mosaïc (X/XX or X/XX/XXX) syndromes, a structurally abnor-

mal X chromosome, or an X autosomal translocation.[3] Manifesting female carriers of DMD usually have a normal karyotype, however, and they express the disease as the result of skewed lyonisation of the X chromosome[11] (inactivation of the normal paternal X chromosome in a large proportion of embryonic cells[17]).

In females without a family history of dystrophy, immunohistochemistry for dystrophin and DNA analysis are key tests to assess dystrophinopathy. The first well documented sporadic female cases of dystrophinopathy were three 10 year old girls who had moderate calf hypertrophy, moderate to severe weakness, raised creatine kinase in serum, and dystrophin deficiency assessed by immunohistochemical study of muscle and immunoblot analysis.[18] In a large multicentre study of 25 sporadic cases of manifesting female carriers of DMD,[15] 40% had proximal limb weakness before the age of 10, 24% had cramps or myalgia, 24% had grossly increased serum creatine kinase, 8% were easily tired, and 4% (one patient) had progressive proximal limb weakness beginning at the age of 45. Female patients initially believed to have sporadic limb girdle dystrophy have occasionally been found to be carriers of DMD,[19 20] as have 27–38% of male patients with sporadic limb girdle dystrophy.[21 22] By contrast, our patient had no weakness at the age of 25. Myalgia on exertion is now well recognised in male patients with dystrophinopathy.[1] Hypertrophy of the calves is a typical sign in up to 77% of manifesting female carriers of DMD.[15] Unusual phenotypic expressions of dystrophinopathies in males include quadriceps myopathy,[23] X linked myoglobinuria,[24] cramps and myalgia,[25] and dilated cardiomyopathy.[26] The clinical range of the disease could even include some entities of obscure pathogenesis such as monomelic hypertrophy with progressive myopathy[27]: these rare patients should be screened for dystrophin because asymmetry of symptoms can be seen in more than 25% of manifesting carriers.[12 28]

Deficiencies in dystrophin associated glycoproteins (DAGs), such as the 50 kDa DAG encoded by chromosome 17, adhalin, can cause autosomal recessive muscular dystrophies mimicking DMD.[29] To what extent adhalin deficiencies may account for clinical symptoms resembling those of DMD carriers in heterozygotes remains to be determined.

We conclude that isolated hypertrophy of the thighs and calves may be the only manifestation of sporadic dystrophinopathy, and this has practical consequences in genetic counselling.

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