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ORIGINAL PAPER

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Becker-like muscular dystrophy in sisters

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Abstract Two sisters with muscular dystrophy of Becker-like clinical features presented. Muscle weakness was most prominent in the pelvic girdle, but in the elder sister the distal muscles of the lower extremities were also affected. The progression was different in the siblings: The older sister showed a more pronounced deterioration than the younger. The family history was negative in four generations including their brother and youngest sister. Serum creatine kinase activities increased considerably. Electromyogram and muscle biopsy specimens revealed myopathic changes characteristic of muscular dystrophy. Chromosomal analysis confirmed normal 46,XX karyotype. DNA analysis with all cDNA probes spanning the entire dystrophin gene failed to reveal any intragenic deletion or duplication on southern blot. Immunohistochemistry for dystrophin using monoclonal antibodies against the rod and C-terminal domains showed normal continuous staining at the sarcolemma of the muscle fibers in the biopsy specimens of both patients. The results practically exclude the possibility of Xp21 myopathy, and it seems reasonable to classify these patients as having autosomal recessive childhood muscular dystrophy.

Key words Autosomal recessive muscular dystrophy · Becker-like phenotype · Dystrophin · cDNA probes

Introduction

Until the past few years the classification of muscular dystrophies was based on the characteristic clinical syndromes, and was refined in the light of creatine kinase (CK) activity, electromyography, and muscle histology (Walton and Gardner-Medwin 1981); however, doubts may arise, firstly in the existence of "limb girdle dystrophy" and secondly, in the possibility of Duchenne or Becker-like dystrophies in girls. Previously, in girls with a negative family history it was almost impossible to make the distinction between patients with autosomal recessive muscular dystrophy and symptomatic carriers of X-linked muscular dystrophy. Localization and mapping of the gene of X-linked dystrophy (Koenig et al. 1987; Kunkel et al. 1985, Monaco et al. 1985) and determination of its protein product (Hoffman et al. 1987) also open up a new prospect in the diagnostic procedures. In this study we present a family with two girls showing the syndrome of Becker-like dystrophy to demonstrate the diagnostic uncertainties and possibilities.

Case reports

Four generations of the family history were negative with regard to any inherited diseases, except the two sisters reported here (Fig. 1). Consanguinity between the parents was excluded. In addition to the girls in question they had one unaffected son and daughter.

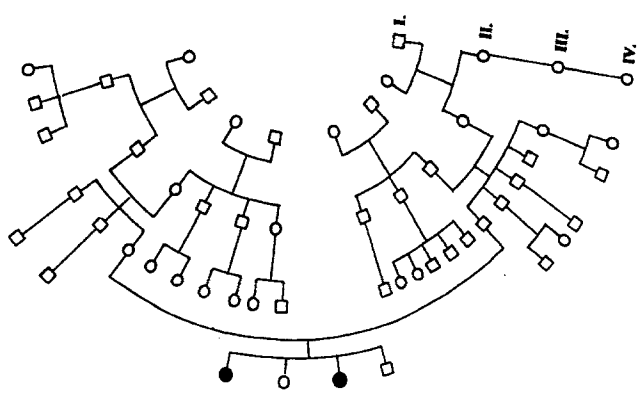

Fig. 1 Pedigree of the family

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Case 1

The elder sister (Fig. 2) was referred to our muscle clinic at age 9 years for fatigue, abnormal changing of her posture, and difficulties in walking. She was born at term after normal pregnancy and delivery. She started to walk at 9 months of age, but she stood and walked on her tiptoes from the beginning. Neurologic examination demonstrated slight weakness in shoulder girdle and moderately severe weakness in lower limbs. The wasting was pronounced symmetrically in the pectoralis, biceps, triceps, brachioradialis, hip flexors and extensors, and quadriceps muscles, whereas deltoids, flexors and extensors of the wrist, hamstrings, and calf muscles were relatively preserved. She had difficulties in running, standing up, and climbing stairs, and walked in a waddling motion with her feet turned downward. Hyperlordotic gait, positive Gowers' sign, slight atrophy of the left thigh, mild calf-muscle hypertrophy, and diminished tendon reflexes were noted. Her serum CK level was high (2485 U/l). She has shown further continuous progression. At the time of the last neurologic control, at 12 years of age she needed considerable help to push herself to an upright position, her walking was very clumsy, she was unable to climb upstairs, her posture became extremely deformed, and she could stand only with some support. Despite the significant deterioration even at the age of 12 years, she was not confined to a wheelchair, rendering a Becker-like dystrophy.

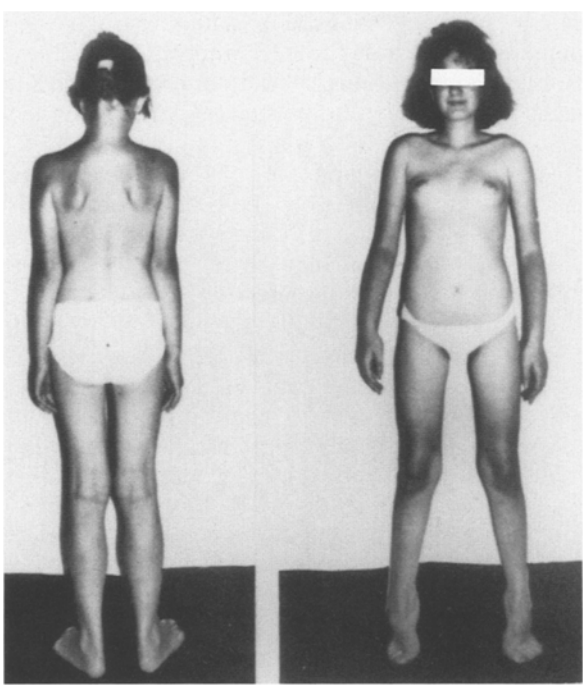

Fig. 2 The affected sisters: case 1 (12 years old; *left*) and case 2 (at age 8 years; *right*)

Case 2

The younger sister was first investigated at age 5 years. She was born after uncomplicated pregnancy and delivery. The early milestones of her mental and motor development were normal. No muscle weakness and wasting were found, but the serum CK activity increased significantly (4510 U/l). Three years later she complained of fatigue and difficulties in climbing stairs. The parents noticed toe-walking. Incipient atrophy in the biceps of the upper extremities and slight calf-muscle hypertrophy was observed, but the bulk of muscles were otherwise normal (Fig. 2). The flexion of arms and hips, and the dorsiflexion of feet, were mildly reduced. There was mild scapular winging. Occasionally positive Gowers' sign was revealed. Tendon reflexes were diminished, but the ankle jerks remained brisk.

Special studies

Genetic studies

Chromosomal abnormalities were excluded, both girls had normal 46,XX karyotype. cDNA analysis for Duchenne gene was performed. Southern blots using all the cDNA probes spanning the entire dystrophin gene (Koenig et al. 1987; Walker et al. 1990) failed to reveal any intragenic deletion or duplication. Serum CK activities of the affected and unaffected family members are shown in Table 1.

Electromyography

In the muscles of the elder sister (case 1) a myopathic pattern was found with a few fibrillation potentials. In the younger girl (case 2) slight focal myopathic changes were revealed with short polyphasic potentials, but no fibrillation potentials.

Muscle biopsies

Muscle biopsy was obtained from the quadriceps muscle of both girls. Fresh frozen cryostat sections were stained

Table 1 Serum creatine kinase (CK) activities in patients and their relatives

	Gender	Age (years)	Clinical affection	CK (U/l)[a]
Case 1	Female	9	Yes	2485
Case 2	Female	5	Yes	4510
Case 2[b]	Female	8	Yes	6465
Sister (unaffected)	Female	6	No	46
Brother (unaffected)	Male	4	No	41
Mother (unaffected)	Female	28	No	82

[a] Normal: 10–80
[b] Repeated determination

with Hematoxylin and Eosin, modified Gömöri trichrome, Sudan Black, PAS, NADH-TR, and myofibrillar ATPase preincubated at pH 9.4, 4.6, and 4.3

In muscle specimens of the elder sister (case 1) the amount of peri- and endomysial connective tissue increased considerably. There was great variation of fiber caliber with both rounded atrophic and markedly hypertrophic fibers (Fig. 3). Necrotic fibers with slight phagocytic invasions were also seen. Clusters of macrophages were present in the interfascicular septa.

The histologic findings in the younger girl (case 2) were similar: There was much variation in fiber size with rounded atrophic fibers, necrotic fibers with phagocytosis, abundant perimysial connective tissue, and somewhat mononuclear infiltration in the perimysial tissue (Fig. 4).

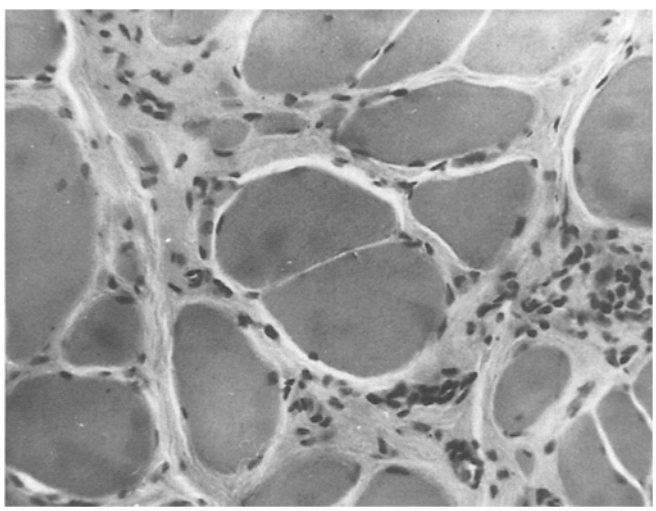

Fig. 3 Muscle biopsy from the quadriceps of case 1 (elder sister). Marked increase of connective tissue, large variation of fiber caliber, split fiber, and necrotic fiber invaded by macrophages (H & E × 200)

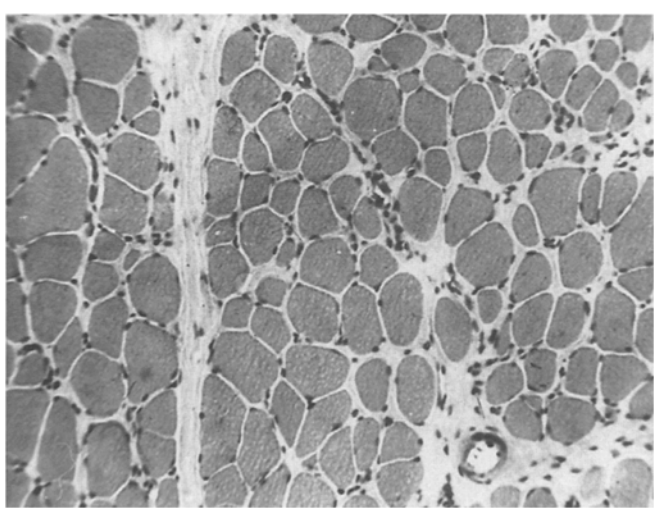

Fig. 4 Quadriceps muscle from case 2 (younger sister). Marked variation in fiber size, abundant interstitial tissue, and split fibers (H & E × 160)

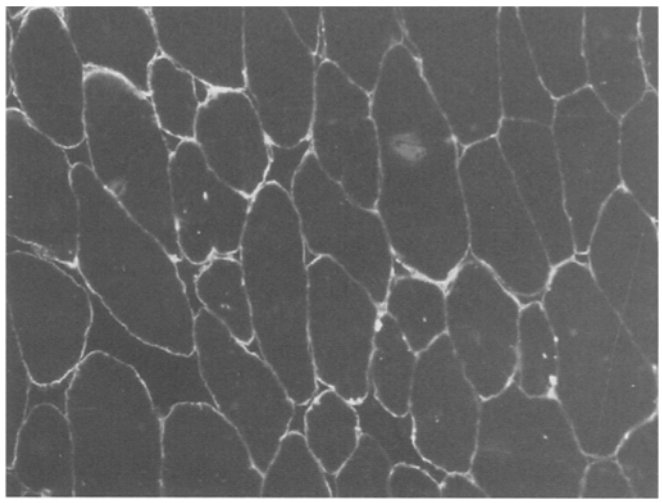

Fig. 5 Immunohistochemical localization of dystrophin in the muscle of case 2 (younger sister; × 160)

Immunohistochemistry for dystrophin using monoclonal antibodies (Novocastra Laboratories, UK) against the rod domain (DYS1) and C-terminal domain (DYS2) was performed. In the muscle specimens normal continuous staining at the sarcolemma of muscle fibers was found (Fig. 5).

Discussion

Muscle dystrophies presented here in the young sisters have given rise to the difficulties in making an exact diagnosis. However, correct classification is essential for reliable genetic counseling. There are two large categories: They may belong to X-linked muscular dystrophies or autosomal recessive limb-girdle dystrophy. Only the new results of molecular genetics and immunohistochemistry brought about the possibility of classifying young female patients correctly.

Regarding the first group, the girls may be manifesting carriers of Duchenne/Becker (DMD/BMD)-type dystrophy. Uneven inactivation of X-chromosome in carriers for a DMD/BMD mutation may be the underlying mechanism for appearance of muscular dystrophy in female heterozygotes (Ayme et al. 1979; Gomes et al. 1977; Moser and Emery 1974; Richards et al. 1990; Yoshioka 1981). Their variable clinical symptoms (frequently slowly progressive proximal myopathy) manifest themselves mostly in the second or third decade of life (Moser and Emery 1974). Sometimes it may appear as early as the first 10 years of age (Ayme et al. 1979; Gomez et al. 1977; Richards et al. 1990). When a symptomatic carrier has an affected boy in the family the diagnosis is simple (Gomez et al. 1977), but in the absence of positive pedigree it may be doubtful. Analysis of DNA indicating deletion in the DMD gene of X-chromosome verifies the diagnosis (Richards et al. 1990); however, point mutations may be overlooked, and consequently, negative cDNA probes do not exclude undoubtedly DMD/BMD mutation.

When cDNA analysis fails to reveal deletion, dystrophin immunohistochemistry may support the detection of carrier status both in manifesting and asymptomatic cases (Arahata et al. 1989a; Bonilla et al. 1988). Mosaicism of fibers with dystrophin positivity and dystrophin deficiency proved to be indicative of carrier status for DMD/BMD gene, and the proportion of dystrophin-deficient fibers in symptomatic carriers was higher as compared with asymptomatic cases (Arahata et al. 1989b; Bonilla et al. 1988). In a recent study three girls with a sporadic myopathy of early onset were presented, none of whom had a verifiable deletion in the DMD/BMD gene, but in muscle specimens a mosaic of fibers with or without dystrophin suggested the carrier state of X-linked dystrophy (Minetti et al. 1991).

Balanced X-autosome translocations affecting the X-chromosome at the Xp21 locus were revealed in girls with characteristic clinical features of X-linked muscular dystrophy (Boyd et al. 1986; Lindenbaum et al. 1979).

The family history of sisters presented here was negative with regard to muscle diseases. The cDNA probes in our patients failed to reveal any deletion in the X-chromosome, and the immunohistochemical observations have shown dystrophin-positive fibers in the muscle specimens. Our patients had normal 46,XX karyotype, and there were no visible structural abnormalities in the chromosomes. Therefore, both karyotypic mosaicism and translocation between X-chromosome and an autosome could be excluded. These findings ruled out any relevance of our patients to X-linked dystrophies, despite the clinical phenotype as well as laboratory and histologic findings similar to BMD.

Some problems arise in distinguishing the autosomal recessive limb-girdle dystrophy from X-linked dystrophies on clinical features. Families with affected members suggestive of Duchenne-type dystrophy, but inherited as an autosomal recessive trait, were presented previously (Hamida et al. 1983; McGuire et al. 1991; Norman et al. 1989; Salih et al. 1983). There are a few clinical features supporting the differentiation: Slightly later appearance of clinical symptoms, lower serum CK activity, and the pedigree may indicate autosomal recessive dystrohpy. The histopathologic pictures may also have some differences. In our elder patient the first symptoms were noticed at a very early age of life. The rise in serum CK activities was considerable in both girls, and in the younger one it was highly increased as early as 5 years of age, when she was apparently free of any clinical symptoms. The histologic changes were also very prominent, but did not contribute to the differentiation. Consequently, only the new methods, i.e., dystrophin identification and DNA analysis, proved to be adequately sensitive in these girls to verify the tpye of their muscular dystrophy as an autosomal recessive form. In the latter group we could not exclude the possibility of a newly discovered type of severe childhood autosomal recessive muscular dystrophy (SCARMD) due to the specific deficiency of the 50 Kd dystrophin-related glycoprotein in the muscle, which would need a special immunohistochemical analysis (Matsumura et al. 1992).

These types of autosomal recessive muscular dystrophies frequently fall under the term limb-girdle muscular dystrophy. However, this form of dystrophy is under extensive discussion, because it may cover a heterogenic group of proximal myopathies. In our cases the symptoms were predominantly proximal, although the elder girl had her distal muscles of the lower extremities affected. Her clinical phenotype seemed more like DMD/BMD. In our opinion, it seems reasonable to consider autosomal recessive childhood muscular dystrophy. The exact classification, first of all the differentiation between manifesting DMD/BMD carriers and young female patients with autosomal recessive inheritance, has practical impact on genetic counseling. Symptomatic women with autosomal recessive trait of inheritance have a much lower chance of delivering an affected offspring compared with the risk of 50% for a carrier of X-linked dystrophy.

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References

Arahata K, Hoffman EP, Kunkel LM, Ishiura S, Tsukuhara T, Ishihara T, Sunohara N, Nonaka I, Ozawa E, Sugita H (1989a) Dystrophin diagnosis: comparison of dystrophin abnormalities by immunofluorescence and immunoblot analysis. Proc Natl Acad Sci USA 86:7154–7158

Arahata K, Ishihara T, Kamakura K, Tsukahara T, Ishiura S, Baba C, Matsumoto T, Nonaka I, Sugita H (1989b) Mosaic expression of dystrophin in symptomatic carriers of Duchenne's muscular dystrophy. N Engl J Med 320:138–142

Ayme S, Pelissier JF, Mattei JF, Giraud F (1979) Duchenne type muscular dystrophy and consanguinity: difficulties in pedigree analysis. J Med Genet 16:393–405

Bonilla E, Schmidt B, Samitt CE, Miranda AF, Hays AP, DeOliveira AB, Chang HW, Servidei S, Ricci E, Younger DS, DiMauro S (1988) Normal and dystrophin deficient fibers in carriers of the gene for Duchenne muscular dystrophy. Am J Pathol 133:440–445

Boyd Y, Buckle V, Holt S, Munro E, Hunter D, Craig I (1986) Muscular dystrophy in girls with X;autosomal translocations. J Med Genet 23:484–490

Gomez MR, Engel AG, DeWald G, Peterson HA (1977) Failure of inactivation of Duchenne dystrophy X-chromosome in one of female identical twins. Neurology 27:537–541

Hamida MB, Fardeau M, Attia N (1983) Severe childhood muscular dystrophy affecting both sexes and frequent in Tunesia. Muscle Nerve 6:469–480

Hoffmann EP, Brown RH, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51:919–928

Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM (1987) Complete cloning of the Duchenne muscular dystrophy cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 50: 509–517

Kunkel LM, Monaco AP, Middlesworth W, Ochs HD, Latt SA (1985) Specific cloning of DNA fragments absent from the DNA of a male patient with an X chromosome deletion. Proc Natl Acad Sci USA 82:4778–4782

Lindenbaum RH, Clarke G, Patel C, Moncrieff M, Hughes JT (1979) Muscular dystrophy in an X;1 translocation female suggests that Duchenne locus is on X chromosome short arm. J Med Genet 16:389–392

Matsumura K, Tomé FMS, Collin H, Azibi K, Chaouch M, Kaplan JC, Fardeau M, Campbell KP (1992) Deficiency of the 50 K dystrophin-associated glycoprotein in severe childhood autosomal recessive dystrophy. Nature 359:320–322

McGuire SA, Fischbeck KH (1991) Autosomal recessive Duchenne-like muscular dystrophy: molecular and histochemical results. Muscle Nerve 14:1209–1212

Minetti C, Chang HW, Medori R, Prelle A, Moggio M, Johnsen SD, Bonilla E (1991) Dystrophin deficiency in young girls with sporadic myopathy and normal karyotype. Neurology 41: 1288–1292

Monaco AP, Bertelson CJ, Middleworth W, Colletti CA, Aldridge C, Fischbeck KH et al. (1985) Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. Nature 316:842–845

Moser H, Emery AEH (1974) The manifesting carrier in Duchenne muscular dystrophy. Clin Genet 5:271–284

Norman AM, Hughes HE, Gardner-Medvin D, Nicholson LVB (1989) Dystrophin analysis in the diagnosis of muscular dystrophy. Arch Dis Child 64:1501–1503

Richards CS, Watkins SC, Hoffman EP, Schneider NR, Milsark IW, Katz KS, Cook JD, Kunkel LM, Cortada JM (1990) Skewed X inactivation in a female MZ twin results in Duchenne muscular dystrophy. Am J Hum Genet 46:672–681

Salih MAM, Omer MIA, Bayoumi RA, Karrar O, Johnson M (1983) Severe autosomal recessive muscular dystrophy in an extended Sudanese kindred. Dev Med Child Neurol 25:43–52

Walker AP, Laing NG, Bartlett RJ, Mechler F, Akkari PA, Chandler DC, Layton MG, Mears ME, Secore SL, Hung WY, Stajich JM, Petricak-Vance MA, Kakulas BA, Roses AD (1990) A TAQ I map of exon-containing fragments of the dystrophin gene. J Neurol Sci 98:163–164

Walton JN, Gardner-Medwin D (1981) Progressive muscular dystrophy and the myotonic disorders. In: Walton JN (ed) Disorders of voluntary muscle. Churchill Livingstone, Edinburgh, pp 481–524

Yoshioka M (1981) Clinically manifesting carriers in Duchenne muscular dystrophy. Clin Genet 20:7–12