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CASE REPORT

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Expression of growth associated protein 43 and neural cell adhesion molecule in congenital fibre type disproportion with interstitial myositis

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Abstract We report on the expression of growth associated protein (GAP)43 and neural cell adhesion molecule (NCAM) in congenital fibre type disproportion (CFTD) with myopathological additional signs of interstitial myositis. We assume that sarcolemmal GAP43 in developmental disordered myocytes plays a role in maintenance of growth morphology. In muscular dystrophy light microscopical evaluation reveals no GAP43 immunoreactivity in regenerating fibres. The expression of GAP43 seems to be a characteristic feature of CFTD. The expression of NCAM, particularly in the sarcolemma of small muscle fibres of CFTD, indicates a functional state of permanent partial denervation. Whether the steroid-responsive interstitial myositis is pathogenetically related to CFTD or a coincidental inflammation is not known. Because of the clinical and myopathological data the differential diagnosis of Emery-Dreifuss muscular dystrophy is considered.

Key words Congenital fibre type disproportion (CFTD) Interstitial myositis · GAP43 · NCAM

Introduction

In 1969, Brooke and Engel [3] found ten children with non-progressive weakness present at birth whose biopsies showed relative smallness of type 1 muscle fibres. Subsequently, Brooke and Engel 1969 termed this condition congenital fibre type disproportion (CFTD). The patients are born floppy with variable weakness which does not progress. Approximately half of the patients have skeletal deformities including foot deformities and kyphoscoliosis [7]. Clancy et al. [5] presented five cases of CFTD in which muscle biopsies satisfied the most important histological and statistical criteria established by Brooke [2], but which differed from the classical clinical expression of the disease. The phenotypic spectrum of these cases ranged from mild to severe grades of hypotonia without the characteristic clinical signs of Brooke's cases. Today, it is assumed that CFTD is a developmental disorder due to an abnormally high rate of embryonic cell death among spinal motor neuroblasts early in gestation [22]. However CFTD is not a clinicopathological entity, but can be found in various congenital myopathies, other muscle disorders and furthermore in diseases of the central nervous system [6, 21, 29].

To assess the functional state of myocytes in CFTD, we studied the expression of growth associated protein (GAP)43 and neural cell adhesion molecule (NCAM; Leu 19) [15, 20] in this condition. The expression of growth-associated protein GAP43 in human striated muscle has not been described previously. GAP43 is a protein of growing interest in studies of neurite formation during neuronal development [13, 26, 27] and regeneration [8, 14, 24, 25]. Leu19 is known to be a marker for regenerating and denervated human muscle fibres and satellite cells [11, 17, 23]. We report a case of CFTD with expression of GAP43 and NCAM in small myocytes and myopathological additional signs of interstitial myositis.

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Case report

A 25-year-old man was admitted to our hospital because of progressive weakness of his legs and arms over the last 3 years. He also complained of myalgia of the right leg. Since birth, he suffered contractures of the ankles and elbows. Neurological examination showed right and proximally accentuated mild atrophic paresis. The patient's family history was unremarkable. Electromyography of the right deltoid and right rectus femoris muscles were myopathic. Occasional positive sharp waves and fibrillations were also noted. Electrocardiography and echocardiography were normal. Serum creatine kinase was elevated (1776 U/l). Muscle biopsy was performed (see below). Immunosuppressant therapy (prednisolone) was initiated. Seven months later follow-up investigation reveals a stationary course of the paresis and a decrease but not normalization of the serum creatine kinase level (367 U/l). La-

ter, electromyography of the right deltoid muscle remained myopathic and a few positive sharp waves and fibrillations were noted. Thus, immunosuppressant therapy was continued.

For controls we carried out conventional histological and immunohistochemical studies in four normal and 11 pathological muscles (3 cases of facio-scapulo-humeral muscular dystrophy, 3 cases of limb-girdle muscular dystrophy, 5 cases of neurogenic atrophy). The diagnosis of the pathological control biopsies was based on conventional criteria considering clinical and myopathological data including immunohistochemistry [1, 7].

Materials and methods

Open muscle biopsy was performed on the left deltoid muscle under local anaesthesia and processed for light microscopy. Specimens were immediately frozen in isopentane cooled in liquid nitrogen, then cut in a cryostat. Sections 8 μm thick were stained with the following methods: haematoxylin and eosin, Gomori's trichrome, periodic acid-Schiff reduced oil red, nicotinamide adenine dinucleotide tetrazolium reductase and metachromatic dye adenosine triphosphatase (ATPase) at pH 9.4 and after incubation at pH 4.3 and 4.5. Semithin sections from Epon-embedded material were stained with toluidine blue.

For immunohistochemical studies in cryostat sections, the following antibodies diluted in TRIS-buffer 0.05 M were used: monoclonal mouse anti-GAP43, 1:5 (Dianova); monoclonal mouse anti-CD56 (Leu19; clone MY11), 1:40 (Becton Dickinson); monoclonal mouse anti-CD8 (Leu2a; clone SK1), 1:40 (Becton Dickinson); monoclonal mouse anti-CD4 (gp32), 1:20 (Biotest); monoclonal mouse anti-CD22 (gp130), 1:10 (Dakopatts); monoclonal mouse anti-CD68 (KP1), 1:100 (Dakopatts). The bound primary antibodies were visualized using the alkaline phosphatase anti-alkaline phosphatase method. Controls for the staining specificity of all primary antibodies have been performed with non-immune immunoglobuline instead of the first antibody. Light haemalaun counterstaining was used to discern cellular structures.

Results

Morphological analysis of the muscle biopsy (ATPase pH 4.3, pH 4.6 and pH 9.4) revealed a small size of type 1 and hypertrophy of type 2 fibres. There was also type 1 fibre predominance (see Table 1, Figs. 1, 2 and Fig. 3). Myotubes and rods were not found. Neurogenic type grouping was not seen. Some necrotic fibres with mononuclear phagocytic cells were detected. In the small hypotrophic and large hypertrophic fibres occasional internal nuclei were seen. Basophilic fibres were not found. There was no liposclerotic change of the muscle.

Immunohistochemically, the great majority of small muscle fibres showed mainly sarcolemmal staining of GAP43 and Leu19 (Figs. 4, 5). Leu19 immunostaining was also positive in some small muscle fibres around centrally placed nuclei. Furthermore, immunohistochemistry showed focally accentuated perimysial-perivascular and endomysial CD8- and CD4-positive inflammatory cells in equal amounts (Fig. 6). Interstitial monocytes (CD68-positive cells) were increased, but B cells (CD22-positive) and natural killer cells (CD16-positive) were not found.

Table 1 Mean fibre diameter and percentage of fibres. The standard measure [12, 18] are in parentheses

	Mean fibre diameter (μm)	Percentage of fibres
Type I	41.41 (51.6)	61 (53.3)%
Type II	99.17 (58.6)	39 (46.7)%

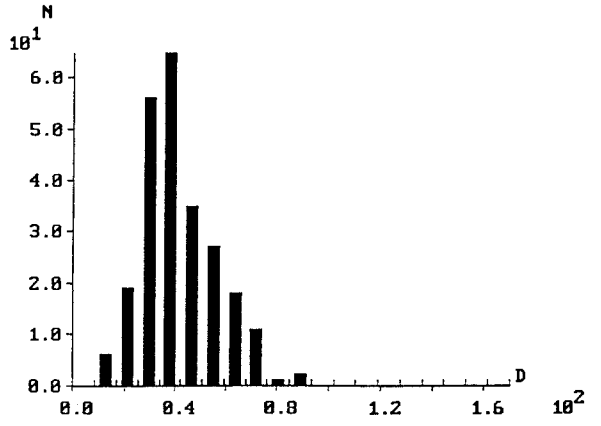

Fig. 1 Histogram of type 1 muscle fibres (*N* number, *D* diameter (μm))

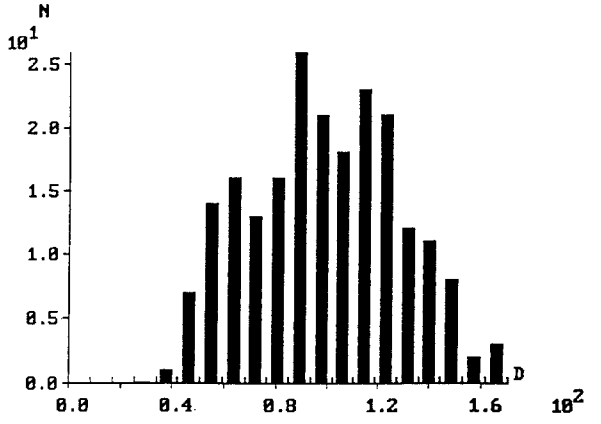

Fig. 2 Histogram of type 2 muscle fibres

The muscle fibres of normal and neurogenic atrophic muscles showed no staining for GAP43 but Leu19 immunostaining in neurogenic disorders was positive in small angular fibres, fibre splitting and a few small round regenerating fibres. In muscular dystrophies no GAP43 immunoreactivity was demonstrated despite some small regenerating fibres express the Leu19 antigen. Satellite cells were seen in all control biopsies. In normal muscle we only found some interstitial CD68-positive mononuclear cells but no other inflammatory cells. In neurogenic atrophy immunostaining showed some CD4- and CD68-positive interstitial cells but never any CD8-positive lymphocyte. In muscular dystrophies there was liposclerotic change of the muscle, pathologic variation in fibre diameter and necrotic fibres were seen. Additionally, there were also some small angular fibres in facio-scapulo-humeral muscular dystrophy. Phenotyping of inflammatory cells in facio-scapulo-humeral muscular dystrophy and

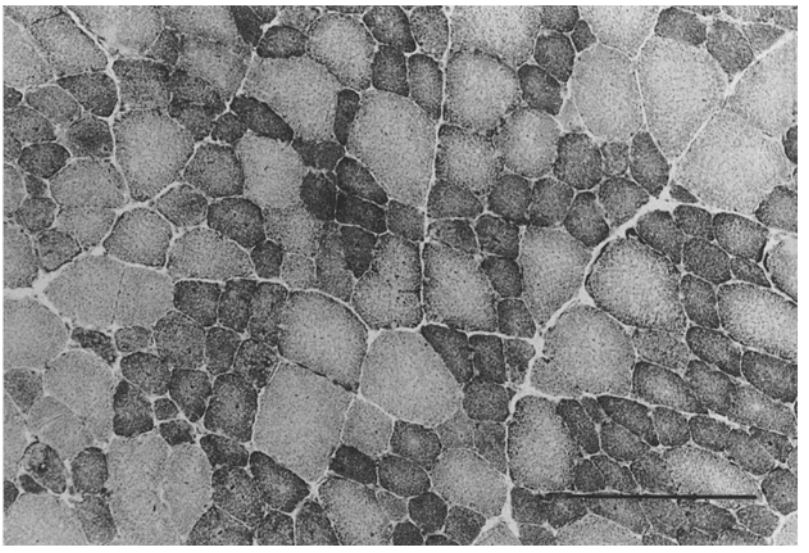

Fig. 3 Small fibres are predominantly of type 1 and more numerous than large type 2 fibres (frozen section, ATPase pH 4.3). *Bar*=180 μm

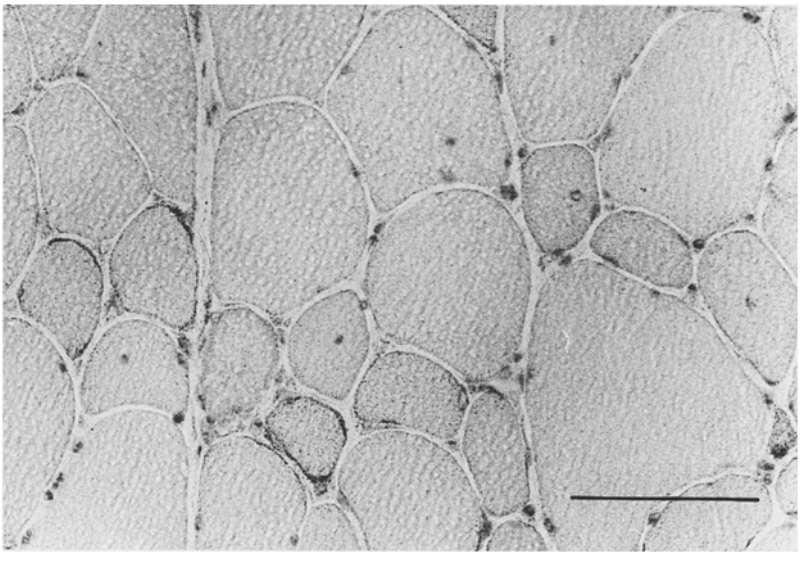

Fig. 4 2 Growth associated protein 43 immunoreactivity in small muscle fibres. Note particularly sarcolemmal staining pattern [frozen section, alkaline phosphatase anti-alkaline phosphatase (APAAP) method]. *Bar*=100 μm

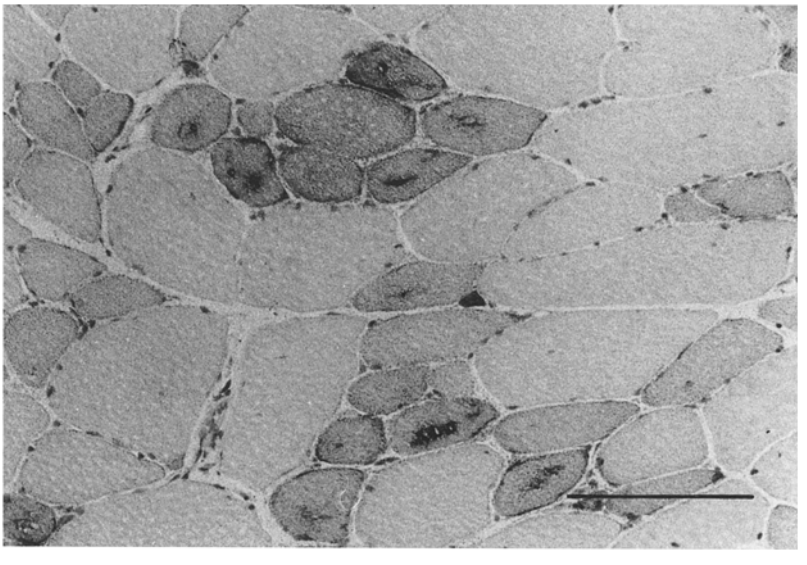

Fig. 5 Leu19 immunoreactivity in small muscle fibres, particularly sarcolemmal and around centrally placed nuclei (frozen section, APAAP method). *Bar*=120 μm

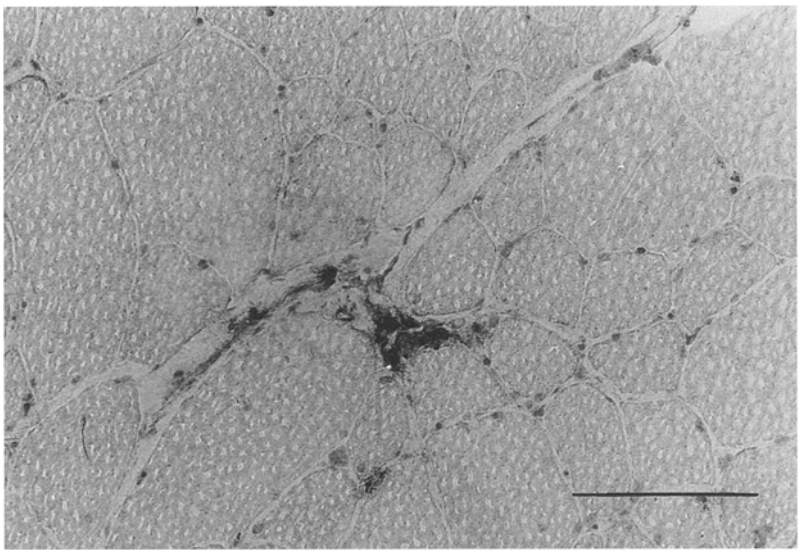

Fig. 6 Perivascular CD8-positive inflammatory cells (frozen section, APAAP method). *Bar*=130 μm

less in limb-girdle muscular dystrophy revealed some interstitial CD4- and CD8-positive cells, but no immunoreactive inflammatory cells surrounding or invading myofibres without signs of degeneration were demonstrable. Furthermore, in muscular dystrophies CD68-positive cells were abundant but CD22-positive cells were not found. There was no complement-mediated angiopathy either in normal nor in dystrophic and neurogenic atrophic muscles. Considering all preparations of immunohistochemistry, controls for the staining specificity with non-immune immunoglobuline instead of the first antibody never shows any specific immunostaining.

Discussion

This case was characterized clinically by long-standing contractures of the ankles and elbows with progressive atrophic paresis in adult life. The latter feature is concluded to be due to pathologically described interstitial myositis, because CFTD is usually a non-progressive disorder. The contractures are typical signs of CFTD. The case presented satisfies the criteria suggested for the diagnosis of CFTD [3, 5, 7]: small size and predominance of type 1 fibres together with hypertrophic type 2 fibres. However because of the clinical picture, especially atrophic paresis together with contractures, and myopathological signs of degeneration we considered the differential diagnosis of a sporadic case of Emery-Dreifuss muscular dystrophy (EDMD) [19]. The feature of fiber type disproportion was previously described in EDMD [10, 21, 29, 30]. In this context the presence of CD4- and CD8-positive peri- and endomysial inflammatory cells may represent a non-specific concomitant inflammatory phenomenon. Otherwise we only found sparse degenerative changes without signs of regeneration in the biopsy. Furthermore, clinical signs of cardiopathy, usually a feature of EDMD [30] were not found, thus the differential diagnosis of EDMD in our case is unlikely. Considering all clinical and myopathological data we made the diagnosis of CFTD together with the unusual finding of interstitial myositis inducing some myopathic changes like central nuclei and necrotic fibres. The picture of hypertrophic type 2 fibres and hypotrophic type 1 fibres could not be related to interstitial myositis (probably due to connective tissue disease) because there we usually find a selective type 2 atrophy [4]. The interstitial myositis is thus an additional sign in the reported case of CFTD. It is not possible to prove whether interstitial myositis is pathogenetically related to CFTD or a coincidental myositis. However long-standing developmental antigenic structure of small myocytes with expression of GAP43 and NCAM might possibly prime the immune system triggering an interstitial myositis, indicating a new form of CFTD associated with onset of interstitial myositis in adult life. To our knowledge there are no reports of myositis in CFTD or vice versa.

Furthermore, for the first time we describe the expression of GAP43 in human muscle cells. Stocker et al. [25] suggested the expression of GAP43 in nonneuronal cells of embryonic chicken limb, some of which may be part of the muscle cell lineage. In the central nervous system and peripheral nervous system GAP43 is known to play a role in growth cone morphology and/or mobility [9, 16]. Verhaagen et al. [28] reported the expression of B-50 (GAP43) in nerve fibres that invade muscle. Particularly in view of sarcolemmal staining pattern, we assume that in the reported case GAP43 is expressed in developmentally disordered myocytes but not in intramuscular nerve fibres. We suggest that GAP43 is important in maintenance of growth morphology of small myocytes in CFTD. Surprisingly, in muscular dystrophies there is no expression of GAP43 in regenerating fibres. So far, the expression of GAP43 seems to be a characteristic feature of CFTD.

In addition to Illa et al. [11] and Knudsen et al. [13] we show that not only regenerating and denervated muscle fibres, satellite cells, the neuromuscular junction and

myoblasts, but also developmentally disordered myocytes express Leu19. From the results of Müller-Felber et al. [17] who recently demonstrated in an experimental study the early (from day 2) but not late fibre type specific expression of Leu19 after denervation, we assume that the expression of Leu19 (NCAM) in small muscle fibres of CFTD indicates a functional state of permanent partial denervation.

The interstitial myositis described responded to immunosuppressant therapy assessed clinically and by laboratory data. This led us to the assumption that CFTD with interstitial myositis is a new steroid-responsive entity.

Our findings indicate that further immunohistochemical studies should be performed regarding the expression of growth-associated protein GAP43 and NCAM in developmental myopathies in order to establish the pathogenesis of these muscle diseases.

To summarize we demonstrate a case of CFTD together with the unusual finding of interstitial myositis. This is the first description of the expression of GAP43 in developmental myopathy.

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