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Origin and significance of small muscle fibres in neuromuscular disease

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Summary. Small muscle fibres, defined as those of less than 40 μm diameter in the male and 30 μm in the female were encountered in muscle biopsies of patients with spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), polymyositis (PM) and myopathy/dystrophy. Excessive reactivity with NADH-TR in small fibres did not discriminate between neurogenic and myopathic disorders. Quantification of perifascicular atrophic fibres, the number of nuclei in atrophic fibres, or the presence of isolated or grouped small fibres without histochemical kinship to their surrounding fibres did not aid recognition of the disease process in the groups studied. Small fibres which reacted strongly both with NADH-TR and ATPase at pH 9.4 (Type 3 fibres) constituted 38% of small fibres in the biopsies of SMA; 25% in ALS; but only 1% and 2.7% in PM and myopathy/dystrophy respectively. Thus, the presence of small Type 3 fibres in muscle biopsies may be a useful marker for neurogenic disorders in adults.

Key words: Small muscle fibres – Neurogenic disease – Polymyositis – Muscular dystrophy – Histochemistry

Introduction

Most muscle fibres in a normal muscle range between 40 to 80 μm diameter in the male and 30 to 70 μm in the female (Brooke and Engel 1969). Fibres of lesser diameter than these limits are thus smaller than normal. Small muscle fibres have also been defined by their circumference and area (Watkins and Cullen 1982). They can be observed in nearly all neuromuscular diseases, in systemic disorders and sometimes in healthy persons (Brooke and Engel 1969; Dubowitz and Brooke 1973). Indeed, it is one of the most common abnormalities encountered in the evaluation of a skeletal muscle biopsy. Most of the histological information on small muscle fibres comes from studies of denervation. Genetically-determined diseases, such as spinal muscular atrophy and Duchenne muscular dystrophy, are often associated with the presence of small fibres in the biopsy (Brooke and Engel 1969; Watkins and Cullen 1982). Small muscle fibres have also been recognized in acquired inflammatory myopathies, such as polymyositis, in myopathies secondary to metabolic or toxic disorders, and also in disuse atrophy.

There are several possible sources of small muscle fibres in neurogenic and myopathic disorders. They may arise from atrophy of previously healthy muscle fibres, due to a decrease in protein synthesis (Griggs et al. 1980), an increase in protein breakdown (Corbett et al. 1982), or both. Fibre splitting may also give rise to small fibres both in myopathies and neurogenic disorders (Swash and Schwartz 1977), although the significance of this phenomenon is still controversial (Schmalbruch 1976; Swash and Schwartz 1977; Swash et al. 1978). Abnormal development and differentiation of muscle fibres, for example in central core disease and nemaline myopathy, may result in small muscle fibres. In addition, it has been suggested that some small muscle fibres may represent the tapered ends of intrafascicularly terminating muscle fibres (Swatland 1983). Fibres undergoing regeneration following necrosis may also approach this size range (Schmalbruch 1976).

The morphometric analysis of muscle biopsy specimens can be useful in diagnosis (Brooke and Engel 1969; Sandstedt et al. 1982; Watkins and

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Table 1. Morphometric data: fibre types based on ATPase classification (Brooke and Engel 1969)

Case	Age	Sex	%	Type 1 fibre		Type 2 fibre	
				diameter (μm) mean + S.D.	atrophy factor	diameter (μm) mean + S.D.	atrophy factor
Spinal muscular atrophy							
1	28	F	11	65.2 + 12.4	186	58.4 + 14.6	462
2	25	M	61	95.4 + 47.0	387	112.9 + 47.6	97
3	17	F	63	85.9 + 40.7	180	67.3 + 35.2	347
4	58	M	25	66.8 + 11.4	15	64.1 + 22.4	361
5	49	F	9	84.7 + 34.5	83	56.1 + 38.3	793
Amyotrophic lateral sclerosis							
6	41	M	54	60.5 + 13.1	31	47.7 + 18.5	556
7	71	M	12	64.4 + 24.7	647	56.6 + 29.6	755
8	67	M	19	61.2 + 12.8	80	49.9 + 24.3	717
9	57	F	71	36.7 + 19.2	620	51.5 + 29.3	683
10	46	M	37	60.4 + 20.7	342	49.1 + 26.4	940
Idiopathic polymyositis							
11	55	F	40	57.4 + 12.6	27	35.4 + 11.1	375
12	50	F	63	47.0 + 10.7	36	47.9 + 10.9	61
13	35	F	42	53.8 + 16.2	109	42.0 + 22.7	743
14	55	M	52	54.4 + 16.9	256	27.4 + 10.6	1639
15	66	M	48	40.3 + 8.5	417	41.8 + 11.2	484
Myopathy/dystrophy							
16	75	M	86	50.2 + 18.4	422	80.9 + 25.1	77
17	22	M	39	77.1 + 14.6	31	83.2 + 23.8	150
18	54	M	64	55.9 + 17.3	224	36.0 + 17.5	1144
19	53	M	59	57.6 + 10.6	41	57.9 + 12.2	44
20	17	M	54	51.2 + 13.2	286	64.6 + 10.7	17
Controls							
1	19	M	53	56.7 + 9.9	21	77.3 + 15.4	8
2	45	M	54	50.4 + 11.4	80	62.4 + 14.2	0
3	33	F	38	58.6 + 11.3	0	53.8 + 12.2	0
4	31	M	43	68.4 + 12.9	0	62.0 + 11.9	0
5	22	M	23	65.2 + 9.9	0	57.4 + 12.7	37

Cullen 1982). We have used this quantitative approach to examine the significance of small muscle fibres in neurogenic and myopathic disorders.

Materials and methods

Patients with neuromuscular diseases. Four groups of muscle biopsies were defined on the basis of site of dysfunction as follows: Group 1, five patients with type III or type IV spinal muscular atrophy (SMA) in whom the basic lesion was in the lower motor neuron; Group 2, five patients with amyotrophic lateral sclerosis (ALS) in whom, in addition to lower motor neuron deficit, the upper motor neuron was also involved; Group 3, five patients with adult-type idiopathic polymyositis (PM), an acquired, inflammatory muscular disorder; Group 4, various metabolic or genetic myopathies including patients with osteomalacia, hypothyroid myopathy, and myotonia congenita respectively, and two unrelated patients with facioscapulohumeral dystrophy. The ages of these 20 patients ranged from 17–75 years (mean 47 yrs).

Control patients. Control data were obtained from muscle biopsies from five patients, four males and one female, aged 19–53 years. These patients were biopsied for various clinical indications, including non-specific muscle pain, as part of negative investigation for suspected polyarteritis, and during an elective orthopaedic procedure. The muscle biopsies in these patients were all histologically normal and no clinical abnormality developed during at least one year's subsequent observation.

Histochemical methods. Open muscle biopsy was performed under local anaesthesia in 21 of 25 patients and needle biopsy was used in four patients. In most cases, the biopsy was taken from the quadriceps, deltoid or biceps brachii, but in two patients, the flexor carpi ulnaris muscle was chosen. Each biopsied specimen was frozen immediately in isopentane which had been cooled with liquid nitrogen. Serial, 6 μm thick, transverse sections were cut in a cryostat.

Routine histological evaluation. A routine assessment of morphological changes was made on each biopsy by using haematoxylin and eosin (H & E) and modified Gomori trichrome stains and a battery of histochemical methods including reduced nicotine adenine tetrazolium reductase (NADH-TR), myosine adenosine triphosphatase (ATPase) preincubated at pH 9.4, 4.6 and 4.3, periodic acid schiff (PAS) and oil red 0 methods (Dubowitz and Brooke 1973). Particular attention was paid to the occurrence of small angular fibres, fibre hypertrophy, grouped

atrophy, fibre type grouping, central nucleation (normal < 3%) (Dubowitz and Brooke 1973), fibre splitting, basophilic fibres, fibre necrosis and phagocytosis, inflammatory changes, endomysial and perimysial fibrosis, and architectural changes.

Quantitative histological evaluation. Each section was examined by light microscopy with a X25 objective, using an eyepiece micrometer. The analyses were performed on muscle fibres in five random microscopic fields. Fibres were classified as Type 1 or Type 2 based on the ATPase reactions (Brooke and Engel 1969). The proportions of each fibre type were recorded and the lesser fibre diameters were measured (Brooke and Engel 1969). The atrophy factor, corrected for sex, was calculated for Type 1 and Type 2 fibres (Brooke and Engel 1969).

Since most normal fibres are between 40 to 80 μm diameter in the male and 30 to 70 μm in the female (Brooke and Engel 1969), we defined small muscle fibres as those with a diameter less than these lower limits. The diameters of these small fibres were measured and their intensity of reaction with ATPase at pH 9.4 or 4.3, and with NADH-TR methods was compared. The location of each small fibre as related to perimysium, endomysium or other muscle fibres of either histochemical type was recorded. The number of isolated and grouped small fibres which were histochemically unrelated to surrounding normal and/or hypertrophic fibres was counted. The number of nuclei in each small fibre was also counted.

Results

Routine histological evaluation

Small angular fibres were present in three of the five patients in the group with SMA. Nearly all the small muscle fibres in the patients with ALS and a majority of the small fibres in three of the five patients with SMA were angular, whereas in the other two patients with SMA the small fibres had a rounded shape. Both rounded and angular small fibres were present in the patients in Group 3 with PM and in patients in Group 4 with myopathy/dystrophy. Fibre hypertrophy and grouped atrophy were prominent in the cases of neurogenic disorders. In Group 4, only the two patients with facioscapulohumeral dystrophy showed fibre hypertrophy. Grouped atrophy was also found in the cases of PM. Central nucleation was particularly prevalent in Group 1 with SMA. Fibre necrosis was present in some cases in all four groups but was most marked in the group with PM. Fibre splitting was seen in six cases in the four groups. Basophilic fibres and phagocytosis were observed in some cases of PM and in the two cases of facioscapulohumeral dystrophy. Inflammatory changes were noted only in biopsies from patients with PM. Fibrosis was prominent in 13 of the 20 biopsies. Marked disorganization of the intermyofibrillar network was noted in two cases of ALS as target fibres, and in two cases of PM and one case of facioscapulohumeral dystrophy as moth-eaten fibres.

Table 2. Numbers of Type 1 and Type 2 small muscle fibres (based on ATPase reaction) reactive with NADH-TR method

Case	No. of small fibres			
	Type 1 fibres		Type 2 fibres	
	NADH-TR Dark	NATH-TR Intermediate	NADH-TR[a] Dark	NADH-TR Intermediate
1	3	0	0	16
2	10	0	1	0
3	9	0	3	9
4	1	0	4	24
5	1	0	43	7
6	3	0	5	34
7	10	0	44	34
8	2	0	17	52
9	194	0	25	25
10	19	0	43	34
11	14	0	4	158
12	33	0	0	28
13	54	0	0	3
14[b]	–	–	–	–
15	66	0	0	84
16	63	0	0	2
17	2	0	2	9
18	18	0	2	48
19	6	0	1	2
20	30	0	0	2

[a] Type 3 fibres
[b] Failure to classify due to technical problem

Morphometric analysis

Morphometric data on the muscle fibres are shown in Table 1. Type 1 fibre predominance was seen in seven cases in the four groups; it was most prominent in Group 4 with myopathy/dystrophy. Four cases in Groups 1 and 2 with ALS or SMA showed Type 2 fibre predominance. All cases, except one in the group with PM and two in the group with myopathy/dystrophy had increased atrophy factors in Type 1 or Type 2 fibres, or in both fibre types. However, a significant difference in the atrophy factor among the four groups of neuromuscular disorders was not proven by the Wilcoxon's rank sum test ($P > 0.1$).

Table 2 shows the reactivity of the small fibres to the ATPase and NADH-TR reactions. All Type 1 small fibres were darkly reactive with the NADH-TR method. Among the 131 small fibres in the group with SMA, 75 (57%) small fibres reacted strongly with NADH-TR; in the group with ALS, 362 (67%) of 541 small fibres reacted densely; 171 (39%) of 444 small fibres reacted darkly in PM; and 124 (66%) of 187 small fibres in the group with myopathy/dystrophy. On the other hand, 51 (38%) small fibres in the group

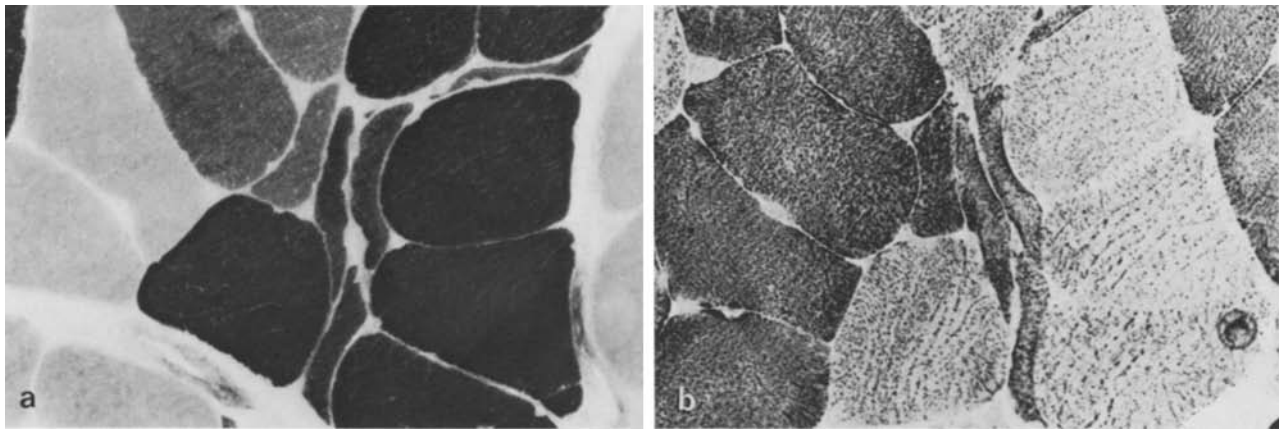

Fig. 1a and b. Small Type III muscle fibres in a patient with ALS. **a** ATPase pH 9.4 **b** NADH-TR ×175

Table 3. Number of isolated or grouped small fibres histochemically unrelated to surrounding fibres in different groups of neuromuscular diseases

Group	No. of isolated small fibres			No. of grouped small fibres		
	unrelated to surrounding fibres	(%)	Total	unrelated to surrounding fibres	(%)	Total
1	22	(16)	140	6	(17)	34
2	7	(12)	60	11	(10)	105
3	41	(26)	159	26	(21)	122
4	14	(13)	105	0	(0)	26

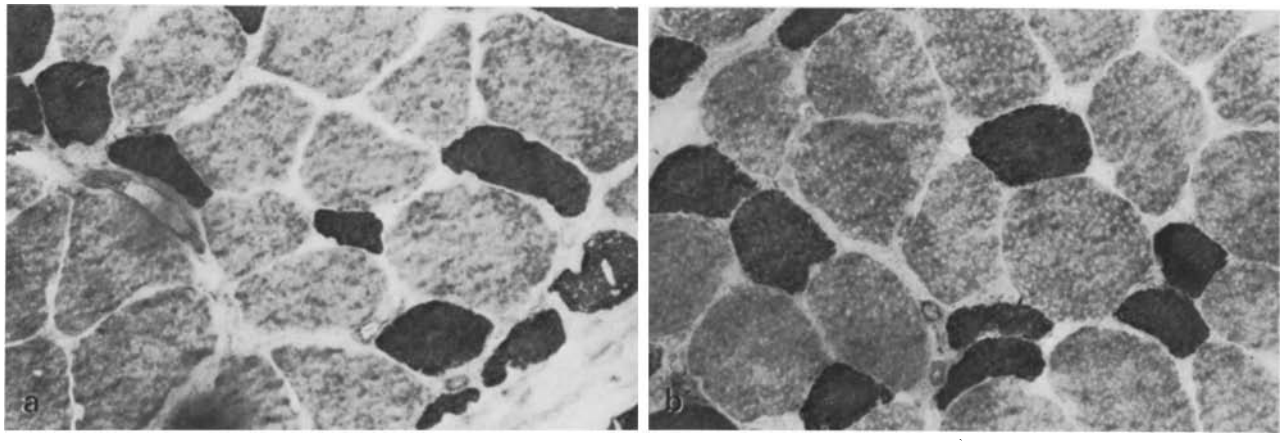

Fig. 2a and b. Small muscle fibres occur **a** alone and **b** in pair unrelated histochemically to their surrounding fibres. ATPase pH 9.4 ×175

with SMA reacted densely both with NADH-TR and ATPase at pH 9.4 (Fig. 1). Similar features were noted in 134 (25%) small fibres in the group with ALS, 4 (1%) small fibres in the group with PM and 5 (2.7%) small fibres in the group with myopathy/dystrophy.

In all of the 20 cases, some of the small fibres were located adjacent to the fascicular margin. The mean percentages of small fibres in this location were 19% in the group with SMA, 17% in ALS, 27% in PM and 14% in myopathy/dystrophy. No statistically significant differences were noted among these groups.

Small fibres were found alone as well as in

groups; they were surrounded by normal and/or hypertrophic fibres. Usually, each group of small fibres consisted of only two to four small fibres, but groups of up to 26 small fibres were seen. Small fibres which appeared unrelated to their surrounding fibres, based on the ATPase reaction, were observed in all four groups of neuromuscular diseases (Table 3 and Fig. 2).

Most small fibres contained less than five nuclei. The arithmetic mean of the number of nuclei per small fibre in the group with SMA was 1.4; in ALS, 2.8; in PM, 2.3; and in myopathy/dystrophy, 2.2. Clumps of nuclei were seen in small fibres in the biopsies with ALS, SMA and PM.

Discussion

In this study, small muscle fibres were defined as fibres with a diameter less than 40 μm in the male and less than 30 μm in the female (Brooke and Engel 1969). All our cases of SMA, ALS, PM and myopathy/dystrophy demonstrated the presence of small muscle fibres. Fibres undergoing necrosis or regeneration were present in 12 of the 20 cases, but they represented an insignificant number of small fibres. Hausmanowa-Petrusewicz et al. (1980) have suggested that hypotrophy, or a defect in the process of myogenesis, may be responsible for the smallness and unusual fibre type characteristics in Type III SMA. On the other hand, shrinkage of previously normal fibres, fibre splitting or both may account for most small fibres in ALS, PM and myopathy/dystrophy.

The degree of change of fibre size can be estimated from the atrophy factor. An abnormal atrophy factor may be a feature of both neurogenic and myopathic disorders (Dubowitz and Brooke 1973). As indicated from our results, no differentiation of the latter is possible from consideration of the atrophy factor alone. All our cases of SMA and ALS had increased atrophy factors. Furthermore, 9 of 10 cases in these two neurogenic groups showed either selective Type 2 fibre atrophy or nonselective Type 1 and Type 2 atrophy. On the other hand, 4 cases in the group with PM and 3 cases in the group with myopathy/dystrophy had abnormal atrophy values. Two of the 3 cases in the group with myopathy/dystrophy showing abnormal values displayed selective Type 1 atrophy.

It has been suggested that angular atrophic fibres react intensely and darkly with the NADH-TR method (Dubowitz and Brooke 1973; Degirolami and Smith 1982). However, in the group with ALS in our study 33% of small fibres, of which most were angular, did not react strongly with NADH-TR. This pattern of reactivity was also found in 40% of the small fibres in 3 of the 5 patients with SMA. In the other 2 patients in this group, small fibres were all of the rounded type. In 61% of the small fibres, of both round and angular types, in the patients with PM, and in 34% in patients in Group 4 with myopathy/dystrophy, the reaction with NADH-TR was weak. Therefore, a large proportion of small atrophic fibres did not react consistently darkly either in neurogenic or myopathic disorders.

Coërs and Telerman-Toppet (1979) concluded from their studies that small angular fibres with excessive activity of oxidative enzymes do not necessarily indicate denervation. This conclusion is supported by our finding that 39% of small, round or angular fibres showed strong reactions with NADH-TR in the group with PM, and 66% in the group with myopathy/dystrophy respectively. Thus, small fibres, reacting intensely with NADH-TR are not a good marker of neurogenic disorders.

Usually, fibres which react strongly with NADH-TR appear pale with ATPase at pH 9.4 and are classified as Type 1. However, small angular fibres, darkly reactive with NADH-TR, can be categorized as either Type 1 or Type 2 on the basis of the ATPase reaction. Those fibres which show high activity for both NADH-TR and ATPase at pH 9.4 have been termed Type 3 fibres (Telerman-Toppet and Coërs 1971). Fibres of this histochemical type have been reported in a number of different diseases, including Duchenne dystrophy (Coërs and Telerman-Toppet 1977), ALS and Charcot-Marie-Tooth disease (Telerman-Toppet and Coërs 1978), limb-girdle muscular dystrophy and SMA (Coërs and Telerman-Toppet 1979), and in oculopharyngeal muscular dystrophy (Dubowitz and Brooke 1973).

In spite of different pathogenic mechanisms, both the small angular fibres which were derived from denervation in the group with ALS and the small round fibres presumed to arise as a consequence of maturational arrest during development of myogenesis in SMA (Hausmanowa-Petrusewicz et al. 1980) could react strongly with NADH-TR and ATPase at pH 9.4. Furthermore, small Type 3 fibres were also found in PM, facioscapulohumeral dystrophy, osteomalacic myopathy, and hypothyroid myopathy. It seems, therefore, that the presence of small fibres of Type 3 histochemical type are not unique features of any specific neuromuscular disorder. However, this fibre type constituted 38% of small fibres in the group with SMA, 25% in the group with ALS, but only 1% in the group with PM and 2.7% in the group with myopathy/

dystrophy. These data thus suggest that in adults quantification of small fibres of Type 3 histochemical type may be an indicator of a neurogenic disorder.

Although perifascicular atrophy is by no means a specific feature of any particular myopathy, it has been recognized as a useful marker for childhood-type dermatomyositis (Mastaglia and Walton 1982; Swash and Schwartz 1984). Such a distribution of atrophic fibers was seen in all four groups of neuromuscular diseases in our study. Among the four groups, cases in the group with PM had the highest mean percentage occurrence (27%) of small fibres located at the periphery of muscle fibre fascicles. However, the mean percentage occurrence of such fibres was not significantly different from that in the other groups. In addition, there was no significant difference in regard to the mean number of nuclei in atrophic fibres in the four groups of neuromuscular disorders.

Isolated or grouped small fibres which were unrelated to their surrounding fibres, based on the ATPase reaction, were seen in all groups. Since the normal distribution of muscle fibres is a mosaic pattern of different fibre types, it is likely that isolated small fibres without histochemical kinship to their surrounding fibres result from shrinkage of previously normal fibres. Groups of small fibres, of the same histochemical type without kinship to surrounding fibres, probably originate either from fibre splitting (Swash and Schwartz 1977) or from atrophy after ineffective reinnervation (Swash and Schwartz 1984). As compared to the other groups, the biopsies of PM had an apparently higher proportion of isolated small fibres which were unrelated to the surrounding fibres, but this did not reach statistical significance and quantification of groups of small fibres lacking kinship to their surrounding fibres also did not discriminate the four groups of disorders.

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References

Brooke MH, Engel WK (1969a) The histographic analysis of human muscle biopsies with regard to fibre types. I. Adult male and female. Neurology (Minneap) 19:221–223

Brooke MH, Engel WK (1969b) The histographic analysis of human muscle biopsies with regard to fibre types. II. Diseases of the upper and lower motor neuron. Neurology (Minneap) 19:378–393

Coërs C, Telerman-Toppet N (1977) Morphological changes of motor units in Duchenne's muscular dystrophy, Arch Neurol 34:396–402

Coërs C, Telerman-Toppet N (1979) Differential diagnosis of limb-girdle muscular dystrophy and spinal muscular atrophy. Neurology 29:957–972

Corbett AJ, Griggs RC, Moxley RT (1982) Skeletal muscle catabolism in amyotrophic lateral sclerosis and chronic spinal muscular atrophy. Neurology (NY), 32:550–552

Degirolami U, Smith TW (1982) Pathology of skeletal muscle diseases. Am J Pathol 107:231–276

Dubowitz V, Brooke MH (1973) Muscle biopsy: A modern approach. Saunders, Philadelphia

Griggs RC, Moxley RT, Forbes GB (1980) 3-Methylhistidine excretion in myotonic dystrophy. Neurology (NY) 30:1262–1267

Hausmanowa-Petrusewicz I, Fidziańska A, Niebrój-Dobosz I, Strugalska MH (1980) Is Kugelberg-Welander spinal muscular atrophy a fetal defect? Muscle Nerve 3:389–402

Mastaglia FL, Walton JN (1982) Inflammatory myopathies, In: Mastaglia FL, Walton JN (eds) Skeletal muscle pathology. Churchill-Livingstone, Edinburgh, pp 360–392

Sandstedt P, Nordell LE, Henriksson KG (1982) Quantitative analysis of muscle biopsies from volunteers and patients with neuromuscular disorders. Acta Neurol Scand 66:130–144

Schmalbruch H (1976) Muscle fibre splitting and regeneration in diseased human muscle. Neuropathol Appl Neurobiol 2:3–19

Swash M, Schwartz MS (1977) Implications of longitudinal muscle fibre splitting in neurogenic and myopathic disorders, J Neurol Neurosurg Psychiat 40:1152–1159

Swash M, Schwartz MS, Sargeant MK (1978) Pathogenesis of longitudinal splitting of muscle fibres in neurogenic disorders and in polymyositis. Neuropathol Appl Neurobiol 4:99–115

Swash M, Schwartz MS (1984) Biopsy pathology of muscle. Chapman and Hall, London, pp 83–85

Swatland HJ (1983) The histochemistry of very small muscle fibres in growing skeletal muscles. J Neurol Sci 61:435–442

Telerman-Toppet N, Coërs C (1971) A third muscle fibre type related to collateral reinnervation in motor neurone and peripheral nerve diseases. Pathol Eur 6:50–55

Telerman-Toppet N, Coërs C (1978) Motor innervation and fibre type pattern in amyotrophic lateral sclerosis and in Charcot-Marie-Tooth disease. Muscle Nerve 1:133–139

Watkins SC, Cullen MJ (1982) Muscle fibre size and shape in Duchenne muscular dystrophy. Neuropathol Appl Neurobiol 8:11–17

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