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Muscle pathology in myotonic dystrophy: light and electron microscopic investigation in eighteen patients

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Myotonic dystrophy (DM) is the most common muscular dystrophy in adults. Two known genetic subtypes include DM1 (myotonic dystrophy type 1) and DM2 (myotonic dystrophy type 2). Genetic testing is considered as the only reliable diagnostic criterion in myotonic dystrophies. Relatively little is known about DM1 and DM2 myopathology. Thus, the aim of our study was to characterise light and electron microscopic features of DM1 and DM2 in patients with genetically proven types of the disease. We studied 3 DM1 cases and 15 DM2 cases from which muscle biopsies were taken for diagnostic purposes during the period from 1973 to 2006, before genetic testing became available at our hospital. The DM1 group included 3 males (age at biopsy 15–19). The DM2 group included 15 patients (5 men and 10 women, age at biopsy 26–60). The preferential type 1 fibre atrophy was seen in all three DM1 cases in light microscopy, and substantial central nucleation was present in two biopsies. Electron microscopy revealed central nuclei in all three examined muscle biopsies. No other structural or degenerative changes were detected, probably due to the young age of our patients. Central nucleation, prevalence of type 2 muscle fibres, and the presence of pyknotic nuclear clumps were observed in DM2 patients in light microscopy. Among the ultrastructural abnormalities observed in our DM2 group, the presence of internal nuclei, severely atrophied muscle fibres, and lipofuscin accumulation were consistent findings. In addition, a variety of ultrastructural abnormalities were identified by us in DM2. It appears that no single ultrastructural abnormality is characteristic for the DM2 muscle pathology. It seems, however, that certain constellations of morphological changes might be indicative of certain types of myotonic dystrophy. (Folia Morphol 2011; 70, 2: 121–129)

Key words: myotonic dystrophy, electron microscopy, nuclear clumps

INTRODUCTION

Myotonic dystrophy (DM) is the most common muscular dystrophy in adults. Two known genetic subtypes include DM1 (myotonic dystrophy type 1, Steinert disease; OMIM 160 900) and DM2 (myotonic dystrophy type 2, proximal myotonic myopathy; OMIM 602 668). DM1 is caused by the expansion of a CTG triplet repetition in the 3′ un-transla-

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ted region of the DM protein kinase gene on 19q13, while DM2 is caused by the expansion of a CCTG tetranucleotide repetition in the first intron of the zinc finger 9 (ZNF 9) gene on 3q21 [18, 19]. These two diseases share many of the same clinical characteristics, such as an autosomal dominant inheritance pattern, myotonia, myopathy, as well as multiorgan involvement affecting the heart, brain, and endocrine system [14, 15, 23, 26, 31, 33]. Certain clinical features, such as typical distal muscle weakness in DM1, proximal muscle weakness in DM2, mild if any facial involvement in DM2, prominent cognitive impairment in DM1, and the absence of a congenital form in DM2, provide some differentiating clinical clues.

However, a considerable number of patients with the genetically proven type of the disease have unusual phenotypic presentations [22, 32, 33]. Thus genetic testing is considered as the only reliable diagnostic criterion in myotonic dystrophies. Whereas the diagnosis of DM1 is relatively easy on clinical grounds and can be easily confirmed by DNA test, the diagnosis of DM2 may often be overlooked because of subtle and non-characteristic phenotype features.

Relatively little is known about DM1 and DM2 myopathology. Muscle histopathology of DM1 is considered rather typical, although no detailed study based on a large series of patients has ever been performed. Muscle biopsy was rarely needed to confirm the clinical diagnosis of DM1. As the genetic confirmation of DM1 became widely available, muscle biopsy was removed from the guidelines for clinical diagnosis of DM1. First reports on skeletal muscle pathology in myotonic dystrophy were published in the late 1960s [3, 12]. A combination of type 1 fibre atrophy and type 2 fibre hypertrophy was found and since then has been regarded as highly specific for myotonic dystrophy as well as rarely seen in other muscle disorders.

Dubowitz [8] in his textbook edited in 1985 reported on the histopathological analysis of 12 adult patients with "classical myotonic dystrophy" since no molecular diagnosis was available at that time. Interestingly, identical observations are presented as "pathological characteristics of DM1" in the latest edition of "Muscle biopsy: a practical approach" [9]. Changes such as multiple internal nuclei (often in long chains), sarcoplasmic masses, and ring fibres were recognised as most common. Subsequently, histopathological changes in early stages of the DM1 were observed. These, according to Dubowitz, were a disparity between the size of type 1 fibres and type 2 fibres with atrophy of type 1 fibres and hypertrophy of the type 2 fibres. Similar abnormalities were described by Karpati et al. [17] in their textbook in 2001 as typical findings for myotonic dystrophy with unstable CTG repetition on chromosome 19q13.3.

Harper, in his monograph [14], also presented data on skeletal muscle pathology in myotonic dystrophy. Among typical changes listed as most important were abundant central nuclei, common ring fibres, and type 1 fibre atrophy. Unfortunately, no detailed analysis of these findings was provided.

Myopathological data are more extensive for DM2 than for DM1. Several papers describing large series of muscle biopsies from DM2 patients [1, 3, 25, 28, 34] as well as many case reports [20, 32, 35] have been published. Most of these studies feature a rather homogenous pattern of muscle pathology in DM2. Multiple internal nuclei, predominant type 2 fibre atrophy and pyknotic nuclear clumps seem to be typical constellations of muscle changes [1]. Only in one report [6] neither characteristic for DM2 abnormalities were found nor were differences between DM1 and DM2 biopsies noted.

We are unaware of any systematic electron microscope examination of myotonic dystrophy muscle biopsies. Several authors have reported on muscle ultrastructure in DM1. Results of early studies are summarised in Harper's monograph [14]. A variety of pathological changes such as degenerative changes of myofibrils and diverse abnormalities of the sarcotubular system [24, 29] were observed, none of them was considered specific for the disease.

For the DM2, electron microscope studies are also scarce. The appearance of non membrane-bound vacuoles consistent with either dilated sarcoplasmic reticulum or a portion of the T-tubule system was presented in one case [32].

The aim of our study was to characterise light and electron microscopic features of DM1 and DM2 in patients with a genetically proven type of the disease.

MATERIAL AND METHODS

Patients

From our group of 135 patients with genetically proven DM subtype (93 DM1 and 42 DM2) we studied 3 DM1 cases and 15 DM2 cases who had muscle biopsy taken for diagnostic purposes during the period from 1973 to 2006, before genetic testing

Table 1. Results of light microscopy analysis in 3 myotonic dystrophy type 1 biopsies

No.	M/F	Age at biopsy	Muscle	Internal nuclei	Fibre type composition		Fibre type diameter [μm]		Nuclear clumps
					Type 1	Type 2	Type 1	Type 2	
1	M	18	Biceps	23%	36%	64%	35 ± 8	61 ± 8	1
2	M	15	Biceps	–	53%	47%	27 ± 13	54 ± 11	–
3	M	19	Deltoid	10%	56%	44%	29 ± 11	52 ± 13	–

became available at our hospital. The DM1 group included 3 males (age at biopsy 15–19). The DM2 group included 15 patients (5 men and 10 women, age at biopsy 26–60).

Molecular genetic analysis

The DNA testing for DM1 and DM2 was performed as previously described by other authors [2, 18, 27, 36].

Muscle biopsy specimens

Three samples were available from the DM1 group, 2 from biceps brachii, and 1 from the deltoid muscle. In the DM2 group 16 muscle samples (13 from vastus lateralis and 3 from biceps brachii) were available from 15 patients. One patient from this group had 2 muscle biopsies taken, the second after an interval of 10 years.

Muscle biopsies were processed using standard histological procedures. One part of the sample was frozen in isopentane, cooled in liquid nitrogen, and sectioned at 6 μm in a cryostat; another part was fixed in 2.5% glutaraldehyde and prepared routinely for electron microscopy.

Light microscopic evaluations were performed on transverse sections conventionally stained with haematoxylin-eosin (H & E), Gomori trichrome, NADH--dehydrogenase, succinie dehydrogenase, (SDH) and ATP-ase after pre-incubation at pH 9.4 and 4.35.

Light microscopy

For the purpose of this study all samples were re-evaluated by two non-blinded authors (A.N.-P. and A.K.). Pathological changes such as central nucleation, selective muscle fibre type, prevalence, and atrophy, nuclear clumps, angulated fibres, sarcoplasmic masses, ring fibres, necrosis, and fibrosis were assessed in every case. The number of muscle fibres evaluated in each sample ranged from 200 to 250. Central nucleation and other structural changes were assessed in selected area H&E staining, whereas fibre type composition and fibre type diameter were assessed in serial sections in the same area in ATPase pH 9.4. A fibre diameter range of 40–80 μm for males and 30–70 μm for females was accepted as normal [4]. As for fibre type composition [4, 5, 16], the following approximate proportions were accepted: type 1 — 30–40% and type 2 — 60–70%. The presence of pyknotic nuclear clumps was evaluated in the same area and calculated per 100 fibres.

Immunohistochemistry was not performed since frozen muscle tissue was not available for the majority of the patients.

Electron microscopy

Ultrastructural examination of muscle samples was performed to evaluate fine structural abnormalities of muscle fibres and to better characterise the pyknotic nuclear clumps. Special attention was paid to the pathological muscle changes indicative of denervation.

RESULTS

Clinical and light microscopic analysis of 3 DM1 and 16 DM2 biopsies are summarised in Tables 1 and 2, respectively.

Histopathological findings

Light microscopy

Sarcoplasmic masses, ring fibres, necrosis, and fibrosis were extremely rare or absent in all examined biopsies (either DM1 or DM2). Our findings in DM1 (Table 1) revealed the presence of substantial internal nucleation in two cases and absence of internal nuclei in one case. Central nuclei were usually single on the cross section and were mostly detected in atrophied fibres. A predominance of type 1 fibres was found in two cases whereas in one case

Table 2. Results of light microscopy analysis in 16 myotonic dystrophy type 2 biopsies

No.	M/F	Age at biopsy	Muscle	Internal nuclei	Fibre type composition		Fibre type diameter [μm]		Nuclear clumps
					Type 1	Type 2	Type 1	Type 2	
1	M	30	VL	2%	28%	72%	31 ± 16	72 ± 10	–
2	F	54	VL	52%	40%	60%	52 ± 13	48 ± 18	14
3	F	51	VL	28%	64%	36%	28 ± 11	78 ± 8	13
4	F	33	Biceps	–	26%	74%	62 ± 19	43 ± 12	2
5a	M	30	VL	28%	41%	59%	75 ± 16	81 ± 13	5
5b	M	40	VL	36%	44%	56%	69 ± 12	80 ± 11	7
6	F	57	VL	40%	18%	82%	59 ± 12	75 ± 9	16
7	F	59	VL	14%	81%	19%	80 ± 9	77 ± 15	28
8	F	50	VL	56%	63%	37%	80 ± 8	30 ± 8	18
9	F	55	Biceps	28%	49%	51%	35 ± 8	65 ± 8	8
10	F	53	VL	48%	66%	34%	50 ± 8	30 ± 8	6
11	F	37	Biceps	10%	38%	62%	45 ± 8	40 ± 8	5
12	M	26	VL	8%	41%	59%	48 ± 9	52 ± 16	4
13	M	34	VL	23%	46%	54%	33 ± 7	61 ± 12	3
14	F	60	VL	15%	22%	78%	62 ± 11	75 ± 14	5
15	M	31	VL	6%	42%	58%	55 ± 13	62 ± 8	6

type 2 fibres were predominant. Preferential type 1 fibre atrophy was observed in all three cases. The diameter of type 2 muscle fibres was within normal limits. A single nuclear clump was seen in one case.

In DM2 (Table 2) we found central nucleation (ranging from 2 to 56%) in all but one biopsy. Central nuclei were usually multiple and occurred frequently in normotrophic as well as hypertrophic fibres. The percentage values of type 1 and type 2 fibres were within the normal range in only two samples. In the majority of specimens type 2 muscle fibre prevalence (from 38 to 81%) was encountered. However, in four muscle biopsies, type 2 muscle fibre decrease (from 62 to 19%) was encountered.

An analysis of muscle fibre diameter showed values from 31 to 81 μm. Atrophy of type 1 affected two males and one female, whereas atrophy of type 2 fibres was noted in two female patients. Hypertrophy of type 1 occurred in two females and hypertrophy of type 2 fibres was found in four females and two males. Single scattered atrophic angulated fibres were encountered in the majority of DM2 biopsies. Pyknotic nuclear clumps (fibres with preserved nuclei and severely reduced cytoplasm) were observed in all but one muscle biopsy (with a frequency of 2 to 28 per 100 fibres). Fibre type grouping or group atrophy was not seen in any biopsy.

Electron microscopy

Myotonic dystrophy type 1 (DM1). Electron microscopy revealed central nuclei in all three examined muscle biopsies. On the longitudinal section such nuclei were often arranged in a row. No other structural or degenerative changes were detected.

Myotonic dystrophy type 2 (DM2). All examined muscle samples showed ultrastructural abnormalities. A variety of pathological but non-specific changes such as central nucleation, atrophied fibres, cytoplasmic bodies, honey comb-like structures, IBM-type cytoplasmic inclusions, and ragged-red fibres were identified. The remaining abnormalities were present in one or two of the examined biopsies and, most often, in single fibres. Only the presence of central nucleation, severely atrophied fibres, and lipofuscin accumulation were consistently found.

Central nuclei were observed in many fibres in all biopsies. Such nuclei were usually multiple within the fibre. Muscle fibres containing internal nuclei

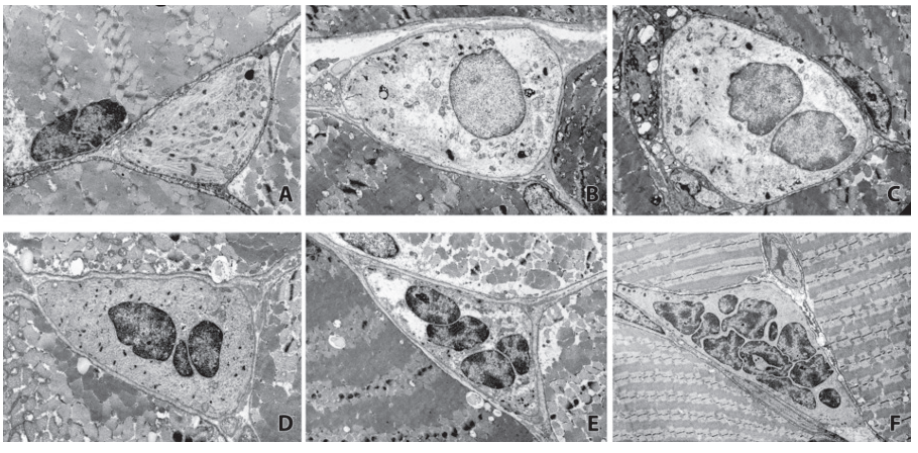

Figure 1. Electron microscopic picture of severely atrophied muscle fibres in DM2 patients; **A.** Muscle fibre devoid of nucleus, ×5000; **B–F.** Muscle fibres containing different numbers of nuclei; B. ×3000; C. ×4000; D. ×4000; E. ×3000; F. ×25 000.

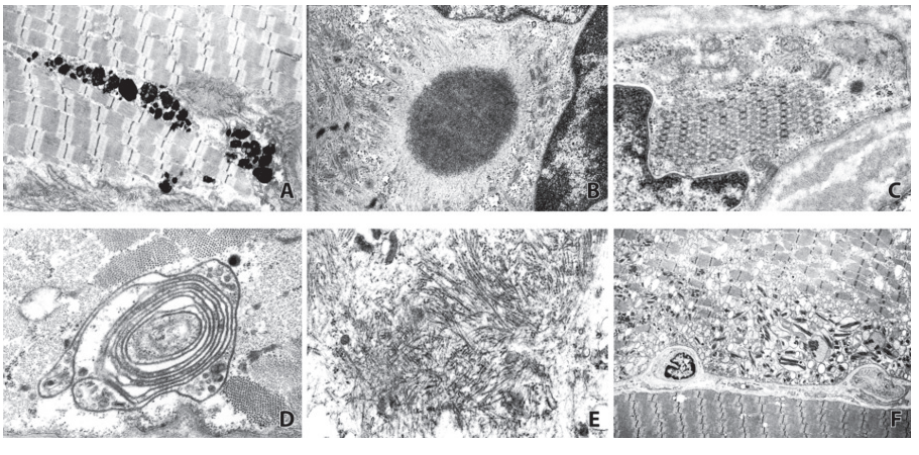

Figure 2. Electron microscopic abnormalities observed in DM2 biopsies; **A.** Lipofuscin accumulation, ×4000; **B.** Cytoplasmic body, ×15 000; **C.** Honey comb-like structure, ×25 000; **D.** Concentric laminated body, ×20 000; **E.** IBM-type cytoplasmic inclusion, ×25 000; **F.** Ragged-red fibre, ×2500.

were not usually atrophied. The appearance of nuclei varied from normal to irregular, hyperchromatic, invaginated, and fragmented. No intranuclear inclusions were noted.

Atrophied fibres (Fig. 1) in the cytoplasm contained almost no contractile material, or fragments of the Z line and only a few scattered mitochondria. Such fibres were devoid of a nucleus (Fig. 1A) or contained one to more than ten nuclei or nuclear fragments (Fig. 1B–F). Nuclei differed in appearance — from large and round (Fig. 1B) to irregular and hyperchromatic (Fig. 1F). The basement membrane of atrophied fibres was regular and not folded. No basement membrane processes or loops were ever observed. Neighbouring fibres showed no features of denervation.

Lipofuscin accumulation occurred in numerous severely atrophied (Fig. 2A) as well as non-atrophied fibres, most often aligned under the sarcolemma. Lipofuscin was present in the form of bodies, which were heterogeneous in composition and contained granules of different sizes and densities. Such bodies were irregular in shape and often limited by a single membrane.

Cytoplasmic bodies (Fig. 2B) were observed in two biopsies both in severely atrophied and moderately atrophied fibres. They consisted of a dense round centre and a peripheral zone of radially oriented filaments. Honey comb-like structures were present as aggregates of highly regular tubular components and were observed in extremely atrophied fibres (Fig. 2C). They were

encountered in two atrophied muscle fibres in two muscle biopsies.

Concentric laminated body fibres (Fig. 2D) were observed as a cylindrical structure of 9 concentric laminae with glycogen granules in the centre.

Electron microscopy revealed non-membrane-bound vacuoles in several muscle fibres in two biopsies. They were located both in the centre and at the periphery of fibers and usually contained membranous whorls, poorly defined debris, and, in one case, IBM-type inclusions.

IBM-type cytoplasmic inclusions were observed in several muscle fibres in one biopsy. They appeared as clusters of randomly-oriented tubulo-filaments measuring 16–21 nm in diameter (Fig. 2E). IBM-type inclusions were often located close to vacuoles, sometimes filling their lumen together with myelin whorls. No such inclusions were identified within muscle nuclei.

Disordered sarcomeres appeared as mini-cores (small unstructured areas within the fibre) and Z-line irregularities.

Ragged-red fibre was observed in only one biopsy as a fibre with marked mitochondrial proliferation (Fig. 2F). Mitochondria were large, irregular in shape, and often contained whorled cristae and paracrystalline inclusions.

Ring fibres were present only in one muscle biopsy. They consisted of a central longitudinal core of normally oriented myofibrils encircled by a thin peripheral band of transversely oriented myofibrils. The normal sarcomere pattern in the peripheral band was preserved, although some sarcomeres were shorter and split. No sarcoplasmic masses were encountered.

DISCUSSION

Our analysis of DM1 biopsies included only three cases. As already mentioned, muscle biopsy is no longer considered necessary in the diagnosis of DM1. The biopsies presented here were taken long before molecular analysis became available. In all three cases the patients were young, and this fact probably explains the scarcity of histopathological alterations and the lack of degenerative changes. According to Dubowitz [9], as the disease progresses, more and more myopathic changes make their appearance. Nevertheless, preferential type 1 fibre atrophy was seen in all three cases, and substantial central nucleation was present in two biopsies. Interestingly, the two changes (ring fibres and sarcoplasmic masses) presented in many textbooks [9, 10, 17] as the most typical, were not found in our material. One might hypothesize that such pathological changes appear in affected muscles with time, as a result of work overload and/or abortive regeneration.

It is worth mentioning that reports on DM1 myopathology are scarce [3, 24, 29, 34] and very often based on small series or even on single cases. Most of them were published before the era of molecular diagnosis. Nevertheless, the findings consistently reported were type 1 atrophy, type 2 hypertrophy, multiple internal nuclei — often in long chains, sarcoplasmic masses, and increased incidence of ring fibres. Why central nucleation should occur is poorly understood. Harper, in his monograph [14], concludes that there is no evidence to support the view that central nucleation results from continuing division of the centrally placed nuclei or as a result of continued inward migration of myonuclei. It is also suggested in the literature [13] that internal nuclei in type 1 muscle fibres in DM1 might indicate its incomplete maturation. In fact, such nuclei are located in the centre, at the early stages of the disease, being single and on the cross section, whereas in the longitudinal section they are often arranged in a row. Such a constellation of findings resembles features of muscle fibre immaturity.

The reason for the selective atrophy of type 1 muscle fibres in DM1 remains unknown. Such atrophy occurs in a variety of congenital neuromuscular disorders. It is suggested [17] that the small size of type 1 fibres may be due to their failing to attain their normal diameter during development. In fact, type 1 muscle fibre atrophy is considered as an early manifestation of the DM1, and it can be present at the pre-clinical stage of the disease. Such preferential atrophy might also be related to pathogenic effects of the CTG repetition-containing RNA in slow and fast muscle fibres.

Early reports on DM2 muscle pathology suggested that the changes were similar to DM1 [7, 26]. Recent muscle biopsy studies, however, have unequivocally reported preferential type 2 muscle fibre atrophy and central nucleation, as well as the presence of angulated fibres and pyknotic nuclear clumps [1, 25, 28, 34].

Our findings revealed a similar spectrum of light microscopic abnormalities. Analysis of general histopathological changes disclosed abnormalities in every examined biopsy. However, the small number of patients, different biopsied muscles, different gender, and age at biopsy made statistical analysis unfeasible. The most frequent pathological alter-

ation in our cohort was central nucleation (15/16 biopsies) and pyknotic nuclear clumps (15/16 biopsies). These data confirm previous reports by other authors [1, 25, 28, 34].

Changes in the sarcolemmal nuclei are also prominent in DM2. Location of nuclei in the centre of muscle fibres is seen in many conditions such as different types of myopathy and chronic neuropathies. Hypertrophied muscle fibres, before they split, contain internal nuclei. Nevertheless, the large number of internal myonuclei is considered a hallmark of myotonic dystrophy. In DM1, internal nuclei were found selectively in small type 1 muscle fibres, whereas in DM 2 most centronucleated type 2 muscle fibres were not atrophic [1]. This may suggest a different, yet unknown pathomechanism(s) of central nucleation of muscle fibres in DM1 and in DM2.

The expression "pyknotic nuclear clumps" is used in the literature for fibres with preserved nuclei and extremely reduced cytoplasm. They are frequently seen in denervation and in different myopathies as an end-stage muscle fibre atrophy.

Their origin in different diseases may be different and not necessarily result from a lack of innervation. It is conceivable that other pathogenic mechanisms (trophic or toxic) are involved in DM2 and, due to factors unknown thus far, pyknotic muscle clumps are formed. In fact, in none of the DM2 biopsies examined by us, typical pathological features of denervation were found.

The observed pathology in our group also included type 2 fibre prevalence (12/16 biopsies). Such a prevalence, although to a lesser extent, was already noted in Schoser' series [28], and it was absent in Pisani's report [25]. The reason for such discrepancies remains obscure and cannot be explained by different patient characteristics or by the morphometric norms adopted.

Preferential type 2 fibre atrophy is considered a typical alteration in DM2 and was reported with variable frequency in the majority of recent papers [1, 25, 28, 34] summarising histopathological findings in larger groups of patients. Vihola et al. [34] found such atrophy in all nine examined patients. Schoser et al. [28] described moderate type 2 atrophy in about two-thirds of his fifty-seven DM2 patients. In Pisani [25] series type 2 fibre atrophy was encountered (ranging from 9% to 23%) in all examined muscles except for the male biceps, whereas Bassez [1] reported such atrophy in all eight examined patients (the muscle was not specified). Normal values for fibre diameters in all cited papers were identical to ours. Interestingly, in our group, preferential type 2 fibre atrophy was found only in 2/16 muscles — in both cases in female VL. Hypertrophy of type 2 fibres occurred in four females and two males.

Type 1 fibres were less affected in the DM2 group — only in two cases type 1 fibre atrophy was noted. Thus it seems that DM2 can be "type 2 muscle fibre disease" characterised by type 2 muscle fibre atrophy, hypertrophy, and central nucleation. Detailed studies by Pisani et al. [25] proved that a high prevalence of central nucleation occurred among hypertrophic type 2 fibres, and this observation had a high predictive value for the diagnosis of DM2. Why preferential involvement of type 2 fibres in DM2 should occur remains unknown. One of the possible pathogenetic mechanisms might be a toxic effect of the CCTG repetition-containing RNA on type 2 muscle fibres.

The results of our study confirm previous observations [1, 25, 28, 34] that DM1 and DM2 are characterised by different myopathological alterations selectively affecting type 1 and type 2 muscle fibres.

There are only a few studies on muscle ultrastructure in myotonic dystrophy. Early studies [24, 29], which were summarised in Harper's monograph [14], inevitably included patients without genetic confirmation. A variety of pathological changes, such as degeneration of myofibrils with the Z and I band affection as well as proliferation of sarcotubular system, were observed. Although none of the reported degenerative alterations was considered specific for the disease, it was assumed that severity of changes was parallel to the degree of muscle involvement.

The authors are aware of only one electron microscopic evaluation of the muscle in genetically confirmed myotonic dystrophy. Toth et al. [32] reported pathological evidence of subsarcolemmal vacuolation in two DM2 patients. They also noted cleft muscle fibre nuclei in both patients as well as angulated atrophic fibres and pyknotic nuclear clumps in one.

The results of our ultrastructural studies in DM1 patients in our group revealed very few abnormalities. This might be due to their young age at biopsy. As a matter of fact, only central nucleation suggested the diagnosis of myotonic dystrophy. Pathological changes considered most typical for DM1 — sarcoplasmic masses and ring fibres — were absent in our material. Neither abnormalities in the sarcotubular system nor degenerative changes in myofibrils were encountered. This is in agreement with the

observations of Mussini [24] and Dubowitz [9] that the severity of the pathological changes parallel the progression of the disease.

Among the ultrastructural abnormalities observed in our DM2 group, the presence of internal nuclei, severely atrophied muscle fibres, and lipofuscin accumulation were consistent findings. Multiple internal nuclei were usually found in normal-size or hypertrophied fibres showing no obvious features of splitting, degeneration, and/or regeneration. These observations exclude already well-known mechanisms of central nucleation and suggest others which remain to be identified.

Our study confirms the previously reported presence of severely atrophied fibres and nuclear clumping in DM2 biopsies. Ultrastructural evaluation showed a population of very small fibres consisting of fibres devoid of nuclei as well as some containing multiple nuclei. It is widely accepted that muscle fibre atrophy may result from both denervation and myopathy. The electron-microscopic picture of chronic denervation is well known, and diverse ultrastructural changes have been described both in experimental animals [11] and in humans [21, 30]. The severely atrophic fibres and pyknotic nuclear clumping identified by us in examined biopsies might suggest denervation as a possible pathogenetic factor in DM2 [34]. However, no other typical pathological features of denervation have been found in electron microscopy. The mechanism leading to muscle fibre atrophy in DM2 remains at the moment unclear.

The other constant finding in the DM2 muscle was lipofuscin accumulation. It is generally accepted that lipofuscin accumulation is prominent in long-lived postmitotic cells. The origin of lipofuscin granules in muscle remains uncertain; they might be lysosomal residual bodies originating in autophagic vacuoles, or they might originate from mitochondrial lipids. Lipofuscin accumulation, however, is considered a nonspecific finding since it was described in different neuromuscular disorders, neurogenic and myopathic, hereditary as well as acquired.

A variety of ultrastructural abnormalities were identified by us in DM2. All of them were present in one or two examined biopsies and usually in one or in very few muscle fibres. To our surprise, only one ring fibre was identified in all the DM2 biopsies. Therefore, it would appear that no single ultrastructural abnormality is characteristic of DM2 muscle pathology. It seems, however, that certain constellations of morphological changes might be indicative of a certain types of myotonic dystrophy. As was pointed out earlier, muscle biopsy is no longer recommended in the diagnosis of typical myotonic dystrophy, but it may still be useful in characterising different, less common phenotypes of myotonic dystrophy.

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