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Muscle Biopsy Findings in Valosin-Containing Protein Multisystem Proteinopathy

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Abstract

Background and Objectives

Valosin Containing Protein-associated multisystem proteinopathy (VCP-MSP) is a progressive, autosomal dominant disorder caused by pathogenic variants in the VCP gene, resulting in a heterogeneous clinical presentation. Muscle biopsy findings are characteristic but not pathognomonic. This study aimed to comprehensively analyse VCP-related myopathology and explore correlations with clinical phenotypes, genetic variants, and disease progression.

Methods

Muscle biopsy images and data were collected retrospectively from adults (≥18 years) with pathogenic or likely pathogenic VCP variants enrolled in the VCP Multicentre International Study. Biopsy data were standardized using the "Common Data Elements for Muscle Biopsy Reporting." Variations in biopsy findings were analysed by biopsy site, time from disease onset, the four most common VCP variants, and clinical phenotypes.

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Glossary

FTD = fronto-temporal dementia; **IBM** = inclusion body myositis; **MHC** = major histocompatibility complexes; **MSP** = multisystemic proteinopathy; **PDB** = paget disease of the bone.

Result

A total of 112 muscle biopsies were included. Most individuals were male (66.0%). The mean age at biopsy was 53.3 years (SD 10.0), with a mean disease duration of 6.5 years (SD 4.5). The most frequent VCP variant was c.464G>A (p.Arg155His) (18.8%). The top clinical phenotypes were isolated myopathy (37.5%), myopathy with Paget disease of bone (17.9%), and myopathy with motor neuron involvement (13.4%). The vastus lateralis was the most common biopsy site (34.8%), and 91% were open biopsies. Histopathologic findings included atrophic fibres (87.5%), rimmed vacuoles (72.3%), endomysial fibrosis (58.0%), and protein aggregates (51.8%), primarily p62 (60.3%) and VCP (36.2%). Degeneration niches with fibrofatty replacement and atrophic fibres were seen in 33.3% of biopsies without frequency differences by clinical phenotypes. There were no differences in biopsy findings among the 4 most common VCP gene variants, except for the absence of degeneration niches in muscle biopsies of 12 patients with c.277C>T (p.Arg93Cys). MRI data from 30 patients showed fat pockets corresponding to these niches and STIR hyperintensity correlated with inflammatory infiltrates in 42.9%. Concordance between clinical phenotype, biopsy, and neurophysiology was observed in only 49.4% of cases, indicating significant heterogeneity.

Discussion

VCP-MSP muscle biopsies consistently show myopathic or mixed patterns with rimmed vacuoles and p62/VCP-positive inclusions, regardless of clinical phenotype, age, or progression. Some lack vacuoles, challenging diagnosis. Discrepancies between clinical, neurophysiology, and biopsy findings should prompt consideration of VCP-MSP to improve detection and management.

Introduction

Valosin-containing protein multisystem proteinopathy, hereafter VCP-MSP, is an autosomal dominant genetic condition produced by pathogenic variants in the valosin-containing protein gene (*VCP* gene).[1] VCP is a member of the AAA-ATPase (ATPases associated with diverse cellular activities protein) family which is involved in the remodeling of molecules using the energy generated by hydrolyzing ATP.[2] VCP is ubiquitously expressed through body tissues representing up to 1% of the cellular proteins.[3] VCP's structure consists of an N-terminal domain that interacts with adapters and cofactors, 2 central D1 and D2 ATPase domains, 2 linker domains (L1 and L2), and a carboxy-terminal domain that also links to some cofactors.[2,4] A VCP monomer assembles into a hexamer with a central cylinder formed by the D1/D2 domains. While the D1 domain is responsible for hexamerization, the D2 domain performs the majority of ATPase activity.[5,6] VCP has a key role in maintaining cell homeostasis participating in protein degradation, autophagy, cell cycle control, and regulation of apoptosis.[2,7]

The first patients with VCP-MSP reported developed a combination of symptoms including involvement of the skeletal muscle, in the form of an inclusion body myopathy,[8] Paget disease of the bone (PDB), and frontotemporal dementia (FTD).[9] Since that original report, diverse clinical presentations have been reported. Currently, VCP-MSP is considered a multisystemic disorder affecting the muscle; bone; and central and peripheral nervous systems including peripheral neuropathy, motor neuron disease, spastic paraparesis, parkinsonism, and dementia.[10-21] It is recognized that there is interindividual and intrafamilial variability in the clinical presentation.[22] Although VCP-MSP was considered to affect only adults, a new phenotype affecting children presenting with cognitive decline has been published, broadening the potential phenotypes even more.[23] As a result, patients with VCP-MSP can be attended by a variety of clinicians and a high level of diagnosis suspicion is required to prevent underdiagnosis and misdiagnosis. In this context, a recently published consensus guideline aiming to homogenize the diagnosis and care of patients with VCP-MSP highlights that ancillary test, such as muscle biopsy and muscle MRI, could aid in differentiating VCP-MSP from other neuromuscular conditions.[24,25] Muscle biopsy reports from patients with VCP-MSP available in the literature describe characteristic, although not pathognomonic, histologic findings. However, because these findings are derived from single cases or relatively small cohorts,[26,27] a large, comprehensive study systematically reappraising *VCP* myopathology has yet to be conducted.

In addition, potential correlations between histologic findings, disease progression, genetic variants, and clinical phenotypes have not been fully described yet. Here, we present the histologic findings from a large cohort of muscle biopsies from patients with genetically confirmed VCP-MSP, as part of the VCP International Multicenter Study, and explore their associations with clinical variables and genotype.[22]

Table 1 Demographic Data

Demographic data	
Total	112
Male/female (103/112)	74 (66.1%)/29 (26.0%)
Age at first symptom	46.6 ± 10.1 (24.0–71.0)
Mean, SD, min, max [y] (97/112)	
Age at genetic diagnosis	56.6 ± 11.0 (33.7–76.0)
Mean, SD, min, max [y] (23/112)	
Age at muscle biopsy	53.3 ± 10.0 (32.0–82.0)
Mean, SD, min, max [y] (97/112)	
Time from first symptom to muscle biopsy	6.5 ± 4.5 (0.0–21.0)
Mean, SD, min, max [y] (99/112)	
Age at last assessment	56.5 ± 10.0 (36.0–82.0)
Mean, SD, min, max [y] (99/112)	
Death % (n)	13.4% (15)
Age at death	66.5 ± 8.8 (50.0–81.0)
Mean, SD, min, max [y] (15/15)	
Time from first symptom to death	17.5 ± 6.0 (9.0–27.0)
Mean, SD, min, max [y] (15/15)	
Variant in the *VCP* gene % (n)	
c.464G>A/p.Arg155His	18.8% (21)
c.463C>T/p.Arg155Cys	17.0% (19)
c.277C>T/p.Arg93Cys	10.7% (12)
c.476G>A/p.Arg159His	6.3% (7)
Others (frequency <5 patients)	42% (47)
Clinical phenotypes % (n)	
Myopathy isolated	37.5% (42)
Myopathy + PDB	17.9% (20)
Myopathy + LMN	9.8% (11)
Myopathy + FTD	7.1% (8)
Myopathy + neuropathy	4.5% (5)
UMN + LMN	3.6% (4)
Classic triad	2.7% (3)
Myopathy + UMN	2.7% (3)
Myopathy + extrapyramidal disorder	1.8% (2)
LMN isolated	1.8% (2)
UMN isolated	1.8% (2)
Neuropathy isolated	0.9% (1)
UMN + LMN + myopathy	0.9% (1)
Not available	7.1% (8)

Classic triad = inclusion body myopathy with Paget disease of the bone and frontotemporal dementia; LMN = lower motor neuron signs; PDB = Paget disease of the bone; UMN = upper motor neuron signs.

Methods

We analyzed muscle biopsies from participants included in the VCP Multicenter International Study, a retrospective, international study that collected clinical, genetic, and ancillary test data from adults (aged 18 years and older) with a pathogenic or likely pathogenic variant in the *VCP* gene.[22] The study involved 52 centers across 24 countries. Genetic variant interpretation was standardized by a geneticist from the John Walton Muscular Dystrophy Research Centre using criteria from the American College of Medical and Genomic Genetics as a guide.[28]

For patients who underwent a muscle biopsy as part of their diagnostic process, clinicians and pathologists were asked to complete a standardized form describing the histologic findings. This form was designed in accordance with the Common Data Elements for Muscle Biopsy Reporting recommendations to ensure consistency in histologic descriptions across different centers.[29] Staining analyses on the biopsies were conducted at the discretion of each center. Collaborators were also asked to provide representative muscle biopsy images with standard histologic staining. A myogenic pattern was defined by the presence of one or more of the following features: increased variability in muscle fiber size, fibers with internal nuclei, signs of muscle fiber regeneration, necrotic fibers, and increased fibrotic and/or fat tissue replacement. A neuropathic pattern was defined by the presence of atrophic fibers of both fiber types often with angulated and flattened atrophic fibers, fiber-type grouping, and/or target formations in muscle fibers.[30]

Standard Protocol Approvals, Registrations, and Patient Consents

The VCP International Multicenter Study obtained Caldicott approval from The Newcastle upon Tyne Hospitals Register Audit (project number 10833, Caldicott Approval: 7918) and institutional review board approvals from the LMU Klinikum at Ludwig-Maximilians University, Munich (project 21-0071); Washington University School of Medicine Institutional Review Board, United States (no 201103416); and the Johns Hopkins Hospital Institutional Review Board, Baltimore, United States (no 00288171). These ethics committees cataloged this study as an audit because it collected retrospective deidentified patient data and no patient inform consent was required.

Statistical Analysis

Data were expressed as number and percentage for categorical variables and as mean ± SD and minimum and/or maximum for quantitative ones. Variations in the frequency of muscle biopsy findings by muscle biopsy site, time from disease onset to muscle biopsy (categorized as <5 years, 5–9 years, and >10 years), and the 4 most common genetic variants in the *VCP* gene were explored by χ^2 test with Bonferroni correction adjustments for multiple comparisons. Differences in the mean time to muscle biopsy from disease onset were explored

Table 2 Muscle Biopsy Data

Total			**112**
Biopsy technique, % (n)			
Open biopsy			91.0% (102)
Conchotome			5.3% (6)
Autopsy			3.5% (4)
Muscle biopsy site, % (n)			
Vastus lateralis			34.8% (39)
Deltoid			27.7% (31)
Biceps brachii			13.4% (15)
Unknown			10.7% (12)
Tibialis anterior			7.1% (8)
Gastrocnemius			3.6% (4)
Trapezius			0.9% (1)
Hamstring			0.9% (1)
Infraspinatus			0.9% (1)
General biopsy pattern by muscle biopsy site, % (n)			
	Vastus lateralis (n = 39)	**Deltoid (n = 31)**	**Biceps brachii (n = 15)**
Myogenic pattern	51.3% (20)	67.7% (21)	60.0% (9)
Myogenic + neurogenic pattern	20.5% (8)	19.4% (60)	40.0% (6)
Neurogenic pattern	20.5% (8)	6.5% (2)	0% (0)
Dystrophic pattern	2.6% (1)	6.5% (2)	0% (0)
Inclusion body myositis like	5.1% (2)	0% (0)	0% (0)

among the 4 most frequent variants in the *VCP* gene and among different clinical phenotypes using analysis of variance. A level of significance of 0.05 was used for hypothesis testing. Statistical analysis was performed using the program IBM SPSS statistics, version 28.

Data Availability

Data are available from the corresponding author upon reasonable request.

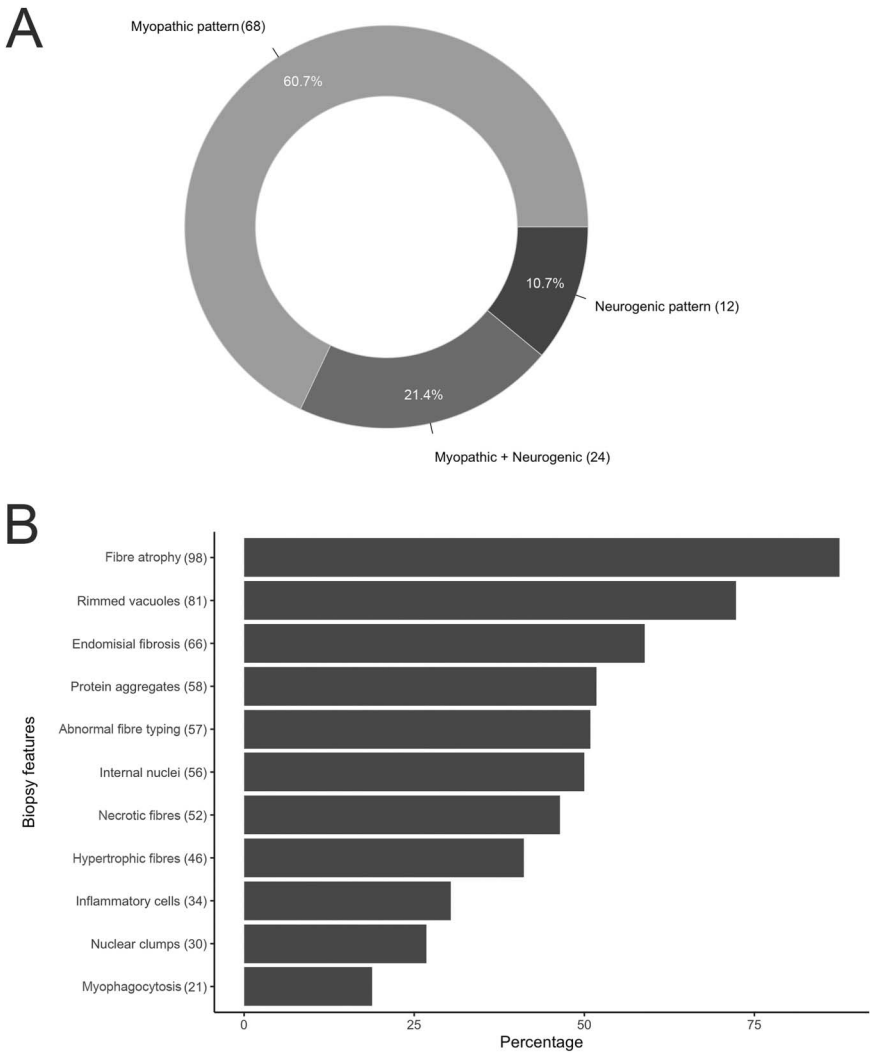
Results

Study Cohort

The VCP International Multicentric Study database includes 271 patients with a genetic diagnosis of VCP-MSP.[22] Of these, 32.1% (87/271) did not have a muscle biopsy, 26.5% (72/271) had a muscle biopsy but no data or images were available for analysis, and 41.3% (112/271) had both biopsy data and images available for analysis and were included in this study. Of these, 12 patients had a muscle biopsy because of undiagnosed asymptomatic hyperCKemia and 1 patient had 2 muscle biopsies performed at different ages. Demographic characteristics of the 271 patients are provided in eTable 1. There were no differences in age at first symptom, age at genetic diagnosis, diagnosis delay, family history, or ambulatory status between patients with and without muscle biopsy (eTables 2–5). However, the proportion of muscle biopsies was higher in patients with a myopathic phenotype [70.7% (133/188)] and in those with a combined myopathic and motor neuron phenotype [100.0% (13/13)] than in patients with an isolated motor neuron phenotype [46.7% (21/45)].

This study focuses on the 112 patients for whom biopsy data and images were available. Table 1 summarizes their demographic, genetic, and clinical characteristics. Sixty-six percent were male, with a mean age at muscle biopsy of 53.3 ± 10.0 years. The most frequent variant in the *VCP* gene was c.464G>A (p.Arg155His), observed in 18.8% (21/112) of patients. A complete list of all *VCP* gene variants is provided in eTable 6. The 3 most common clinical phenotypes among patients in this study were isolated myopathy (37.5%, 42/112), myopathy associated with PDB (17.9%, 20/112), and myopathy associated with a motor neuron phenotype (13.4%, 15/112). A motor neuron phenotype was defined based on clinical signs and symptoms observed at the last patient

Figure 1 Frequency of Findings on Muscle Biopsies of Patients With VCP-MSP Disease



(A) Frequency of general patterns identified in muscle biopsies of the study. (B) Frequency of histologic abnormalities observed in the muscle biopsies included.

follow-up. Upper motor neuron signs included increased deep tendon reflexes (even in weak muscles or with spread), pathologic reflexes (Hoffman, Babinski, crossed adductor, and snout), and spasticity. Lower motor neuron signs included muscle weakness, muscle wasting, fasciculations, and/or EMG abnormalities, such as chronic neurogenic changes (large, prolonged, and polyphasic motor unit potentials), ongoing denervation (fibrillation or positive sharp waves), and fasciculation potentials.[31]

Muscle Biopsy Findings

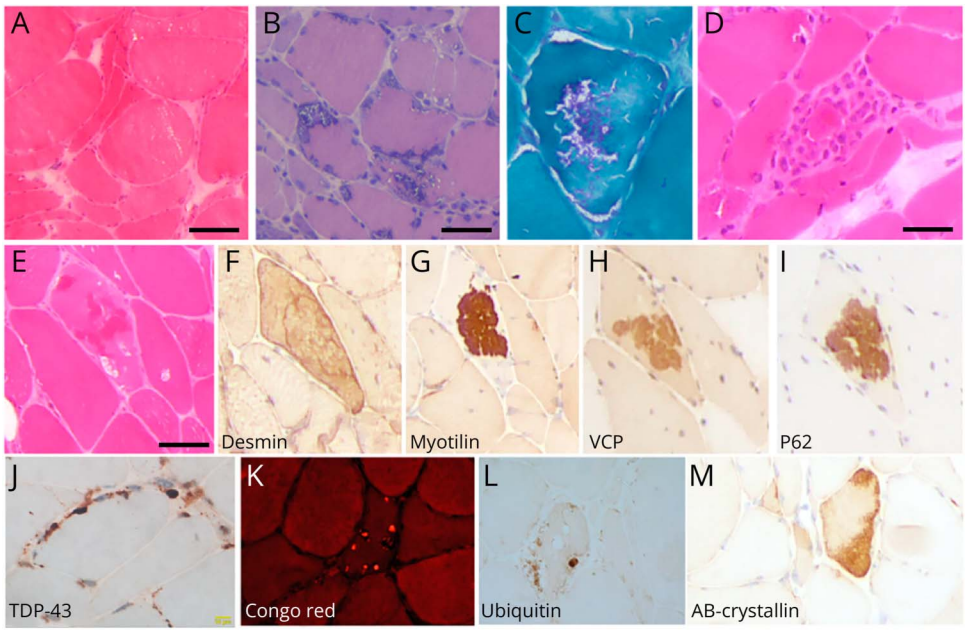
Ninety-one percent of the biopsies were open biopsies (102/112), with the vastus lateralis being the most common biopsy site (34.8%, 39/112; Table 2). There were no differences in time from symptom onset to muscle biopsy among the 3 most frequently biopsied muscles (eTable 7).

The most frequent histopathologic pattern was a myogenic pattern, accounting for 60.7% (68/112) of the samples, regardless of the biopsy site (Figure 1 and Table 2). This was followed by a mixed pattern, which combined myogenic and neurogenic features, observed in 21.4% (24/112) of patients.

The most frequent muscle biopsy findings included the presence of atrophic, either angulated or rounded, fibers in 87.5% (98/112). Atrophy of fiber types I and II was found in 73.5% (72/98) of patients, exclusively fiber type II atrophy in 17.3% (17/98) and exclusively fiber type I atrophy in 9.2% (9/98). Rimmed vacuoles were reported in 72.3% (81/112), endomysial fibrosis in 58.0% (65/112), and protein aggregates in 51.8% (58/112) (Figures 1 and 2).

Owing to the retrospective nature of the study, the antibodies used to identify the components of the protein aggregates observed in the sarcoplasm varied between laboratories. However, most centers reported the presence of inclusions that were immunostained for p62 (60.3%, 35/58) and VCP (36.2%, 21/58; Figure 2). Protein aggregates also included sarcomeric and intermediate-filament proteins such as myotilin (7.0%, 4/58), desmin (10.3%, 6/58), or αβ-crystallin

Figure 2 Examples of Histologic Findings Observed in Muscle Biopsies of Patients With VCP-MSP Disease



(A) Hematoxylin and eosin staining showing angulated atrophic fibers, rounded fibers, and prominent variability in fiber size. (B and C) Rimmed vacuoles identified on hematoxylin and eosin staining (B) and trichrome Gomori staining (C). (D) Hematoxylin and eosin staining showing a necrotic fiber undergoing phagocytosis. The necrotic fiber is in the center; it has lost its normal polygonal shape and appears eosinophilic (pink) because of protein denaturation. Individual nuclei are not identifiable because they may have disappeared or fragmented. Numerous macrophages, appearing as cells with foamy cytoplasm and dark nuclei, surround and invade the necrotic fiber. (E) Hematoxylin and eosin staining showing a muscle fiber with protein aggregates seen as eosinophilic inclusions in the sarcoplasm. These protein aggregates stained positive for the following proteins on immunohistochemistry: desmin (F), myotilin (G), VCP (H), and p62 (I). (J) TDP-43 aggregates in the sarcoplasm of a muscle fiber identified on immunohistochemistry. (K) Amyloid structures identified with Congo red staining. (L) Ubiquitin-positive inclusions and (M) AB-crystallin–positive protein aggregates identified on immunohistochemistry. Calibration bar: 20 × 50 µm for images A and B and 40 × 20 µm for the rest.

(8.6%, 5/58). TDP-43 staining and ubiquitin staining were identified in 2 and 1 biopsy, respectively,[32] although these antibodies were not routinely used in all laboratories. Finally, congophilic inclusions, as revealed with Congo red staining, were identified in only 1 patient studied.

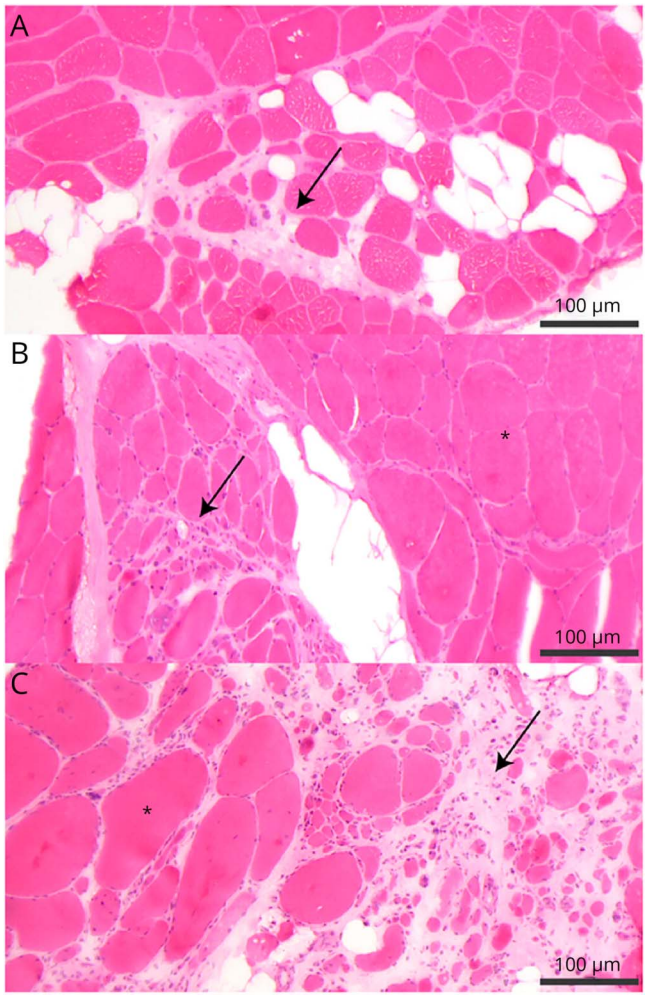
Inflammatory cells were detected in 30.3% (34/112) of the muscle samples. Most of the inflammatory cells observed were macrophages surrounding or phagocytizing necrotic fibers. Expression of major histocompatibility complex I, when detected, was restricted to few fibers. The biopsy findings did not differ by muscle biopsy site (χ^2 test with Bonferroni corrections for multiple comparisons, $p > 0.05$ in all patients [eFigure 1]).

Of interest, 31.3% of the biopsies (35/112), on low-magnification optic microscopy (wider field of view), showed demarcated focal areas or foci characterized by the presence of fibers with alterations in the muscle fiber structure (internal nuclei, p62 inclusions) and markedly atrophied fibers embedded in fibrotic and fatty tissue. The degree of atrophy of the fibers in these regions was variable, but we usually found several rounded fibers that were extremely atrophic (Figure 3). These fibers may contain vacuoles and protein aggregates as well. Inflammation was not frequently observed in these foci. These foci were located within areas of apparently normal muscle tissue or areas showing less prominent abnormalities. We described these foci as degeneration niches. These degeneration niches were identified in all muscles biopsied, including the quadriceps in 23.1% (9/39), deltoid in 58.1% (18/31), brachial biceps in 13.1% (2/15), tibialis anterior (3/8), and gastrocnemius (1/3). We did not see significant differences in the frequency of degenerative niches among clinical phenotypes, suggesting that this finding is common across all clinical presentations (χ^2 test $p > 0.05$, data not shown).

Progression of Histologic Changes

There was variability in the severity of the histologic changes observed in the muscle biopsies that allowed us to infer what could potentially be the natural history of the muscle degeneration process in VCP-MSP (Figure 4). In the mildest cases, muscle biopsies displayed only scattered fibers closely located showing abnormalities, such as fiber atrophy, internal nuclei, or vacuolization often associated with p62/VCP-positive aggregates. A second group of biopsies was characterized by a larger number of abnormal fibers displayed in foci, which contained sometimes inflammatory cells, fibrosis, and adipose tissue. The size of these foci increased progressively affecting sometime an entire fascicle. At that stage, it was not unusual to find areas of the biopsy severely affected mixed with areas completely normal or showing minor changes. Severe atrophy of muscle fibers was a prominent feature in these areas. In the most advanced stages, we could observe larges areas of the tissue that were disrupted, sometimes completely replaced by fibrotic and adipogenic tissue, but even at this stage, we could always find small regions of the biopsy showing normal muscle fibers or muscle fibers with minor abnormalities.

Figure 3 Examples of Degenerative Niches Identified in Muscle Biopsies of Patients With VCP-MSP Myopathy



(A, B, and C) Three examples of degenerative niches (arrows) characterized by areas of the muscle containing atrophic fibers embedded in fibrotic and fat tissue closely located to areas of the muscle with clearly lower involvement (asterisks). Calibration bar: 10 × 100 µm.

Associations Between Biopsy Findings and Genotype, Phenotype, and Neurophysiology Studies

There were no differences in the mean time from symptom onset to muscle biopsy among the 4 most common *VCP* gene variants or clinical phenotypes (ANOVA, $p > 0.05$, eTable 7C). There were no differences in the frequency of biopsy findings among the 4 most common *VCP* gene variants, except for the absence of degeneration niches in the muscle biopsies of the 12 patients with the c.277C>T (p.Arg93Cys) variant (χ^2 test with Bonferroni corrections for multiple comparisons, $p = 0.01$; eFigure 1B).

In patients presenting with a clinical phenotype indicative of myopathy, the predominant pattern in the muscle biopsy was myogenic, accounting for 65.3% (49/75) of patients. Notably, 20.0% (15/75) exhibited a mixed pattern, including neurogenic changes, despite not having any clinical sign of motor neuron involvement as depicted in Figure 5. Of interest, half of the patients displaying both a myopathic and motor neuron phenotype on clinical examination showed a myogenic pattern in their muscle biopsies with no clear neurogenic involvement. However, patients with the isolated motor neuron phenotype primarily exhibited a predominant neurogenic pattern in their biopsy. The only patient who was diagnosed with an axonal sensory motor polyneuropathy exhibited a muscle biopsy consistent with a neurogenic pattern. These results highlight the potential discrepancies in phenotype and histologic patterns in patients with VCP-MSP.

A neurophysiology study was available for 87 of the 112 patients who underwent a muscle biopsy. The study revealed concordance between the clinical phenotype and patterns identified in both the biopsy and needle EMG, classifying them as either myogenic or neurogenic, in 49.4% (43/87) of the patients. In the rest of the patients, discrepancies in ancillary tests were identified in relation to the clinical phenotype, as reported in Figure 5 and eTable 8, emphasizing the diversity in both clinical phenotypes and complementary study results in VCP-MSP.

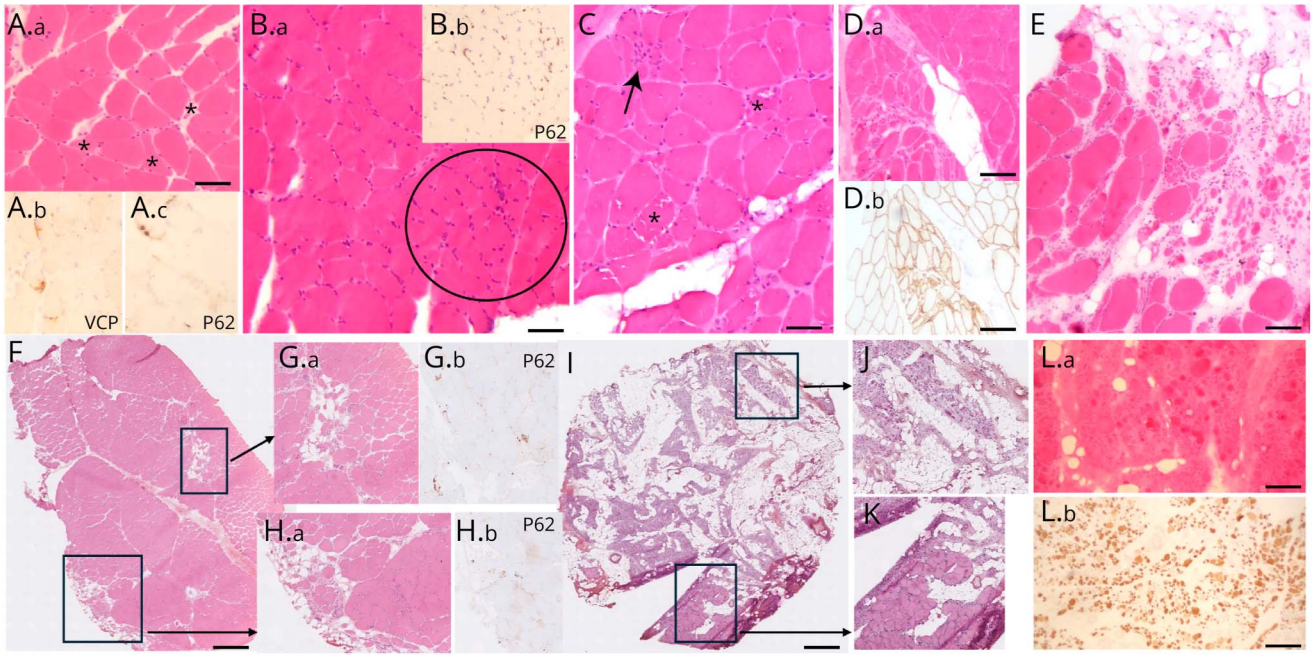
Correlation Between Biopsy Findings and Muscle MRI Findings

A muscle MRI was available for 30 of the 112 patients included in the study. The muscle MRI was performed within a 1-year window relative to the muscle biopsy. In most patients (43.3%, 13/30), muscle biopsies were obtained from muscles with minimal fat replacement on MRI (Mercuri score 1 or 2) while in 20.0% (6/30), biopsies came from muscles with significant fat replacement (Mercuri score 3 or 4). In 36.6% (11/30), biopsies were obtained from upper limb muscles, while MRI focused on the lower limbs limiting the possibilities of association analysis. We have already described the presence of fat pockets on muscle MRI consisting of rounded areas of fat replacement located inside otherwise normal muscle,[33] which could resemble the degenerative foci observed in the muscle biopsies. Fat pockets were observed in 46.7% (14/30) patients with a MRI, of which 2 of them had degenerative foci identified in their biopsies. In addition, 21 of 30 muscle MRIs (70.0%) showed an increased signal on STIR sequencing compatible with the presence of muscle edema, which has been described in a variety of situations, including acute denervation or muscle inflammation. Of these 21 patients, 9 (42.8%) exhibited inflammatory infiltrates and 7 (33.3%) showed the presence of macrophages.

Discussion

This study summarizes key histologic findings from muscle biopsies of patients with VCP-MSP. Most samples showed muscle fiber atrophy, rimmed vacuoles with protein aggregates, degenerative foci, and increased fibrosis and fat tissue. Findings were consistent across biopsy sites and phenotypes.

Figure 4 Natural History of Pathologic Progression in Muscle Biopsies of Patients With VCP-MSP Myopathy



(A) Example of a very mild case where only few atrophic scattered fibers (asterisk) were identified. VCP (A′) and p62 (A″) inclusions were identified in isolated fibers. (B) Mild case showing a group of small atrophic fibers (circle) with p62 inclusions (B′). (C) More advanced case displaying several groups of atrophic fibers closely located (arrow) and vacuolated fibers (asterisk). (D) Degenerative foci containing atrophic fibers staining positive for caveolin (D′) embedded in expanded fibrotic tissue surrounded by an area of normal muscle. (E) More advanced case with a large degenerative foci. (F) Hematoxylin and eosin staining at low resolution where 2 degenerative niches were identified (G and H) with several fibres containing p62-positive inclusions (G′ and H′) while the rest of muscle biopsy was almost nonaffected. (I) Severe case where the muscle biopsy was almost completely replaced by fat and fibrotic tissue (J), except an area (square) showing milder anomalies (K). (L) End-stage case where muscle architecture was lost, and extremely atrophic fibers were observed staining positive for embryonic myosin heavy chain (L′) embedded in expanded fibrotic tissue.

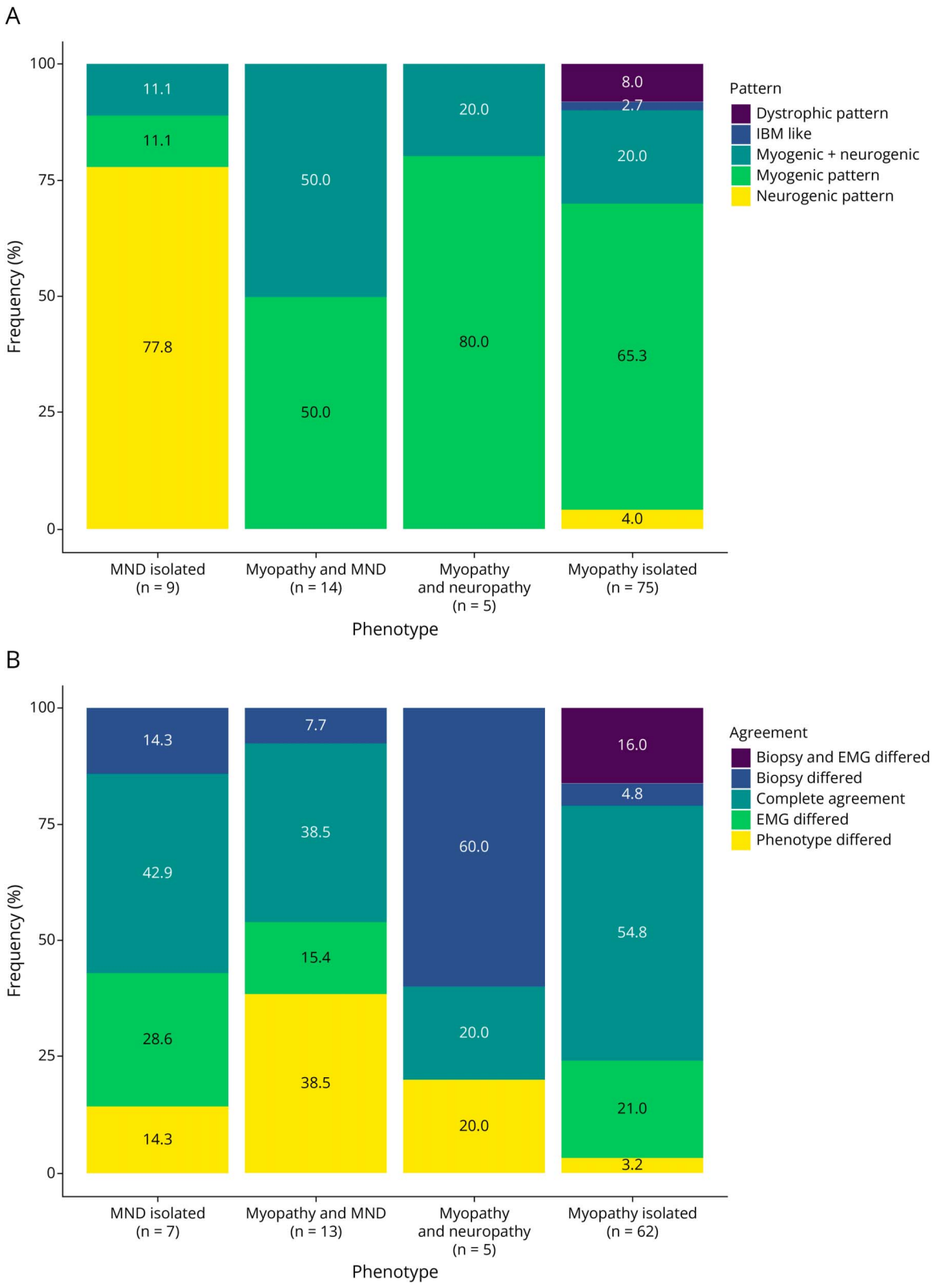
These observations should be discussed in light of VCP's known functions.

Skeletal muscle fibers are postmitotic syncytial cells with a low renewal rate, relying on satellite cell activation for regeneration.[34] Essential cellular processes such as autophagy, protein quality control, and stress response maintain homeostasis by removing damaged proteins and organelles.[4,35,36] Dysfunction in these processes is particularly harmful to low-turnover cells such as muscle fibers, neurons, and osteoclasts, potentially leading to cell death. Multisystemic proteinopathies (MSPs) arise from genetic variants affecting these pathways, contributing to conditions such as dementia, ALS, muscle disease, and PDB.[37] A hallmark of MSPs is the accumulation of protein aggregates, vacuoles, or stress granules, which drive disease progression.[26,37]

VCP interacts with ubiquitinated substrates and components of the Ubiquitin Proteasome System, including E3/E4 ligases and deubiquitylating enzymes.[38] Under normal conditions, VCP's ATPase domains help unravel ubiquitinated proteins for degradation by the proteasome.[39,40] It plays a crucial role in clearing ubiquitinated proteins from the membranes of the endoplasmic reticulum, mitochondria, and lysosomes.[41] In addition, VCP aids in resolving stress granules containing RNA and proteins and contributes to chromatin repair.[41,42] Dysfunctional VCP hexamers fail to recruit and degrade ubiquitinated targets, leading to their accumulation in muscle fibers, inside rimmed vacuoles, or freely in the cytosol—often bound to VCP, as observed in muscle biopsies from this study.[1] VCP also regulates autophagy, including autophagosome formation, maturation, and lysosomal clearance.[43-45] Variants in VCP can impair autophagy, causing the accumulation of p62, LC3 substrates, and dysfunctional lysosomes in muscle fibers, as demonstrated in this study.

Cellular stressors such as starvation, oxidative stress, viral infection, DNA damage, and heat shock trigger the formation of stress granules—cytoplasmic complexes containing RNA, RNA-binding proteins, ribosomal components, and transcription factors.[46] Stress granules inhibit protein translation and must dissolve after stress.[47] VCP facilitates their clearance,[48] but its dysfunction leads to persistent stress granules, potentially forming beta-amyloid-prion–like structures detectable by Congo red staining,[46] disrupting RNA biogenesis[49] and contributing to neurodegenerative and myopathic diseases. Stress granules have also been described in muscle biopsies of patients with other myopathies such as *TIA1* gene-related myopathies and in neurons of patients with neurodegenerative diseases such as FUS or TDP-43–linked ALS/FTD.[50] TDP-43 is also a common component of persistent stress granules. Here, TDP-43 has not been systematically stained, but we identified several

Figure 5 Correlations Between Muscle Findings, Phenotype, and EMG Changes



(A) Frequency of general muscle biopsy patterns in patients with VCP-MSP with 4 different phenotypes. (B) Level of agreement between the phenotypic description, EMG, and muscle biopsy results. MND = motor neuron disease; n = number of patients.

patients who had TDP-43 inclusions in the sarcoplasm underscoring these granules' role in disease pathophysiology.

An intriguing finding is our study is the presence of highly atrophic muscle fibers in some patients. The mechanism by which VCP dysfunction leads to muscle fiber atrophy remains unexplored. Atrophy may occur because of impaired autophagy, similar to lysosomal diseases such as Pompe disease, where unresolved autophagy triggers starvation and activates atrogenes such as *MURF1* and *ATROGIN1*.[51,52] Alternatively, proteasome dysfunction or unresolved stress granules inhibiting protein translation could contribute. Another possible cause is motor neuron loss because atrophic, angulated fibers often appear in VCP-MSP biopsies, hinting at neuronal involvement.[53]

Another notable finding is the active process of muscle degeneration occurring in foci that progressively expand. This process begins with isolated fibers exhibiting rimmed vacuoles and p62 inclusions, which then leads to the formation of atrophic fiber foci that are gradually lost and replaced by fibrotic and fatty tissue. Although the exact mechanism driving this degeneration and its expansion remains unclear, we hypothesize that intercellular communication among muscle fibers may play a role, potentially through direct contact or secreted factors, similar to mechanisms seen in prion diseases.[54,55] Whether this is dependent on or triggered by the accumulation of stress granules and protein aggregates remains to be elucidated.

The findings in this report are not specific to VCP-MSP and can also occur in other diseases. Rimmed vacuoles are found in various myopathies, both acquired and inherited. Inclusion body myositis (IBM) is characterized by rimmed vacuoles, angulated fibers, ragged-red fibers, necrotic fibers, and inflammatory infiltrates of CD8 lymphocytes and macrophages.[8,56] While some of these features overlap with VCP-MSP, generalized expression of major histocompatibility complexes (MHCs) 1 and 2 in muscle fibers, common in IBM, is absent in VCP-MSP, where MHC1 expression is limited to a few fiber.[57] Rimmed vacuoles and protein aggregates are common in MSP caused by mutations in *SQSTM1, HNRNPA2B1*, and *HNRNPA1*, as well as in myofibrillar myopathies and those linked to variants in the *TTN* gene.[58-61] In addition, neurogenic diseases can present groups of angulated or rounded atrophic fibers, sometimes affecting entire fascicles, as seen in spinal muscle atrophy and ALS.[53]

One of the most intriguing aspects of the disease is the pathologic mechanism of VCP variants. Because VCP is a dominant disease, muscle fibers contain 1 healthy allele encoding a normal VCP monomer and 1 diseased allele encoding a dysfunctional VCP monomer. The expected consequence of having only 1 mutated allele is that the resulting hexamer will consist of a combination of functional and dysfunctional monomers. This could lead to conformational changes that compromise the binding of adapters and cofactors, impairing VCP's cellular localization and function.[4] Dysfunctional VCP monomers can lead to a dominant negative effect, or they may lead to a toxic gain of function of the protein.[62] The functional consequences of the pathogenic variants in ATPase activity of VCP can be studied in vitro using a protein assay and have already been described elsewhere.[63] Most of the variants reported here lead to an increase in the ATPase activity in vitro, and only a minority do not affect ATPase activity or reduce it.[22,64] However, our results show that the pathologic features observed in the skeletal muscles are the same regardless of the *VCP* variant, suggesting that ATPase activity does not influence the pathologic changes observed.

Being a retrospective study, there was variability in the types of staining performed on muscle biopsies across different countries and centers, which may have led to underreporting of certain findings such as the presence of TDP-43 or ubiquitin aggregates or congophilic inclusions. Another key limitation of this study was the inability to determine whether the presence of degenerative niches is exclusive to VCP-MSP because muscle biopsy samples from other genetic (e.g., GNE myopathy and other multisystem proteinopathies) and inflammatory (e.g., inclusion body myositis) conditions were not available for comparison. While literature searches and expert consultations suggest that these features have not been reported in other disorders, further validation is needed. Further international collaboration, which extends the study to additional MSPs, may provide the opportunity for direct histopathologic comparisons in the future. In addition, we have not directly studied muscle biopsies from our cohort to confirm prion-like degeneration. However, previous research has shown that VCP plays a crucial role in suppressing protein aggregation and seeding in neurons, with VCP inhibition or VCP variants increasing α-synuclein and TDP-43 aggregation in cells, neurons, and mouse models.[55] In addition, TDP-43 seeding has been shown to be specific to IBM in muscle biopsy lysates from patients with IBM, IMNM, and ALS.[65] It remains to be determined whether this seeding phenomenon occurs throughout all muscle fibers in patients with VCP-MSP or originates in focal areas, like the degenerative niches described in this study.

In summary, our study outlines the key features observed in patients with VCP-MSP disease. These findings enhance our understanding of the disease's pathophysiology and open new avenues for research into the muscle degeneration mechanisms caused by dysfunctional VCP. Furthermore, these insights can aid in diagnosing new patients, particularly those without a family history.

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