# Motor neuron involvement in multisystem proteinopathy

## Implications for ALS

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### ABSTRACT

**Objective:** To explore the putative connection between inclusion body myopathy, Paget disease, frontotemporal dementia (IBMPFD) and motor neuron disease (MND).

**Methods:** Clinical, genetic, and EMG characterization of 17 patients from 8 IBMPFD families.

**Results:** Limb weakness was the most common clinical manifestation (present in 15 patients, median onset age 38 years, range 25–52), with unequivocal evidence of upper motor neuron dysfunction in 3. EMG, abnormal in all 17, was purely neurogenic in 4, purely myopathic in 6, and mixed neurogenic/myopathic in 7. Cognitive/behavioral impairment was detected in at least 8. Mutations in *VCP* (R155H, R159G, R155C) were identified in 6 families, and in *hnRNPA2B1* (D290V) in another family. The genetic cause in the eighth family has not yet been identified.

**Conclusion:** Mutations in at least 4 genes may cause IBMPFD, and its phenotypic spectrum extends beyond IBM, Paget disease, and frontotemporal dementia (FTD). Weakness, the most common and disabling manifestation, may be caused by muscle disease or MND. The acronym IBMPFD is, therefore, insufficient to describe disorders due to *VCP* mutations or other recently identified IBMPFD-associated genes. Instead, we favor the descriptor multisystem proteinopathy (MSP), which encompasses both the extended clinical phenotype and the previously described prominent pathologic feature of protein aggregation in affected tissues. The nomenclature MSP1, MSP2, and MSP3 may be used for *VCP*-, *HNRNPA2B1*-, and *HNRNPA1*-associated disease, respectively. Genetic defects in MSP implicate a range of biological mechanisms including RNA processing and protein homeostasis, both with potential relevance to the pathobiology of more common MNDs such as amyotrophic lateral sclerosis (ALS) and providing an additional link between ALS and FTD. ***Neurology*® 2013;80:1874–1880**

### GLOSSARY

**ALS** = amyotrophic lateral sclerosis; **IBM** = inclusion body myopathy; **IBMPFD** = inclusion body myopathy with Paget disease and frontotemporal dementia; **LMN** = lower motor neuron; **MSP** = multisystem proteinopathy; **MUAP** = motor unit action potential; **PrLD** = prion-like domain; **UMN** = upper motor neuron; **VCP** = valosin-containing protein.

Inclusion body myopathy (IBM) with Paget disease and frontotemporal dementia (IBMPFD) is a rare multisystem degenerative disorder named after the organ systems originally recognized to be affected—muscle, bone, and brain. Mutations in the valosin-containing protein (*VCP*) gene were the first identified cause of IBMPFD,[1,2] but reports of families without linkage to chromosome 9 established the genetic heterogeneity of the disorder.[3,4] It has recently emerged that mutations in the *HNRNPA2B1* (chromosome 7) and *HNRNPA1* (chromosome 12) genes account for some families with IBMPFD.[5] We first raised the possibility of a connection between IBMPFD and amyotrophic lateral sclerosis (ALS) after mutations in the *VCP* gene were identified in patients with familial ALS.[6] Interestingly, the original report of IBMPFD[7] almost 30 years ago described it as a "familial disorder of combined lower motor neuron degeneration and skeletal disorganization"; the presence of fasciculations, EMG evidence of chronic reinnervation, and muscle biopsy showing grouped atrophy all pointing toward a primarily neurogenic process. This family was subsequently found to have an R155Q mutation in the *VCP* gene.[8] Since the original description, various reports of families with IBMPFD have made some reference to ALS,

**Supplemental data at www.neurology.org**

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either explicitly (e.g., by the mention of "family member with ALS") or implicitly (e.g., through clinical and electrophysiologic findings that indicate motor neuron involvement, such as fasciculations, spasticity, hyperreflexia, extensor plantar responses, chronic reinnervation changes on EMG, and prolonged central motor conduction time).[8,9] Invariably, these reports overlooked the possible direct connection between *VCP* mutations and ALS. We have therefore conducted the present study to more clearly define the relationship between IBMPFD and motor neuron disease.

## METHODS

**Study population.** See appendix e-1 on the *Neurology*® Web site at www.neurology.org. Participants for this study were recruited from across the United States through physician referrals and patient self-identification. Seventeen individuals from 8 families, each with at least 2 family members affected by some combination of muscle weakness (presumed due to IBM or ALS), Paget disease, and cognitive/behavioral impairment, were enrolled. The institutional review board of the University of Miami approved the study protocol and all participants provided written informed consent.

**Genetics.** Genetic testing (figure) established the cause of disease to be mutations in the *VCP* gene (10 individuals from 6 families), the *HNRNPA2B1* gene (4 individuals from 1 family), or an unknown gene (3 individuals from 1 family). All 3 patients in whom the causative gene has not been identified tested negative for mutations in *VCP*, heterogeneous nuclear ribonucleoprotein A2B1 (*HNRNPA2B1*), *HNRNPA1*, and *C9ORF72*. Mutations in other genes known to cause familial ALS were excluded through analysis of whole-exome data.

**Clinical evaluation.** The same neurologist (M.B.) examined 16 of the 17 study participants, either at the study center (n = 8) or at the patients' homes (n = 8, who were too ill to travel). The remaining subject (family 3, III-3) was examined by a second neurologist (B.O.). Evaluations included a careful history, neuromuscular examination, and bedside cognitive evaluation specifically directed at detecting signs of frontotemporal dysfunction (attention, verbal fluency, executive dysfunction, and memory). Nerve conduction studies and routine EMG was performed in all subjects and by the same neurologist (M.B.) in all but 2 cases; EMG in one of these 2 cases was performed by a coauthor (B.O.) and EMG in the other was performed by an electromyographer at the Mayo Clinic. Careful semiquantitative evaluation of motor unit action potentials (MUAPs) was performed to distinguish neurogenic from myopathic processes. Early recruitment of short-duration, low-amplitude, polyphasic MUAPs was interpreted as a sign of myopathy. Reduced recruitment of long-duration, large-amplitude, polyphasic MUAPs was interpreted to indicate a neurogenic process. The potential for late-stage development of large -amplitude, polyphasic MUAPs in chronic myopathies was recognized, and particular emphasis was placed on analysis of MUAP duration and the recruitment pattern to distinguish chronic myopathy from a primarily neurogenic process—i.e., long duration and reduced recruitment indicating a neurogenic disorder. Medical records, including alkaline phosphatase, creatine kinase, bone x-rays, radionucleotide bone scans, and muscle biopsies, were reviewed where available. The diagnosis of ALS was based on the finding of progressive upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction in multiple body regions, according to the principles laid out in the El Escorial criteria.[10]

**Neuropsychological assessment.** As mentioned above, bedside cognitive evaluations were performed on all study subjects. In addition, 4 of the participants underwent a 2- to 3-hour detailed neuropsychological test battery to assess cognitive and behavioral functioning. Logistical constraints precluded the administration of this battery to the other participants. The battery consisted of standardized measures that have been shown to be clinically and empirically sensitive to the cognitive and behavioral changes observed in patients with frontotemporal dementia. As part of the evaluation, caregivers were also asked to complete brief paper and pencil questionnaires assessing the participant's cognitive/behavioral symptoms.

**Statistics.** Median age at onset or diagnosis was estimated by product-limit method when individuals who had not exhibited a particular clinical manifestation (weakness, Paget disease, or cognitive/behavioral impairment) by the time of evaluation were included in the calculation.

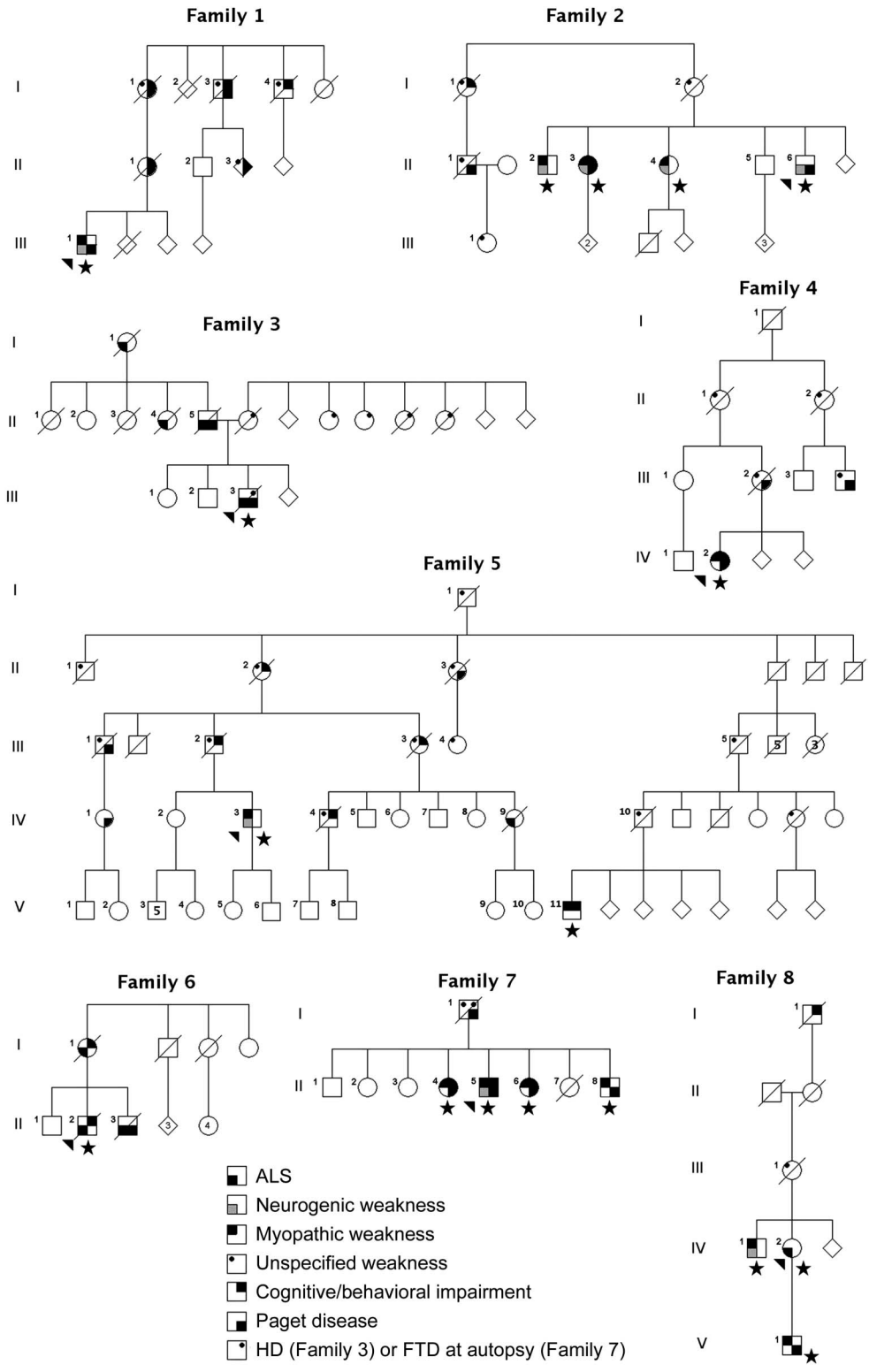
## RESULTS

The study population comprised 17 individuals (11 male, 6 female) from 8 apparently unrelated families, including 6 families with mutations in the *VCP* gene (R155H in 4 families, R155C in 1 family, and R159G in 1 family); 1 family with a D290V mutation in the *hnRNPA2B1* gene; and 1 family in which mutations in the *VCP*, *hnRNPA2B1*, *hnRNPA1*, and *C9orf72* genes, as well as in other genes known to cause familial ALS, were excluded (table). Median age at appearance of first clinical manifestation was 34 years (range 25–52), with weakness being the most common initial symptom (n = 10, median age at onset 38 years, range 25–52) and Paget disease of bone the next most frequent initial diagnosis (n = 7, median age at onset 37 years, range 30–54).

Cognitive impairment was identified in 4 subjects through bedside evaluation alone, cognitive/behavioral impairment in another 4 through both bedside cognitive evaluation as well as detailed neuropsychological testing. In this latter group, detailed testing revealed cognitive impairment in 2 and behavioral impairment in all 4. Impairments were typically of the frontotemporal variety with executive dysfunction (e.g., poor performance on the antisaccade test) and impaired verbal fluency, as well as apathy and disinhibition. Due to the limited sensitivity of bedside testing and its exclusive focus on cognitive (rather than behavioral) impairment, the prevalence of cognitive/behavioral impairment among our study subjects (8/17 = 47%) is likely an underestimate.

Among the 15 subjects who had weakness, it was asymmetric at onset in 8. Weakness remained asymmetric in 4 of these patients by the time of study evaluation, which occurred 0.5, 2, 15, and 28 years after onset of weakness. Weakness typically appeared first

**Figure** Multisystem proteinopathy pedigrees




Pedigrees of the 6 VCP families (families 1–6), the *HN*RNPA2B1 family (family 7), and the gene unknown family (family 8). Arrowhead indicates the proband. Star indicates family members who were examined. Phenotype status is denoted by symbols as indicated. Pedigrees have been altered to protect privacy. ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia.

in the limbs (n = 14) rather than in the bulbar muscles (n = 1). Limb weakness first appeared in the arms in 3 patients (all proximal), in the legs in 10 patients (n = 6 proximal, n = 4 distal), and simultaneously in the arms and legs in 1 patient (proximal). After extended follow-up (median latency from onset of weakness to examination = 15 years), in the majority of patients weakness remained most

**Table** Summary of clinical features

| | | | | Symptom history | | | | | Clinical examination findings | | | | | | | | EMG | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family no. | Generation: individual no. | Gene name (mutation) | Sex | Weakness (onset age, y) | Paget (onset age, y) | Cognitive/ behavioral | Initial weakness | Progression of weakness | Age at examination, y | UMN findings | Bulbar findings | Respiratory weakness | Distribution of weakness | Pattern of weakness | Symmetry | Ongoing denervation | MUAP morphology |
| 1 | III-1 | *VCP* (R155H) | M | Y (41) | Y[a] (37) | N | Distal leg (right) | Rapid | 44 | N | N | Y | Arm < leg | Proximal < distal | N | Y | Mixed |
| 2 | II-6 | *VCP* (R155H) | M | Y[a] (29) | Y (39) | N | Proximal arm (left) | Slow | 55 | N | N | N | Arm < leg | Proximal > distal | N | Y | Neurogenic |
| 2 | II-3 | *VCP* (R155H) | F | Y[a] (41) | Y (54) | Y | Proximal arm (left > right) | Slow | 62 | N | N | N | Arm > leg | Proximal > distal | Y | Y | Mixed |
| 2 | II-4 | *VCP* (R155H) | F | Y[a] (48) | N | N | Proximal legs (bilateral) | Slow | 60 | N | N | N | Arm < leg | Proximal = distal | Y | Y | Mixed |
| 2 | II-2 | *VCP* (R155H) | M | Y[a] (32) | N | N | Proximal legs (bilateral) | Slow | 64 | N | N | Y | Arm < leg | Proximal > distal | Y | N | Mixed |
| 3 | III-3 | *VCP* (R155H) | M | Y (47) | Y[a] (30) | Y | Bulbar | Rapid | 48[d] | Y | Y | Y | Arm < leg | Proximal > distal | N | Y | Neurogenic |
| 4 | IV-2 | *VCP* (R155C) | F | Y (43) | Y[a] (33) | Y[c] | Proximal arms + legs (bilateral) | Slow | 44 | N[b] | N | N | Arm < leg | Proximal > distal | Y | N | Myopathic |
| 5 | IV-3 | *VCP* (R155H) | M | Y[a] (28) | N | N | Proximal legs (right) | Slow | 59 | N[b] | N | N | Arm < leg | Proximal > distal | Y | Y | Mixed |
| 5 | V-11 | *VCP* (R155H) | M | Y[a] (35) | N | Y | Proximal arm (right) | Slow | 49 | N | N | N | Arm = leg | Proximal = distal | N | N | Myopathic |
| 6 | II-2 | *VCP* (R159G) | M | Y[a] (52) | N | Y | Proximal legs (bilateral) | Slow | 65[d] | Y | N | Y | Arm = leg | Proximal > distal | Y | Y | Neurogenic |
| 7 | II-5 | *HNRNPA2B1* (D290V) | M | Y[a] (38) | Y (46) | Y[c] | Distal leg (right) | Slow | 52 | N | N | N | Arm < leg | Proximal > distal | Y | Y | Mixed |
| 7 | II-4 | *HNRNPA2B1* (D290V) | F | Y (38) | Y[a] (35) | Y[c] | Distal leg (left) | Slow | 53 | N | N | N | Arm = leg | Proximal > distal | Y | Y | Myopathic |
| 7 | II-6 | *HNRNPA2B1* (D290V) | F | N | Y[a] (37) | Y[c] | NA | NA | 50 | N | N | N | None | None | NA | Y | Myopathic |
| 7 | II-8 | *HNRNPA2B1* (D290V) | M | Y (38) | Y[a] (34) | N | Distal leg (right) | Slow | 45 | N[b] | N | N | Arm < leg | Proximal < distal | Y | N | Myopathic |
| 8 | IV-2 | Unknown[e] | F | Y[a] (25) | N | N | Proximal legs (bilateral) | Slow | 56 | Y | N | N | Leg only | Proximal > distal | Y | N | Neurogenic |
| 8 | IV-1 | Unknown[e] | M | Y[a] (32) | N | N | Proximal legs (bilateral) | Slow | 62 | N[b] | N | N | Leg only | Proximal > distal | Y | N | Mixed |
| 8 | V-1 | Unknown[e] | M | N | Y[a] (34) | N | NA | NA | 36 | N | N | N | None | None | NA | N | Myopathic |

Abbreviations: > = more severely than; < = less severely than; MUAP = motor unit action potential; NA = not applicable; UMN = upper motor neuron; *VCP* = valosin-containing protein.

[a] Designates initial symptom.

[b] Pathologically brisk reflexes, but isolated (i.e., no other signs of UMN dysfunction).

[c] Underwent detailed neuropsychological testing (in addition to the bedside cognitive testing that all subjects received).

[d] Died ~1 year after examination.

[e] Negative for *VCP*, *HNRNPA2B1*, *HNRNPA1*, and *C9ORF72*.

severe in the legs, and proximal muscles were typically affected to a greater extent than distal muscles.

Manifestations of both UMN and LMN dysfunction were unequivocally present in 3 patients, with UMN dysfunction evidenced clinically by spastic dysarthria, brisk jaw jerk, limb spasticity, hyperreflexia, and extensor plantar responses (family 3, III-3); limb spasticity, hyperreflexia, and an extensor plantar response (family 6, II-2); and hyperreflexia, positive Hoffman sign, and extensor plantar responses (family 8, IV-2); pathologically brisk reflexes were evident in 4 additional patients. Among the 3 patients with both UMN and LMN dysfunction, EMG showed reduced recruitment of chronic neurogenic MUAPs in all 3 and widespread ongoing denervation in 2 of the 3 (table). In addition to these 3 patients, EMG findings were purely neurogenic in 1 other patient, whereas 6 patients evinced purely myopathic features on EMG and 7 patients demonstrated a mixed pattern of both neurogenic and myopathic findings. Overall, therefore, there was evidence for involvement of motor neurons or their axons in 11/17 (65%) study participants. Two of the 3 patients with both UMN and LMN dysfunction died of the disease 1.5 and 14 years after the onset of weakness. The third patient with both UMN and LMN dysfunction is still alive 30+ years after weakness first appeared.

Weakness generally progressed slowly over a period of decades, although 2 subjects reported periods of very rapid decline in muscle strength. One of these subjects (family 3, III-3) was already described above. The second subject (family 1, III-1) presented with a mixed neurogenic-myopathic picture without UMN signs, but weakness (including respiratory muscle involvement) progressed rapidly over a period of <2 years. There was no apparent pattern in the relationship between the site of initial weakness (arm, leg, proximal, distal, or bulbar) and the subsequent progression of disease, the underlying genotype, or physiology (neurogenic vs myopathic).

Notwithstanding the relatively long latency from onset of weakness to study evaluation and the cross-sectional nature of this study, we found evidence that either myopathic or neurogenic processes may dominate early in the course of disease. In 2 patients (family 7, II-6, and family 8, V-1) who reported no weakness and had normal strength on confrontation testing (i.e., their muscles are at most minimally affected), the EMG was myopathic. Furthermore, one individual (family 8, IV-1) complained only of weakness in the legs and confrontation testing showed normal arm strength; EMG of the legs showed chronic neurogenic changes, but EMG of the arms showed early recruitment of clearly myopathic MUAPs. Perhaps contrasting these observations is the finding of a primarily neurogenic process in patients evaluated soon (0.5–2 years) after onset of weakness (e.g., family 1, III-1, and family 3, III-3) and whose disease has since progressed rapidly. The finding of neurogenic changes early in the course of disease also mitigates the possibility that such findings are solely attributable to a longstanding and severe primary muscle disease.

There was no clear association between the mutated gene (*VCP*, *HNRNPA2B1*, unknown) or the specific mutation within the *VCP* gene (R155H, R155C, R159G) on the one hand, and the motor phenotype on the other. Lower motor neuron neurogenic pathology was evident in subjects with mutations in *VCP* (R159G), *HNRNPA2B1*, and the unknown gene; motor neuron disease (defined on the basis of combined UMN and LMN dysfunction) was observed in subjects with 2 different mutations in the *VCP* gene and a mutation in a third unknown gene.

**DISCUSSION** With the goal of exploring the putative connection between IBMPFD and motor neuron disease, we have performed careful clinical and EMG evaluations of 17 consecutive patients from 8 apparently unrelated IBMPFD families. Motor neuron involvement was apparent in the majority of study participants, including 3 patients with unequivocal UMN findings and 4 additional patients with subtle UMN findings. Importantly, the neurogenic phenotype was observed across families with different genes (*VCP*, *HNRNPA2B1*, and an as yet unidentified gene) and different mutations of the same gene (e.g., *VCP*) (table). The rate of progression in 2 of the 3 patients with ALS was relatively slow, although atypical, similarly slow rates of progression have been reported in both sporadic[11] and familial[12] forms of ALS.

These observations strongly support our hypothesis that weakness in IBMPFD is not solely due to myopathy, but at least in part due to a neurogenic process that may implicate the motor neuron. The phenotypic spectrum of IBMPFD, therefore, is broader than previously recognized, extending to include motor neuron disease. As such, the term IBMPFD seems overly restrictive. IBMPFD-ALS is similarly cumbersome and probably inadequate given the rare, but well-described, involvement of other organ systems including cardiac,[13] hepatic,[14] visual,[14] auditory,[15] sensory,[16] and autonomic systems,[17] as well as emerging evidence that mutations in *VCP* may infrequently manifest as Parkinson disease,[18–20] hereditary spastic paraplegia,[21] or cerebellar ataxia.[22] Instead, we propose to change the name of this syndrome from IBMPFD to multisystem proteinopathy (MSP), using the nomenclature of MSP1 for disease caused by mutations in *VCP*, MSP2 when due to mutations in *HNRNPA2B1*,[5] MSP3 when caused by *HNRNPA1* mutations,[5] and MSP4 when due to mutations in the as yet unidentified gene. This nomenclature emphasizes the multisystem nature of the degenerative

process (most prominently nerve, muscle, bone, and brain) as well as the conspicuous deposition of TDP-43, hnRNPA2B1, and hnRNPA1 protein in affected tissues of patients with this multisystem disease independent of genetic etiology.[5,6,23,24] The neuropathology of MSP1, for example, is characterized by TDP-43 and ubiquitin-positive neuronal intracytoplasmic inclusions and dystrophic neurites in the neocortex,[23] TDP-43-, hnRNPA1-, and hnRNPA2B1-positive cytoplasmic inclusions in muscle,[5,24] and nuclear extrusion of TDP-43 accompanied by accumulation of TDP-43-positive cytoplasmic inclusions in the hypoglossal nucleus.[6] Similarly, MSP2 and MSP3 are characterized by clearance of TDP-43 as well as hnRNPA2B1 and hnRNPA1, respectively, from myonuclei, with accumulation of large cytoplasmic inclusions that are immunoreactive against TDP-43, hnRNPA1, and hnRNPA2B1.[5] Although there has been no report of TDP-43 pathology in Pagetoid bone from patients with IBMPFD (perhaps no one has yet looked), ultrastructural analysis of bone from patients with IBMPFD demonstrates tubulofilamentous inclusions similar to the inclusions identifiable in muscle tissue.[1,8,13,25] The role of TDP-43, hnRNPA2B1, and hnRNPA1 in disease pathogenesis is strongly supported by the fact that missense mutations in any of the 3 genes is sufficient to cause ALS or MSP.[5,26–28]

The significance of defining the phenotypic overlap between IBMPFD and ALS is best understood in the context of the recent discovery that mutations in *HNRNPA2B1* and *HNRNPA1* are novel genetic causes of MSP.[5] Along with TDP-43 and FUS (both of which are hnRNPs), hnRNPA2B1 and hnRNPA1 have conserved prion-like domains (PrLDs). Indeed, PrLDs are present in ~250 human proteins and are particularly enriched in proteins that undergo dynamic assembly/disassembly into non-membrane-bound organelles or "granules."[29] Recently it was determined that the PrLDs in TDP-43, FUS, hnRNPA2B1, and hnRNPA1 mediate fibrillization that underlies assembly into cytoplasmic RNA granules.[5,30,31] Interestingly, mutations in these genes that cause MSP tend to cluster within these PrLDs, enhance the fibrillization properties of these proteins, and show increased recruitment into cytoplasmic RNA granules with presumed impairment in metabolism of client RNAs. The discovery that mutations in *HNRNPA2B1* and *HNRNPA1* may also cause ALS, therefore, adds to the growing body of evidence supporting perturbed RNA metabolism as an important pathophysiologic mechanism relevant to motor neuron degeneration in ALS. Furthermore, this discovery emphasizes the importance of prion-like mechanisms in ALS pathophysiology, at least in patients with ALS due to certain genetic causes, and lends support to the idea that cell-to-cell spread through protein conformational change may underpin the "spread by contiguity" that has been reported in ALS.[32] Although the specifics of how this might occur remain unclear, one speculative idea is that PrLD-containing proteins are extruded through exosomes into the cytoplasm and then engulfed by neighboring cells through a process of macropinocytosis.

Recognizing that the phenotype of MSP extends to include dysfunction of the motor neuron, including typical ALS, is important for a number of reasons. First, findings of motor neuron degeneration/dysfunction should not detract from the diagnosis of MSP. Second, patients with MSP may benefit from the infrastructure and sophistication of the multidisciplinary care that is now recommended for patients with ALS. Moreover, the genetic defects in MSP implicate a range of biological mechanisms—particularly altered protein homeostasis, PrLD-mediated self-aggregation, and RNA metabolism—that are likely relevant to the pathobiology of more common motor neuron degenerative diseases such as ALS.

## AUTHOR CONTRIBUTIONS

Michael Benatar contributed to all aspects of the work described in this manuscript including study concept and design, obtaining funding, acquiring data, study supervision, and drafting/revising the manuscript. Joanne Wuu contributed to study concept and design, obtaining funding, study supervision, and drafting/revising the manuscript. Catalina Fernandez contributed to acquiring data. Conrad C. Weihl contributed to acquiring data and drafting/revising the manuscript. Heather Katzen contributed to acquiring data and drafting/revising the manuscript. Julie Steele contributed to acquiring data. Bjorn Oskarsson contributed to acquiring data and drafting/revising the manuscript. J. Paul Taylor contributed to all aspects of the work described in this manuscript including study concept and design, obtaining funding, acquiring data, and drafting/revising the manuscript.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## DISCLOSURE

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