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Valosin-containing protein disease: inclusion body myopathy with Paget's disease of the bone and fronto-temporal dementia

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Abstract

Mutations in valosin-containing protein (VCP) cause inclusion body myopathy (IBM) associated with Paget's disease of the bone (PDB) and fronto-temporal dementia (FTD) or IBMPFD. Although IBMPFD is a multisystem disorder, muscle weakness is the presenting symptom in greater than half of patients and an isolated symptom in 30%. Patients with the full spectrum of the disease make up only 12% of those affected; therefore it is important to consider and recognize IBMPFD in a neuromuscular clinic. The current review describes the skeletal muscle phenotype and common muscle histochemical features in IBMPFD. In addition to myopathic features; vacuolar changes and tubulofilamentous inclusions are found in a subset of patients. The most consistent findings are VCP, ubiquitin and TAR DNA-binding protein 43 (TDP-43) positive inclusions. VCP is a ubiquitously expressed multifunctional protein that is a member of the AAA+ (ATPase associated with various activities) protein family. It has been implicated in multiple cellular functions ranging from organelle biogenesis to protein degradation. Although the role of VCP in skeletal muscle is currently unknown, it is clear that *VCP* mutations lead to the accumulation of ubiquitinated inclusions and protein aggregates in patient tissue, transgenic animals and *in vitro* systems. We suggest that IBMPFD is novel type of protein surplus myopathy. Instead of accumulating a poorly degraded and aggregated mutant protein as seen in some myofibrillar and nemaline myopathies, *VCP* mutations disrupt its normal role in protein homeostasis resulting in the accumulation of ubiquitinated and aggregated proteins that are deleterious to skeletal muscle.

1. Introduction

IBMPFD is an autosomal dominantly inherited disorder with variable penetrance of three predominant phenotypic features [1]. (1) 90% of patients develop disabling weakness with a mean onset of 45 years of age at this same mean age (2) 51% of affected patients have osteolytic lesions consistent with PDB. Finally (3) 32% develop a typical FTD manifested by prominent language and behavior dysfunction with a mean onset of 54 years old [2]. Other phenotypic features have been reported as well, including dilated cardiomyopathy, hepatic fibrosis, cataracts and sensory-motor axonal neuropathy [3-5]. How common these features are in affected patients is not known.

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Familial syndromes phenotypically similar to IBMPFD had been described prior to the identification of *VCP* as the causative gene in 2004 [1]. Four previous reports described an autosomal dominant syndrome with weakness and PDB with and without dementia [6-9]. Most notably, a multisystem myotonic disorder with frontotemporal dementia was described that subsequently was recharacterized as IBMPFD once *VCP* gene testing was performed [3]. In addition, a family with lower motor neuron degeneration and PDB reported in 1982 by Tucker et al [8] was found to have an R155P gene mutation in *VCP* [1]. Whether the two other previous syndromes were in fact IBMPFD remains unknown.

2. Clinical features of IBMPFD myopathy

Muscle weakness is an isolated symptom in ~30% of patients and the presenting symptom in greater than half of patients with IBMPFD suggesting that IBMPFD may be commonly seen in a neuromuscular clinic without its other syndromic features [10]. Because of this, it is important for clinicians to take a careful family history of dementia and PDB in a patient with a suspected IBM. Patients can present with both proximal and distal muscle weakness. Commonly described patterns include proximal lower extremity weakness involving the limb girdle muscle groups [3,4,11-14]; scapulohumeral weakness with prominent scapular winging [5,11,13,14]; axial weakness with head ptosis and marked lumbar lordosis [3,5,11,13,14]; distal lower and upper extremity weakness [5,11-14] or a mixture of these patterns. Future evaluations using larger cohorts of IBMPFD patients will be needed to identify the predominant phenotypic pattern. In addition there have been reports of facial and tongue weakness [14]. Weakness can be symmetric or more commonly asymmetric suggesting a “patchy” involvement of muscle groups [13,14]. Sensory exam is normal although deep tendon reflexes are generally absent or reduced likely due to the patient's weakness [14]. As expected the distinct muscle weakness patterns have led to the misdiagnosis of these patients as limb girdle muscular dystrophy (LGMD), fascioscapular humeral dystrophy (FSHD), distal myopathies (i.e. Welander and Miyoshi) and amyotrophic lateral sclerosis (ALS) [13].

The clinical presentation of muscle weakness within similar mutations and even within families is strikingly variable making phenotypic characterization of IBMPFD more difficult [13,15]. Patients progress slowly over years necessitating assistive devices and wheelchair. Many die of respiratory insufficiency or complications stemming from end stage dementia [13,16].

Electromyography of affected muscles reveals findings consistent with an “irritable myopathy” with brief small polyphasic action potentials and irritative features that include fibrillations, and positive sharp waves, although the most consistent feature is myopathic potentials [14]. Nerve conduction studies are typically normal [14]. Elevated CK levels of > 220u/L were found in 13/73 (18%) patients with the majority having normal values (20-220u/L) [13]. Muscle MRI has been reported in some patients and demonstrates widespread signal changes in multiple muscle groups including axial muscles [5,12].

Other important clinical and laboratory studies include careful attention to focal musculoskeletal pain and pre-senile dementia as these may be signs of underlying PDB and FTD respectively. Therefore all patients with suspected IBMPFD need a careful mental status examination with special attention to behavioral alterations (e.g., personal/social unawareness, perseveration, abulia, disinhibition), early expressive or receptive language dysfunction, and relative preservation of memory, orientation, or praxis [17]. In addition, a serum alkaline phosphatase is a useful screening measure for PDB. However any patient with a complaint of pain or pathologic fractures needs a detailed radiologic survey to evaluate for PDB.

3. Muscle pathology in IBMPFD

Reported muscle biopsy histopathology is consistent with IBM in a subset of patients [1,3-5,11,12,14]. These patients have histologic features consistent with IBM including ubiquitin positive and tubulofilamentous inclusions seen by EM [5,11]. However, many patients have no rimmed vacuoles and have been characterized as a non-specific myopathy [13]. A recent review of 49 patients from nine different families noted that of the 18 patients with muscle biopsies performed only seven (39%) had evidence of vacuoles and only two of the nine families had been correctly identified as having a hereditary IBM [13].

IBMPFD muscle biopsies have variable myopathic features. These features include variation in muscle fiber size and increased endomysial connective tissue in patchy regions throughout the biopsy [5,14]. Notably some patients have large regions of myopathic grouping that include prominent vacuoles, a feature unusual in sporadic inclusion body myositis (sIBM) [5,14] (Figure 1A). Vacuoles are both sarcoplasmic and subsarcolemmal. They are typically irregularly shaped and can be rimmed with basophilic debris (Figure 1B and 1C) [5,11,14]. Eosinophilic bodies have also been reported in scattered fibers of some patients [13]. There have been no reports of birefringent congophilic amyloid after congo red staining using polarized light or texas red enhanced fluorescence associated with vacuolar structures. Although one report described a muscle biopsy from one IBMPFD patient as having “IBM” pathology in addition to a large number of congophilic nuclei, in both vacuolated and non-vacuolated muscle fibers [18]. There is no endomysial inflammation as confirmed via immunohistochemistry for MHCI and T cells [14]. Dystrophy related immuno-stains are normal and include dystrophin, emerin, α, β, γ, δ sarcoglycan, and α dystroglycan [14]. Oxidative enzyme stains are normal as well, indicating no obvious mitochondrial pathology [14].

Immunohistochemical staining for β-amyloid using the 6E10 antibody, SMI31, and desmin highlight scattered regions of focal accumulation within myofibers [5,14]. Antibodies to ubiquitinated proteins demonstrate inclusions in most muscle biopsies [5,12,18]. Ubiquitinated inclusions are most prominent in the myonuclei as well as subsarcolemmal (Figure 1D). Ubiquitin immunostaining can be seen throughout the sarcoplasm and around and within rimmed vacuoles as well (personal observations). However, Guyant-Marechal et al reported two patients with an R155C mutation that had scarce subsarcolemmal rimmed vacuoles and an absence of ubiquitinated inclusions [3].

The localization of VCP in normal skeletal muscle has been reported to be diffusely sarcoplasmic, intramyonuclear and perinuclear [1,3,19]; in contrast, VCP forms sarcoplasmic and myonuclear inclusions in IBMPFD patient tissue [1,3,5]. The inclusions are present in vacuolated and non-vacuolated fibers (Figure 1E). VCP can also be increased diffusely in scattered myofibers throughout the sarcoplasm [3]. VCP inclusions often co-localize with ubiquitin [5]. The presence of VCP inclusions is not specific for IBMPFD and is found in other muscle disorders with prominent protein aggregate pathology including sIBM [1].

Electron microscopy of skeletal muscle from IBMPFD patients demonstrates disordered myofibrils, vacuolation, and myeloid bodies. In addition, there are tubulofilamentous inclusions within myonuclei and myofibers [5,13,20]. Electron micrographs of affected skeletal muscle from one patient harboring an L198W mutation in VCP demonstrated abundant randomly oriented 15-21nM in diameter tubulofilamentous inclusions within myonuclei [11]. These inclusions appeared to be present in the sarcoplasm only when adjacent to degenerating myonuclei suggesting they are nuclear in origin [11].

These biopsy findings suggest that vacuoles and congophilic amyloid inclusions are not necessary for a diagnosis of IBMPFD since up to 60% of IBMPFD biopsies failed to show vacuoles [13] and several reports describe scarce vacuoles in many patients [3,5,12]. In our own personal experience of reviewing 10 IBMPFD patient biopsies, we found evidence of vacuoles in only 7/10 patients (unpublished observations). Perhaps the most consistent and diagnostically helpful finding is ubiquitin positive myonuclear and sarcoplasmic inclusions, VCP inclusions and TDP-43 inclusions (see below).

4. Overlapping pathology with fronto-temporal dementia (FTD)

Patients with VCP dementia have prominent ubiquitin positive intranuclear neuronal inclusions classifying it as a novel type of FTD [21,22]. Rare intranuclear neuronal VCP inclusions are also present [21,22]. FTDs are a group of neurodegenerative disorders associated with degeneration of the prefrontal and anterior temporal lobes. They are histopathologically distinguishable from other dementias due to the lack of Alzheimer disease type pathology [23]. ~50% of affected patients report a family history of dementia suggesting that genetic causes of FTD are important. Of those familial cases, 30-40% are due to mutations in tau and have classic tau histopathology (named tauopathies) [23]. However, a large number of FTDs fall into the classification of fronto-temporal dementia with ubiquitinated inclusions (FTD-U) (>50%). IBMPFD was the first genetically identified FTD-U [1,21,22]. The FTD-Us are distinct from the tauopathies in that they lack any tau pathology and have the presence of ubiquitin and TDP-43 positive intraneuronal inclusions [24]. TDP-43 is an RNA binding protein that mediates exon skipping of pre-mRNA species [25]. The accumulation of TDP-43 inclusions in FTD-U as well as ALS brain tissue is thought to be specific for these disorders and play a key role in their pathogenesis [25].

TDP-43 positive inclusions are found in IBMPFD affected brains and co-localize with ubiquitin [26]. TDP-43 inclusions are both intranuclear and within the cytosol of affected neurons [26]. It is speculated that a hyperphosphorylated TDP-43 species seen only in FTD-U patient tissue is a molecular signature for this disease [25]. Consistent with this, IBMPFD patient brain tissue has a similar phosphorylated TDP-43 species in insoluble brain extracts [26].

In the 6 IBMPFD patient's muscle examined, all had prominent TDP-43 inclusions that co-localized with ubiquitin [27]. These inclusions were predominantly subsarcolemmal and sarcoplasmic (Figure 1F). In addition, a TDP-43 immunoblot from IBMPFD patient muscle tissue demonstrated a similar molecular signature to that of FTD-U brain having a 43kDa and slightly larger migrating species consistent with an unmodified and phosphorylated form of TDP-43 [27]. TDP-43 positive inclusions were not exclusive to IBMPFD patient muscle, but were also seen in 78% of sIBM patient muscle biopsies, while 0% of normal muscle and 8% of steroid responsive polymyositis patient muscle biopsies had similar TDP-43 inclusions [27].

5. The biology of VCP

VCP is a member of the AAA+ protein family [28]. Dysfunction in this family of proteins is associated with many inherited diseases including hereditary spastic paraparesis due to mutations in the proteins spastin and paraplegin [29,30] and juvenile onset generalized dystonia associated with mutations in torsinA [31]. The typical features of a AAA+ protein are one or two tandemly encoded AAA+ protein domains (two in the case of VCP) [28]. These domains are essential for ATP binding and hydrolysis. Other features that are less conserved among AAA+ family members include an N and C terminal domain. These domains allow for substrate and cofactor selectivity. The active form of most AAA+ proteins is a macromolecular complex consisting of six monomers formed in a ring around a

central pore. In the case of VCP both AAA+ protein domains (D1 and D2 domains) sit on top of each other making a stacked or double ring structure (Figure 2).

VCP has been implicated in multiple cellular processes ranging from mitosis, cell death, organelle biogenesis, membrane fusion and protein degradation [32]. However, it is increasingly clear that these processes are likely related to VCP's regulatory role within protein degradation pathways [33]. VCP associates with many members of the ubiquitin-proteasome system (UPS) including polyubiquitin, E3/E4 ubiquitin ligases, and deubiquitinating enzymes [33]. VCP is essential for endoplasmic reticulum associated degradation (ERAD) of misfolded ER, transmembrane and secreted proteins [33]. VCP resides at the cytosolic face of the ER and facilitates the extraction of ERAD substrates via interactions with the channel protein derlin-1 which serves as the “retrotranslocon” [33]. Once removed from the ER, misfolded ERAD substrates are ubiquitinated and sent to the 26S proteasome for degradation [33]. This step is also mediated by VCP [33]. VCP participates in the delivery of degradation destined ubiquitinated cytosolic proteins to the 26S proteasome as well [34,35].

VCP may also be involved in protein inclusion formation [36-38]. VCP preferentially associates with aggregate prone polyglutamine containing proteins [39]. VCP is also present in the inclusions from multiple disease including ALS, Parkinson's disease and Huntington disease [40,41]. More recently it was shown that VCP is essential for aggresome formation [36-38]. Histone deacetylase 6 (HDAC6), an aggresome essential protein, binds to ubiquitinated proteins and VCP [42]. HDAC6 is responsible for the trafficking of misfolded proteins formed in the setting of UPS impairment to the aggresome where they are degraded via autophagy [43,44]. Overexpression of HDAC6 decreases protein aggregate toxicity, enhances the autophagic degradation of protein aggregates, and rescues model organisms from neurodegeneration [36,43-45].

Genetic knockdown or expression of ATPase inactive forms of VCP lead to an increase in ubiquitinated proteins, ER vacuolization, and impaired degradation of both ER derived and cytosolic proteins emphasizing the critical role for VCP in the UPS [38,46,47]. The specific proteins affected by the loss of VCP activity in these experiments are unknown and likely are dependent upon the cell type and with what adaptor protein(s) VCP is associated. Several VCP specific substrates have been proposed in mammalian cells, all with varying levels of biochemical and genetic evidence (Table 1). A VCP substrate, in contrast to a cofactor or adaptor, binds to VCP and the substrate's degradation or function is reliant on that interaction.

The exact mechanism by which VCP associates with substrates is unclear. Initial studies suggested that the N terminal domain, where many *VCP* mutations reside, bound preferentially to polyubiquitinated (chains containing 4-6 ubiquitins) substrates [48]. More recent studies suggest that the N terminal domain binds to ubiquitin like motifs such as UBA, UBX and UBL domains on specific adaptor proteins such as Npl4, p47 or UBXD7 [49,50]; whereas the central pore domain residues are essential for substrate binding and ER retrotranslocation [51].

6. VCP in skeletal muscle

VCP is an abundant and ubiquitously distributed protein. It has been reported to be similarly expressed in most mammalian tissues [52] and targeted deletion of *VCP* in mice is embryonic lethal [53]. The normal role of VCP in mammalian skeletal muscle is not known. One study found that the localization of VCP in differentiated skeletal muscle tissue is intranuclear [19]. Our studies in addition to others, find VCP throughout the sarcoplasm but also perinuclear and within endomysial vessels in skeletal muscle [1,3]. In cultured

myotubes VCP is predominantly found throughout the cytoplasm and excluded from the nucleus [5,54] (personal observations). This may suggest that VCP alters it localization and perhaps its function depending upon the differentiation state of the cells. The perinuclear localization of VCP is intriguing since this is an area rich in protein synthetic function via the secretory pathway [55-57]. This suggests that VCP is participating in the degradation of misfolded ER synthesized proteins via ERAD in skeletal muscle.

VCP is also a component of inclusion bodies in muscle disease. In particular, VCP accumulates as protein aggregates in sIBM and myofibrillar myopathies ([1] and unpublished data). Whether VCP is intrinsically aggregating in these diseases or facilitating the degradation of other protein aggregates is not known.

7. IBMPFD mutant VCP

To date there have been thirteen unique IBMPFD mutations in *VCP* associated with eight different residues with an arginine at residue 155 having three different mutations each (Figure 2) [13]. These residues cluster within the N and D1 domain interface (Figure 2). This is a region that has been proposed to associate with ubiquitin and ubiquitin-like domain containing proteins [48,50]. Most notably using NMR spectroscopy, it was shown that ubiquitin-like domain of the ERAD essential protein Npl4 binds VCP in a region containing residues R93, R95, and R155 which are all mutated in IBMPFD [50]. Other studies examining mutant VCP structure demonstrate that the most common IBMPFD mutation, R155H, retains ATPase activity and stable hexamer formation [58]. In addition, *in vitro* binding of mutant VCP to the UBX domain containing cofactors Ufd1, Npl4, or p47 appears to be unchanged [5,59]. These initial studies suggest that VCP mutations do not result in large conformational changes that affect co-factor binding or ATPase activity. However, the presence of subtle changes in structure, binding affinities or ATPase activity needs to be more fully explored.

In contrast to the biochemical assays detailed above, expression of IBMPFD associated VCP mutations R155H or R95G in cultured myoblasts leads to an increase in both ER associated and cytosolic ubiquitinated inclusions that in some cases co-localizes with mutant VCP [36,58]. This finding is similar to that seen with expression of an ATPase inactive dominant negative VCP or following proteasome inhibition [58]. Consistent with this, the degradation of a prototypical misfolded ERAD substrate, the ΔF508 cystic fibrosis transmembrane regulator (ΔF508CFTR), is slowed in VCP mutant expressing cells [58]. The undegraded ΔF508CFTR co-localizes with mutant VCP in ubiquitinated inclusions [54].

Similarly, an aggregate prone expanded polyglutamine containing protein (polyQ80) failed to be degraded as efficiently in VCP mutant expressing cells [36]. PolyQ80 inclusions were more numerous and VCP mutant cells accumulated insoluble aggregates [36]. This was due to a failure in protein aggregate trafficking to an aggresome [36]. Aggresomes are microtubule dependent cellular structures containing sequestered misfolded and aggregated proteins [60]. Aggresomes contain the machinery necessary for autophagic degradation of these protein aggregates [61]. The impaired aggresome formation resulted in a decrease in the autophagic clearance of protein aggregates and cell death in VCP mutant expressing cells [36]. Overexpression histone deacetylase 6 (HDAC6) rescued polyglutamine induced cell death in these cells [36].

Two independent transgenic mouse lines that overexpress the most common *VCP* mutant R155H in mouse skeletal muscle under the control of muscle creatine kinase promoter develop progressive weakness in a gene dose dependent manner between six and nine months [62]. By nine months of age they have disorganized internal architecture with vacuolation. In addition, they have prominent ubiquitinated inclusions and congophilic

amyloid [62]. High molecular weight ubiquitinated proteins were present after one month of age, several months prior to the onset of animal weakness or IBM-like pathology which occurs after 6 months [62]. This finding has been confirmed in patients as well. Tissue lysates from 2 patients with an R155C gene mutation had an increase in undegraded high molecular weight ubiquitinated proteins when compared with normal control tissue lysates [3].

Further support for dysregulation in protein degradation comes from genetic studies in *c. elegans*. Janiesch et al found that VCP mutations selectively impaired the proteasomal degradation of the myosin chaperone, unc-45 [54]. VCP mutant R155H or R95G expression stabilized the level of mammalian unc-45b similar to that seen with the addition of proteasome inhibitor MG132 [54]. A dysregulation in unc-45b degradation may lead to a decrease in myosin content and subsequent disordered myofibrils in IBMPFD patients [54].

Another study by Hubbers et al found that transient and stable expression of VCP mutants R93C, R155C and R155H in HEK293 and C2F3 myoblasts did not result in an increase in ubiquitinated proteins [5]. They found no difference between the ubiquitin immunostaining pattern of normal patient and IBMPFD mutant patients cultured myoblasts [5]. The differences between these studies may relate to cell type or expression level of mutant VCP and may highlight the tissue selectivity of these mutations.

8. IBMPFD a disorder of protein homeostasis

Fundamentally, VCP is involved in protein homeostasis or maintaining the proper balance between protein synthesis and protein degradation necessary for all cells [63]. This balance is disturbed in many disease states associated with protein accumulation [63]. *VCP* mutations may affect the normal function of VCP in protein homeostasis. For example, VCP is necessary for the retrotranslocation of misfolded ER synthesized proteins [51]. A failure in this activity would result in defective ERAD, the accumulation of ubiquitinated inclusions and activation of the ER stress response. Over time, the accumulation of undegraded ER proteins may secondarily affect the entire UPS as has been previously reported [64]. We previously demonstrated that mutant VCP fails to degrade a prototypical ERAD substrate as efficiently in cultured myoblasts [58].

This mechanism has been postulated in another multisystem disorder with a rimmed vacuole myopathy and neurodegeneration, Marinesco-Sjogren Syndrome (MSS). MSS is due to loss of function mutations in the ER co-chaperone, SIL1 [65,66]. SIL1 associates with the ER luminal chaperone Grp78/BiP and facilitates Grp78/BiP nucleotide exchange [67]. This function promotes proper folding of ER synthesized proteins [67]. Loss of SIL1 in mice leads to a neurodegenerative phenotype with ubiquitinated neuronal inclusions and activation of the ER stress response [68].

VCP also shuttles ubiquitinated substrates to the 26S proteasome [48]. It binds to polyubiquitinated proteins and the 26S proteasome [35,48]. Loss of functional VCP leads to the accumulation of high molecular weight ubiquitinated proteins and accumulation of model UPS substrates [47,48]. We demonstrated the accumulation of high molecular weight ubiquitinated proteins and cytosolic ubiquitinated inclusions in VCP mutant expressing cells and transgenic animals [36,58,62]. This accumulation of undegraded ubiquitinated proteins may lead to further UPS dysfunction and impair other ubiquitin dependent cellular pathways such as chromatin remodeling and/or membrane trafficking [69,70].

VCP is also essential for aggresome formation perhaps through its interactions with HDAC6 [36-38]. We found that under conditions of proteasome impairment or expression of an aggregate prone protein, VCP mutant expressing cells failed to generate aggresomes [36].

This resulted in the accumulation of insoluble protein aggregates that were not degraded as efficiently via autophagy [36]. The consequence of impaired aggresome formation is the accumulation of ubiquitinated proteins and protein aggregates such as TDP-43 (an autophagy substrate), seen in IBMPFD muscle tissue [27,71].

Finally, the degradation of specific UPS substrates is mediated by VCP. These substrates include IκB, Unc-45b and hypoxia inducible factor 1 α [34,35,54] (see Table 1). In the case of Unc-45b (the only substrate tested under conditions of VCP mutant expression), loss of VCP and expression of mutant VCP leads to the accumulation of Unc-45b protein [54]. However alterations in Unc-45b do not explain the bone and nervous system pathologies seen in IBMPFD since Unc-45b is exclusively expressed in skeletal muscle [72]. Perhaps a specific subset of VCP substrates is mishandled by mutant VCP in IBMPFD diseased muscle, brain and bone. This mechanism is reminiscent of another multisystem disease, myotonic dystrophy type 1 (DM1) [73]. Although the pathogenesis of IBMPFD is likely different from DM1, in which a “toxic” RNA species sequesters mRNA splicing machinery leading to the mis-splicing of multiple mRNA transcripts. Both disorders may lead to a composite phenotype affecting multiple organs via unique subsets of mishandled mRNA in the case of DM1 or protein in the case of IBMPFD. Alternatively, one specific substrate common to muscle, brain and bone is mishandled such as TDP-43.

9. Conclusions

IBMPFD is an autosomal dominant multisystem disorder due to mutations in the multifunctional chaperone *VCP* [1]. Muscle weakness typically presents in the 3rd to 4th decade of life and is phenotypically heterogeneous with both proximal and distal weakness owing to the confusion in the diagnosis as being FSHD or LGMD. Muscle biopsies often show vacuoles in a subset of patients but more commonly have prominent ubiquitinated sarcoplasmic and myonuclear inclusions in association with VCP and TDP-43 inclusions. Since >70% of patients with IBMPFD do not have features of dementia, it is important to consider the diagnosis of IBMPFD in patients with isolated muscle weakness and vacuolar pathology. More importantly, a careful family history of dementia, Paget's disease and progressive weakness is helpful since not all affected present with the same phenotypic features.

While the underlying pathogenic mechanism is still unclear for IBMPFD, recent studies suggest that perturbations in protein homeostasis (i.e. UPS, ERAD and protein aggregate handling) may underlie the cause of disease [36,58,62]. This would be a novel mechanism of disease for an autosomal dominant protein aggregate myopathy. These diseases have traditionally been thought to be due to the accumulation of a mutant aggregate prone protein. For example many myofibrillar myopathies are due to autosomal dominant mutations in aggregate prone proteins such as desmin and myotilin [74,75]. These proteins accumulate as large inclusions in diseased skeletal muscle. In some cases it has been shown that desmin accumulation secondarily leads to UPS dysfunction [76].

However, we suggest an alternative mechanism for protein surplus myopathies; one in which a mutant VCP monomer incorporates into a VCP hexamer leading to a dysfunctional VCP protein complex that fails to perform its normal function in protein homeostasis. This dysfunction secondarily leads to the accumulation of ubiquitinated and TDP-43 positive protein inclusions which are detrimental to skeletal muscle leading to disease. Therapies aimed at improving protein homeostasis by enhancing the UPS or clearance of protein aggregates may prove beneficial in the treatment of IBMPFD.

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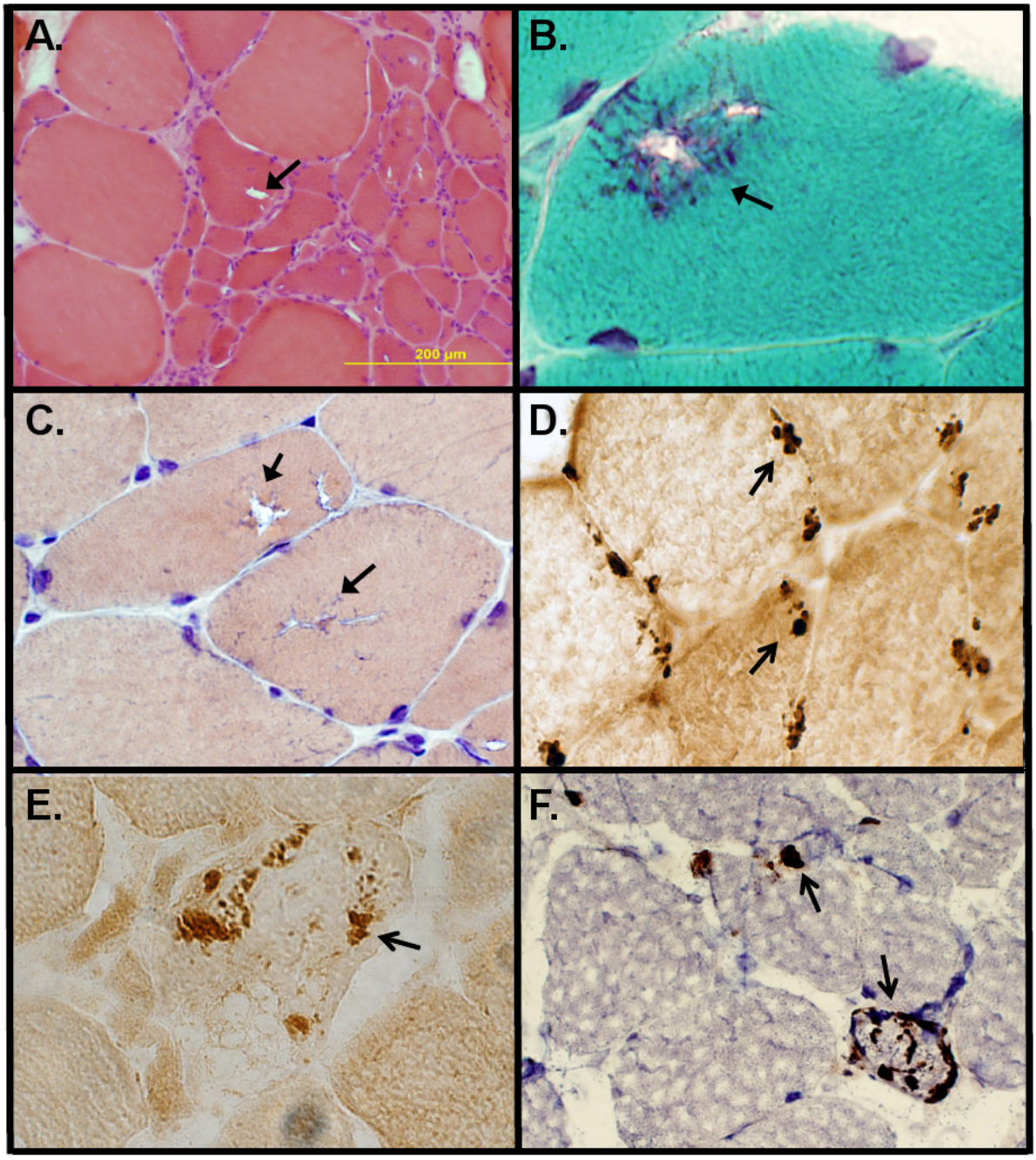

Figure 1.
Histochemical and immunohistochemical stains of IBMPFD patient muscle biopsies. A) hematoxylin and eosin demonstrating large regions of grouped atrophy with smaller fibers containing rimmed vacuole, B) A fiber with a prominent rimmed vacuole seen with modified gomori trichrome stain, C) congo red staining demonstrating fibers with vacuoles and basophilic debris. D) Immunostaining with an anti-ubiquitin antibody (FK2) demonstrates large inclusions that are myonuclear and subsarcolemmal. E) A vacuolated fiber with VCP inclusions. F) TDP-43 immunostaining sarcoplasmic inclusions. Closed arrows highlight vacuoles and open arrows denote inclusions

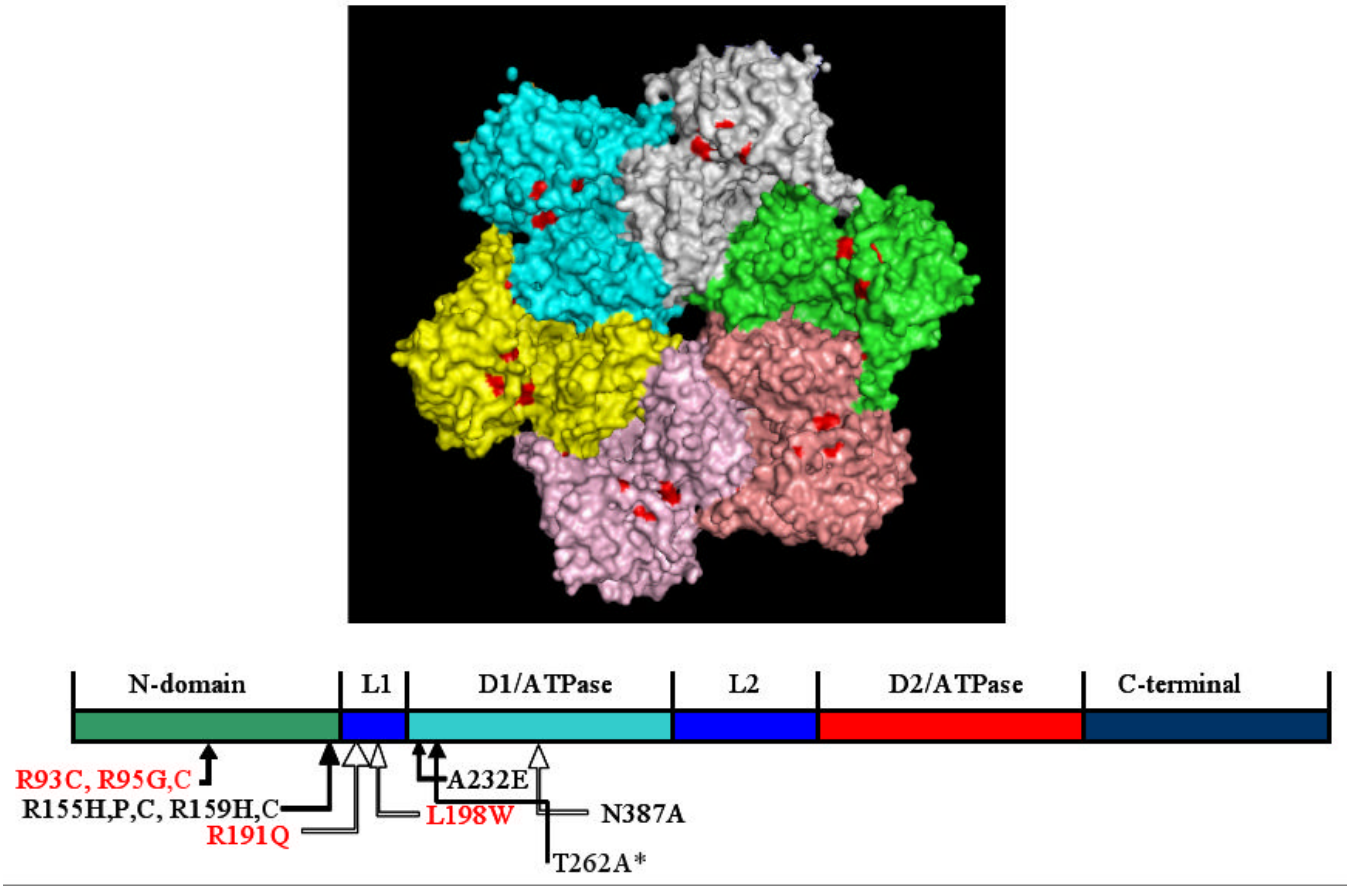

Figure 2.

A) Space fill diagram of a VCP hexamer (top) with each monomer colored individually and a linear diagram of a single VCP monomer showing the N-domain, L1 and L2 linkers, D1 and D2 ATPase domains and the C-terminal domain (bottom). Red space fill in hexamer represents residues that are mutated in IBMPFD. *Mutation T262A is unpublished (Spina S and Ghetti B, 2007 American Academy of Neurology Meeting).

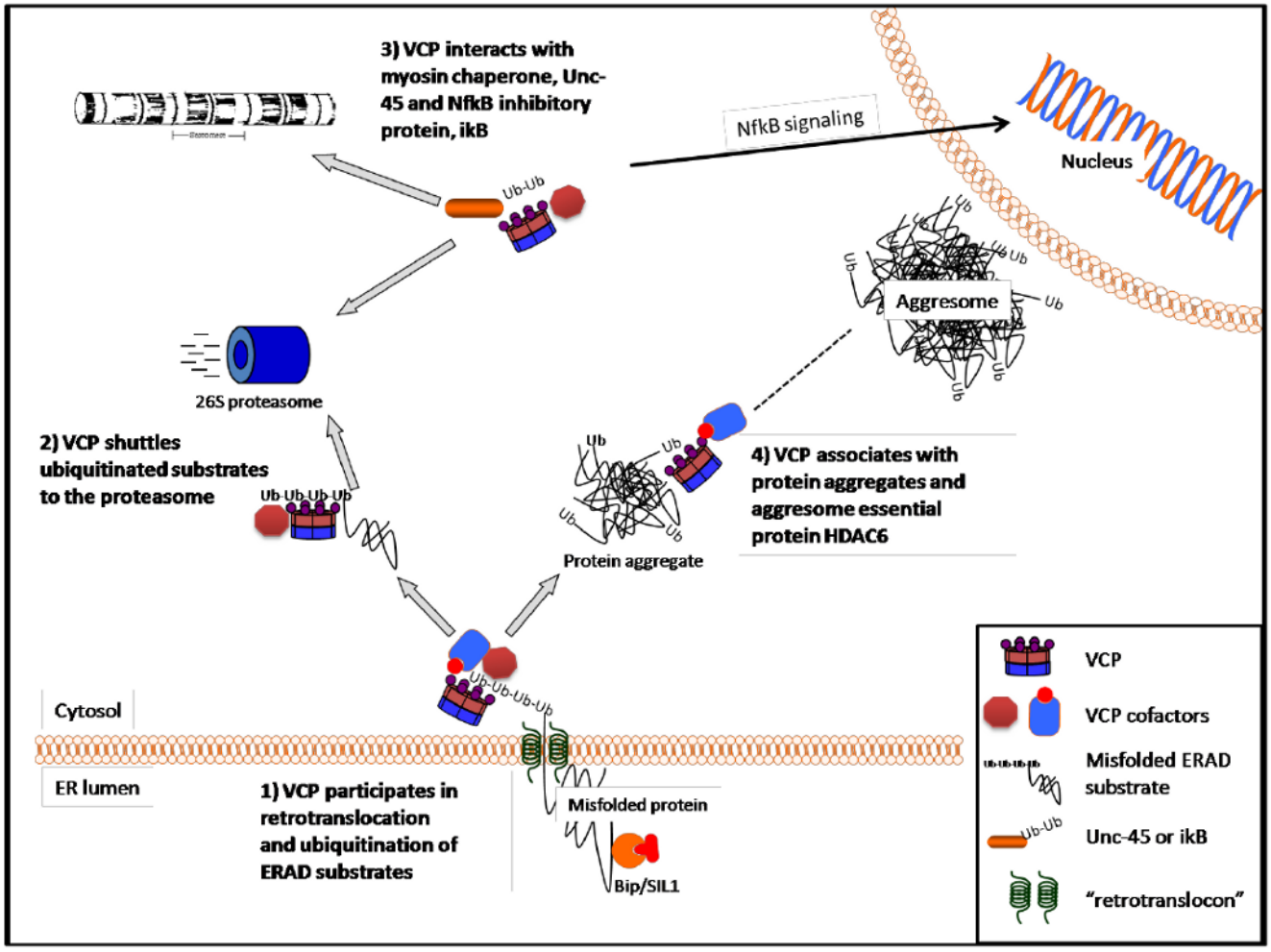

Figure 3.

Proposed functions of VCP. 1) VCP participates in the retrotranslocation and subsequent ubiquitination of ERAD substrates via binding to derlin-1 and one of several ER associated E3 ubiquitin ligases such as Hrd1 or gp78. 2) VCP shuttles ubiquitinated protein substrates to the 26S proteasome via interactions with rad23. 3) VCP mediates degradation of many short-lived signaling molecules such as Unc-45b and IκB. Unc-45 is responsible for myosin folding and myofibrillogenesis and IkB mediates NFkB signal transduction. 4) In the setting of UPS dysfunction or protein misfolding, VCP associates with small ubiquitinated protein aggregates and facilitates their sequestration into "aggresomes" via direct interactions with HDAC6. Aggresomes are then degraded via the autophagosome-lysosome system.

Table 1

Putative VCP substrates:	**Function**	**Citation**
ERAD		
? All ERAD substrates	Facilitates quality control through secretory pathway	[46,77,78]; and others
Dysferlin	Mutated in LGMD2B; sarcolemmal protein involved in membrane repair	[79]
HMG-CoA reductase	Rate controlling enzyme in cholesterol synthesis; target of statins	[80]
inositol 1,4,5-trisphosphate receptors	Facilitates intracellular Ca^{+2} release from the ER	[81]
Cystic fibrosis transmembrane receptor (CFTR)	Mutated in cystic fibrosis; plasma membrane localized Cl-channel	[46]
Polycystin-2	Mutated in autosomal dominant polycystic kidney disease; Ca^{2+}-permeable nonselective cation channel	[82]
α1-antitrypsin	Mutated in α1-antitrypsin deficiency; secreted serine protease inhibitor	[83]
Cytoplasmic		
? All N-end rule and ubiquitin fusion degradation pathways substrates	Facilitates degradation of multiple model substrates	[47]
ikB (inhibitor of kappa B)	Inhibitor of NFkB signaling pathway	[35]
Unc-45B	Skeletal muscle specific myosin chaperone (UCS domain protein)	[54]
cyclinE	Binds Cdk2, which regulates transition from G_1 to S phase	[48]
Hypoxia inducible factor-1α (Hif1α)	Hypoxia induced transcriptional regulator	[34]
Ataxin-3	Polyglutamine containing protein associated with machado-joseph disease; deubiquitinating enzyme	[39,84]
Nuclear		
Werner protein	Nucleolar helicase mutated in Werner syndrome (progeria)	[85]
Slow wallerian degeneration (Wld-S)	Mutant protein found associated with Wld-S mouse	[86]
Breast cancer associated protein 1 (BRCA1)	Mutated in some forms of breast cancer, involved in DNA repair	[87]
Aurora B kinase	Facilitates chromatin condensation and nuclear envelope fusion	[88]