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REVIEW

Valosin-containing protein and the pathogenesis of frontotemporal dementia associated with inclusion body myopathy

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Abstract Frontotemporal dementia with inclusion body myopathy and Paget's disease of bone (IBMPFD) is a rare, autosomal dominant disorder caused by mutations in the gene *valosin-containing protein* (*VCP*). The CNS pathology is characterized by a novel pattern of ubiquitin pathology distinct from sporadic and familial frontotemporal lobar degeneration with ubiquitin-positive inclusions without VCP mutations. Yet, the ubiquitin-positive inclusions in IBMPFD also stain for TAR DNA binding protein, a feature that links this rare disease with the pathology associated with the majority of sporadic FTD as well as disease resulting from different genetic alterations. VCP, a member of the AAA-ATPase gene family, associates with a plethora of protein adaptors to perform a variety of cellular processes including Golgi assembly/disassembly and regulation of the ubiquitin–proteasome system. However, the mechanism whereby mutations in VCP lead to CNS, muscle, and bone disease is largely unknown. In this report, we review current literature on IBMPFD, focusing on the pathology of the disease and the biology of VCP with respect to IBMPFD.

Keywords Frontotemporal dementia · Inclusion body myopathy · Neurodegenerative disease · Valosin-containing protein · TDP-43

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Introduction

Frontotemporal dementia (FTD) associated with inclusion body myopathy (IBM) and Paget's disease of bone, or IBMPFD, is a rare autosomal dominant disorder characterized by variable penetrance of this unusual triad of clinical features [1]. Myopathy is the most common clinical feature, present in 80–90% of affected individuals and is characterized by adult-onset (~44 years) proximal and distal muscle weakness clinically resembling limb girdle muscular dystrophy [1–3]. Paget's disease of bone, observed in 43–51% of IBMPFD is similar to the sporadic counterpart with a typical distribution of pathology in the spine, pelvis, and skull, and elevated alkaline phosphatase, but with an earlier age of onset (~42 years) [1–3]. The dementia associated with IBMPFD presents later than both the IBM and Paget's disease with a mean age of 54 years at onset and, consequently, is only present in 31–37% of affected individuals [1, 3]. The dementia associated with IBMPFD is typical of FTD [4, 5] and is characterized by language and/or behavioral dysfunction with relative preservation of memory [1, 2, 6]. Notably, many patients report visual and auditory hallucinations [1]. Single case reports have also reported the presence of a dilated cardiomyopathy [7] and hepatic fibrosis [8], although it is unclear if these disorders are part of the clinical spectrum of IBMPFD.

A genome-wide screen of four large families showed linkage of IBMPFD to a 1.08–6.46 cM critical interval on chromosome 9p13.3-12 [1]. Subsequently, using a candidate gene approach, six mutations were identified in the gene *valosin-containing protein* (*VCP*) in 13 families with IBMPFD (Fig. 1) [9]. Arginine 155 was by far the most common amino acid affected, present in 10 out of 13 families. To date, a total of nine *VCP* gene mutations have been reported in more than twenty kindred [6–12], with $^{R}155^{H}$ and $^{R}155^{C}$ remaining the most frequent mutations identified. *ApoE4* is a potential modifier gene of the phenotypic expression of FTD in IBMPFD [13], although the molecular basis for this link is unclear.

Biology of valosin-containing protein

VCP, also known as p97, and its homologs *TER94* (*Drosophila*), *CDC48p* (yeast), and *VCP-like ATPase* (bacteria), are member of the AAA-ATPase gene superfamily (ATPase Associated with diverse cellular Activities) [14, 15]. The expressed protein functions as a molecular chaperone in a plethora of distinct cellular processes including ubiquitin-dependent protein degradation, stress responses, programmed cell death, nuclear envelope reconstruction, and Golgi and endoplasmic reticulum (ER) assembly. Notably, many of these activities are directly or indirectly regulated by the ubiquitin proteasome system (UPS). To perform these activities, VCP functions as a homohexamer [16, 17], binding to multiple ancillary proteins associated with UPS function. Specifically, the VCP complex binds to polyubiquitin chains and untethers ubiquitinated proteins from their binding partners thereby facilitating transport to the UPS. This is supported by genetic and biochemical studies which implicate VCP in these protein degradation pathways. Mutations in the yeast homolog of *VCP*, *CDC48p*, or depletion of VCP from mammalian cells leads to the accumulation of polyubiquitinated proteins [18]. Similarly, treatment of cell lines with RNAi specific for VCP results in the accumulation of high molecular weight conjugates of ubiquitin, a finding that correlates with inhibition of UPS function [19].

VCP contains two ATPase domains (designated D1 and D2; Fig. 1), an amino-terminal domain (designated N) and a carboxy-terminal domain (designated C) as well as linkers L1 and L2 that join the N-D1 and D1–D2 domains, respectively [14, 15]. VCP functions as a homohexamer composed of dual stacked rings formed by the D1 and D2 ATPase modules [16, 20–22]. The D1 domain is primarily responsible for VCP hexamerization and this molecular assembly is not dependent on nucleotide binding [16, 22, 23]. The D2 domain confers the major ATPase activity to VCP [21, 22] that enables VCP to function as a molecular chaperone interacting with a diverse group of adaptors to perform specific cellular tasks. The N-domain, the least conserved module in the AAA-ATPase family, mediates the majority of VCP cofactor binding function, as well as substrate specificity including interactions with ubiquitinated target proteins [18, 24, 25]. Most IBMPFD-associated mutations cluster in the N-domain [9]. The $^{A}232^{E}$ *VCP* mutation is an exception. Located in the amino-terminus of the D1-domain, this mutation may be associated with a more severe clinical course [9]. The C domain is poorly characterized, but this region includes the major tyrosine phosphorylation site implicated in the regulation of ER assembly [26, 27].

A wide array of proteins of seemingly unrelated function interacts with VCP primarily through the N domain, including the well-characterized adaptors p47 and the Ufd1–Npl4 complex [28]. VCP interacts with p47 through a UBX domain and is required for proper post-mitotic assembly of Golgi stacks as well as fusion of the ER membrane [29, 30].

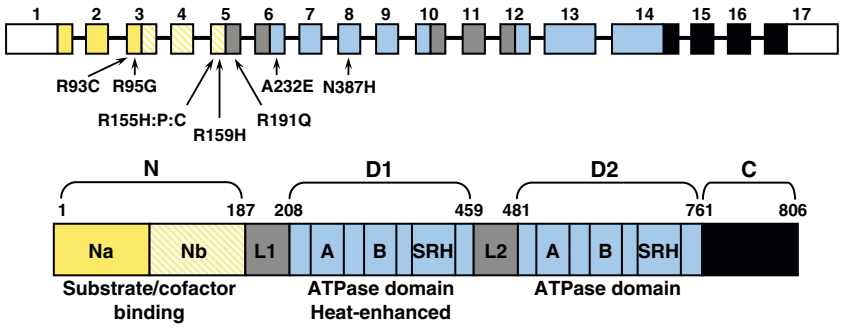

Fig. 1 Schematic of VCP gene (*top*) and protein (*bottom*) structure. Exons are numbered 1–17. Positions of mutations in *VCP* gene are indicated. VCP consists of 2 ATPase domains (D1 and D2) and an N-terminal domain (N) that provides substrate specificity (Na and Nb indicate sub-domains of the 'N' domain). These domains are separated by flexible linkers (L1 and L2). Functional domains are color-coded in both the gene and protein structure. Both D1 and D2 are required for activity of the VCP hexamer. The N and D1 domains are both required for binding of ubiquitin and p47. A, B, and SRH indicate the Walker A, Walker B, and 2nd region of homology motifs in the ATPase domains

The highly homologous adaptor protein p37 also binds VCP through a UBX domain, but instead functions in Golgi and ER biogenesis during interphase and at the end of mitosis [31]. In addition to its role in managing Golgi and ER fusion activities, p47 has also been implicated in VCP-dependent protein degradation through the UPS, possibly by aiding in the delivery of substrates to the proteasome [32, 33]. VCP is essential for ER-associated degradation (ERAD), a pathway in which defective or abnormally folded and short-lived ER proteins are degraded [34]. This participation of VCP in ERAD is dependent on its binding partners Ufd1 and Npl4 [35–38]. Specifically, substrates destined for proteasomal degradation are retrotranslocated from the ER lumen to the cytosolic face of the ER where they are bound by the VCP/Ufd1–Npl4 complex [25, 36, 38, 39]. The ERAD substrates are subsequently ubiquitinated and transferred to the proteasome for degradation [40, 41]. Current research efforts are directed towards determining whether mutations associated with IBMPFD affect these or other related biological processes.

The relationship of IBMPFD to FTLD-U

Multiple neuropathological abnormalities are associated with the clinical syndrome of FTD that are broadly grouped into two categories, tauopathies, defined by the presence of tau pathological inclusions and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) [4, 5]. FTLD-U is the most common pathology underlying the clinical syndrome of FTD [42, 43] and is characterized by ubiquitin-positive neuronal cytoplasmic and nuclear inclusions as well as dystrophic neurites that are not detected with antibodies recognizing other cellular proteins including tau, α-synuclein, β-amyloid, neuronal intermediate filaments, and expanded polyglutamine tracts [44–47]. Moreover, distinct patterns of ubiquitin pathology have been described in FTLD-U that may correlate with mutations in different genes [48–50].

Until recently, there was only limited information on the brain pathology associated with IBMPFD. The initial report of the neuropathology associated with IBMPFD described non-specific degenerative changes [1]. More specific pathological features were described in a recent case report of a 55-year-old German patient with IBM and FTD and a heterozygous $^{R}155^{C}$ missense mutation in the *VCP* gene [12]. Postmortem examination of this patient revealed prominent frontotemporal and striatal atrophy associated with neuronal intranuclear inclusions that were immunostained with antibodies to both ubiquitin and VCP. Biochemically, no alteration in the expression of VCP was identified. We recently performed a detailed, systematic analysis of the neuropathologic changes in eight patients from five families with three different *VCP* gene mutations [6]. Of these eight patients, six had clinical FTD or an uncharacterized dementia. A novel pattern of ubiquitin pathology was identified in IBMPFD that was distinct from sporadic and familial FTLD-U without *VCP* gene mutations. IBMPFD neuropathology was characterized by abundant ubiquitin-positive neuronal intranuclear inclusions and dystrophic neurites; only rare intracytoplasmic inclusions were identified (Fig. 2a). Both the neuronal nuclear inclusions and dystrophic neurites were most numerous in the upper cortical layers, but the pathology was also present in neurons through the entire cortical thickness. A subset of the inclusions was also detected with antibodies to SUMO-1 and PML (promyelocytic leukemia) protein [51]. In addition, scattered glial intranuclear inclusions were also noted within white matter. In contrast to Schröder et al. only rare inclusions were detected with antibodies to VCP and there was no biochemical alteration in the VCP protein [6]. The ubiquitin pathology was abundant in the neocortex, less

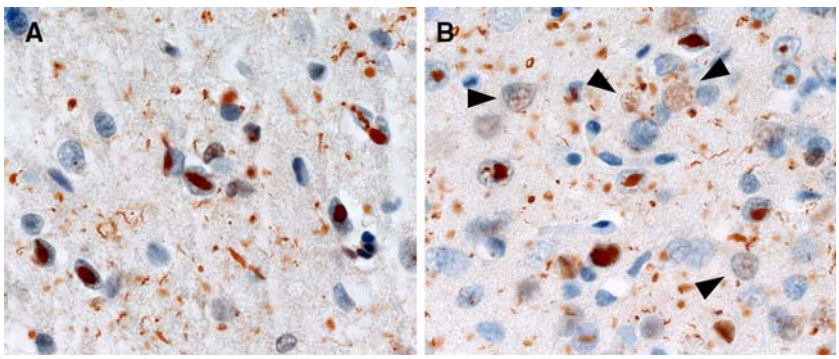

Fig 2 Ubiquitin- and TAR DNA binding protein 43 (TDP-43)-positive pathology in inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD). Immunohistochemistry with anti-ubiquitin (**a**) and TDP-43 (**b**) reveal robust staining of intranuclear inclusions and dystrophic neurites in affected cortical regions in IBMPFD. The intranuclear inclusions had round, lentiform, or rod shapes that were either straight or slightly curved. Note the reduced nuclear TDP-43 staining compared to physiological nuclear TDP-43 immunoreactivity in adjacent non-affected neurons (*arrowheads* in **b**)

robust in limbic and subcortical nuclei, and conspicuously absent in the dentate gyrus, a brain region commonly affected in both sporadic and familial FTLD-U. Of note, the ubiquitin pathology was present in all six IBMPFD patients with dementia, as well as one of the two subjects who was cognitively intact at the time of his last clinical examination. In this non-demented individual, the ubiquitin pathology had the same morphology and distribution as that seen in patients with FTD, but was less robust, suggesting a preclinical stage of disease. A similar pattern of FTLD-U pathology was also reported in a large Belgian family with a $^{R}159^{H}$ *VCP* gene mutation [10, 52]. Three-dimensional confocal reconstructions of the intranuclear inclusions demonstrated a flattened, leaf-like morphology that ultrastructurally were composed of straight 10–18 nm filaments [10]. However, neuropathological analysis of three patients from large kindred with the $^{R}155^{C}$ *VCP* mutation did not detect ubiquitin- or VCP-positive inclusions [8]. Interestingly, these authors noted prominent perinuclear cytoplasmic vacuoles that were detected with antibodies to MG160, a normal constituent of the Golgi apparatus. As discussed above, VCP plays a key role in Golgi assembly/disassembly, suggesting that Golgi dysfunction may be a potential mechanism for the pathogenesis of VCP-mediated neurodegeneration.

TAR DNA binding protein 43 (TDP-43) was recently identified as a major disease protein in the ubiquitinated inclusions characteristic of both sporadic and familial FTLD-U as well as sporadic amyotrophic lateral sclerosis [53]. Pathological TDP-43 was hyperphosphorylated, ubiquitinated, and cleaved to generate C-terminal fragments and was recovered only from affected central nervous system regions, including hippocampus, neocortex, and spinal cord. To determine if the ubiquitin pathology associated with mutations in VCP is characterized by the accumulation of TDP-43 we analyzed TDP-43 in the CNS pathology of five patients with *VCP* gene mutations [54]. Immunostaining for TDP-43 revealed robust pathology consisting of intranuclear inclusions and dystrophic neurites in affected brains regions similar to that detected with immunostaining for ubiquitin (Fig. 2b). Furthermore, accumulations of TDP-43 co-localized with the ubiquitin pathology in IBMPFD including both the intranuclear inclusions and dystrophic neurites. In neurons and glia without ubiquitin pathology, both in unaffected and affected brains regions, physiologic TDP-43 was robustly detected in the nuclei of neurons and glia (Fig. 2b). In contrast, there was a dramatic reduction in the labeling intensity of nuclear TDP-43 in affected cortex. A biochemical analysis of IBMPFD brain tissue revealed insoluble phosphorylated TDP-43 only in extracts from affected brain regions similar to sporadic FTLD-U. The identification of TDP-43, but not VCP, within ubiquitin-positive inclusions supports the hypothesis that *VCP* gene mutations lead to a dominant negative loss or alteration of VCP function culminating in impaired metabolism of TDP-43.

The myopathy associated with IBMPFD is characterized by adult-onset proximal and distal muscle weakness, clinically resembling limb girdle myopathy [1, 2]; yet, muscle biopsies from affected individuals demonstrate highly variable pathology. Most muscle biopsies show non-specific myopathic changes including variation in muscle fiber size, mildly increased endomysial connective tissue, and focal regions with "myopathic grouping" [1, 2, 7, 8]. Rimmed vacuoles similar to that observed in sporadic IBM have been reported in a subset of patients [1, 2, 7–9]. Ultrastructural analysis of the rimmed vacuoles reveals them to be autophagic vacuoles containing filamentous material [7]. Immunohistochemical analysis reveals small and large cytoplasmic aggregates of VCP in scattered muscle fibers including those without rimmed vacuoles or other morphological changes, a pattern of immunostaining not seen in sporadic IBM [7–9]. Furthermore, in one muscle biopsy, subsarcolemmal and cytoplasmic areas were observed with increased labeling for αB-crystallin and desmin [7]. In this biopsy, intranuclear inclusions were detected in myocytes with antibodies to VCP that ultrastructurally were composed of filamentous material [7]. Multiple fibers also displayed foci with granulofilamentous material similar to the myofibrillar myopathies. Nonetheless, additional work is clearly necessary to define the myopathy associated with *VCP* gene mutations. Moreover, it has yet to be determined whether TDP-43 metabolism is altered in affected muscle in IBMPFD similar to that observed in the brain.

VCP and the pathogenesis of neurodegenerative disease

Prior to recognition that mutations in VCP were causative of IBMPFD, VCP was implicated as playing a role in neurodegenerative disease. VCP has been detected in a small proportion of the disease-defining inclusions of a broad array of neurodegenerative diseases, including senile plaques in Alzheimer's disease, Lewy bodies in Parkinson's disease, neuronal intranuclear inclusions in CAG/polyglutamine diseases and ubiquitin-positive inclusions in amyotrophic lateral sclerosis [55–57]. A more direct role for VCP in the pathogenesis of neurodegenerative disease is derived from studies in *Drosophila melanogaster* and *Caenorhabditis elegans* [58, 59]. An unbiased genetic screen in *Drosophila* identified null mutations in *TER94* as a suppressor of polyglutamine induced toxicity [59]. Seemingly in contrast with these results, over-expression of human *VCP* in the fly suppressed neurotoxicity associated with polyglutamine-expanded ataxin-3 [60]. In *C.elegans*, overexpression of *C41C4.8* and *C06A1.1*, worm homologs

of human *VCP*, suppresses the formation of aggregates resulting from expanded polyglutamine repeats, but the impact on neurodegeneration was not reported [58].

The mechanism whereby mutations in VCP result in IBMPFD, as well as the basis of selective vulnerability of brain, muscle, and bone, remains unknown. The autosomal dominant pattern of inheritance in IBMPFD suggests a dominant mechanism—either toxic gain of function or dominant negative activity. The possibility of a dominant negative mechanism of pathogenesis is particularly intriguing since VCP functions as a hexameric complex and at least some mutant forms of VCP retain the ability to form hexamers [61]. Recent work has started to address the mechanism of pathogenesis of IBMPFD, although the results are far from conclusive. The most common mutation in VCP, $^{R}155^{H}$, does not affect ATPase activity [61]. However, the presence of ubiquitinated inclusions in the brains of IBMPFD patients and the role of VCP in UPS function suggest that these mutations may somehow disrupt normal protein degradation pathways leading to disease [6, 10, 12]. Because the majority of mutations in VCP associated with IBMPFD are localized to the substrate-binding domain (Fig. 1), one possible mechanism of disease may be the disruption of binding between VCP and protein adaptors essential for regulating UPS activity. However, no differences were observed in the binding of wild type verses mutant VCP with the adaptors Ufd1, Npl4, p47, and ataxin-3 ([7] and Guinto JB, Taylor JP, Forman MS, unpublished observations). Alternatively, impairment of ERAD has been implicated in IBMPFD pathogenesis. Specifically, the expression of mutant VCP in a myoblastic cell line was associated with increased immunodetection of ubiquitin-conjugated proteins [61]. In addition, in a subset of transfected cells, IBMPFD mutations were associated with the formation of aggregates that contain VCP, ubiquitin, and ER resident proteins, and elevated steady state levels of exogenously expressed mutant CFTR (cystic fibrosis transmembrane regulator), a protein degraded by ERAD. However, another study did not confirm the cell culture findings described above. In this study, analysis of primary human myoblasts from control and IBMPFD subjects did not reveal a similar increase in staining for ubiquitin or ubiquitinated protein aggregates [7]. Alternatively, a complex of VCP, UFD-2, and CHN-1 was recently implicated in myosin assembly and myofibril organization by regulating the expression of UNC-45, a molecular chaperone implicated in myosin assembly [62]. The expression of IMBPFD-associated VCP mutations in skeletal muscle cells reduced UNC-45 degradation that correlated with severe myofibril disorganization in myotubules. This study suggests a possible mechanism for the selective vulnerability of skeletal muscle in IBMPFD; however, the implication for the pathogenesis of FTD is unclear. Recently, a transgenic mouse model of the muscle pathology associated with IBMPFD was reported [63]. Transgenic mice expressing the $^{R}155^{H}$, but not wild type VCP developed age-dependent muscle weakness that was associated with abnormal muscle architecture and autophagocytic vacuoles, both features of IBM. Ubiquitin-positive inclusions and high molecular weight protein aggregates were also observed prior to the onset of clinical weakness. One limitation of this study was that the expression of mutant VCP was significantly greater than that of wild-type protein. Furthermore, there was no discussion of whether VCP or ERAD substrates were present in this ubiquitinated protein aggregates. Additional model systems will need to be developed to resolve these discrepancies.

Summary

Clearly, elucidating the mechanism of VCP-mediated IBMPFD requires further investigation. While disruption of ERAD and/or UPS function is an attractive hypothesis, mutations in VCP may affect other cellular activities regulated by VCP such as Golgi, ER or myofibril assembly. Alternatively, mutations in VCP may lead to a novel, toxic gain of function that is not otherwise predicted based on its known functions. Future studies should include the development of model systems that recapitulate all aspects of the pathology observed in IBMPFD. An understanding of the pathogenesis of FTLD-U in general will have to integrate the biology of multiple distinct genetic elements in addition to VCP. For example, FTD-causing mutations in *progranulin* are also characterized by ubiquitin- and TDP-43-positive inclusions [52]. Thus, TDP-43 appears to be a pathologic substrate linking a variety of distinct patterns of FTLD-U pathology caused by different genetic alterations. Finally, IBMPFD is a multisystem disease affecting the brain, muscle, and bone. Understanding the selective vulnerability of these tissues as well as the variability of penetrance of these phenotypes will ultimately lead to a better understanding of the IBMPFD as well as the sporadic counterparts including FTD, IBM, and Paget's disease of bone.

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