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CHAPTER 15

Clinical spectrum of VCP myopathy, Paget disease, and frontotemporal dementia: experimental models and potential treatments

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Introduction

Clinical features of VCP hereditary inclusion-body myopathy

Hereditary inclusion-body myopathy (h-IBM) is a heterogeneous group of disorders associated with rimmed vacuoles and cytoplasmic and intranuclear inclusions of 15–21-nm filaments [1]. An autosomal recessive quadriceps-sparing form of the disorder with onset in early adulthood prevalent among the Iranian Jewish population is associated with mutations in the UDP-N-acetylglucosamine-2 epimerase/N-acetylmannosamine kinase (*GNE*) gene [2, 3]. Nonaka inclusion-body myopathy is an allelic disorder with a similar phenotype [4].

Inclusion-body myopathy associated with Paget disease of the bone and frontotemporal dementia (IBMPFD; OMIM 167320), first reported in 2000, is an autosomal dominant, progressive, and ultimately lethal condition with onset typically in the 20s to 30s. Physical exam reveals muscle weakness and atrophy of the pelvic and shoulder girdle, marked scapular winging, and difficulty walking up stairs [5–7]. Muscle disease typically progresses to involve other limb and respiratory groups; ultimately, individuals die in their 50s to 60s from progressive muscle weakness, and cardiac and respiratory failure [5, 8]. Electromyography shows both myopathic and neurogenic changes suggestive of myopathy, and serum creatine kinase concentration is usually normal to mildly elevated (range, 40–1145 U/L; normal range, 20–222 U/L).

Histologically, patients show the presence of rimmed vacuoles and inclusion bodies in the muscle fibers (see Plate 15.11). Electron micrographs of affected skeletal muscle demonstrate prominent 15–21-nm tubulofilamentous inclusions within myonuclei. Weihl et al. [9] identified large TAR

Muscle Aging, Inclusion-Body Myositis and Myopathies, First Edition. Edited by Valerie Askanas and W. King Engel.

DNA-binding protein 43 (TDP-43)-positive ubiquitinated inclusions in muscle cytoplasm in IBMPFD patients, thus adding h-IBMs to the growing list of TDP-43-positive inclusion diseases. Kimonis et al. [5] reported cardiomyopathy in three out of 11 individuals in the original family. Hubbers et al. [10] reported that mutant valosin-containing protein (*VCP*) leads to a novel form of dilatative cardiomyopathy with inclusion bodies.

Paget disease of the bone in IBMPFD

Paget disease of the bone (PDB) is a common condition characterized by increased and disorganized bone turnover, which can affect one or several skeletal regions (Figure 15.1). These abnormalities disrupt normal bone architecture and lead to various clinical complications such as bone pain, osteoarthritis, pathological fracture, and bone deformity. Genetic mutations play an important role in PDB by disrupting normal signaling in bone remodeling. The nuclear factor κB (NFκB) signaling pathway is one such pathway identified as being important in PDB. To date there are four gene mutations or polymorphisms in the NFκB signaling pathway associated with increased risk of PDB. These include TNFRSF11A, which encodes receptor activator of NFκB ligand (RANK), TNFRSF11B, which encodes osteoprotegerin, VCP, and SQSTM1 [11, 12], the latter of which encodes the signaling adaptor p62, a multidomain protein implicated in the activation of the transcription factor NFκB [13–16]. Recently variants in optineurin (OPTN) was found to be a risk factor for Paget's disease in a genome-wide association study [15]. Interestingly OPTN mutations have also been found in patients with amyotropic lateral sclerosis [16]. Thus these mutations are likely to predispose to PDB by disrupting normal NFκB signaling. NFκB plays a critical role in cell survival, in addition to regulating bone turnover.

Early-onset PDB is seen in 49% of IBMPFD patients [5, 7], and typically begins in the 30s to 40s, the mean age of onset being 42 years. The diagnosis of PDB is based on serum alkaline phosphatase (ALP) concentration, urine concentrations of pyridinoline (PYD) and deoxypyridinoline (DPD), and radionuclide scans or skeletal radiographs. Zoledronic acid is a potent bisphosphonate that has recently been licensed for the treatment of established PDB. A single injection results in sustained biochemical remission in over 95% of subjects for up to 2 years [17]. It is therefore feasible and appropriate to identify these patients, since they represent a high-risk group who might gain benefit from early therapy.

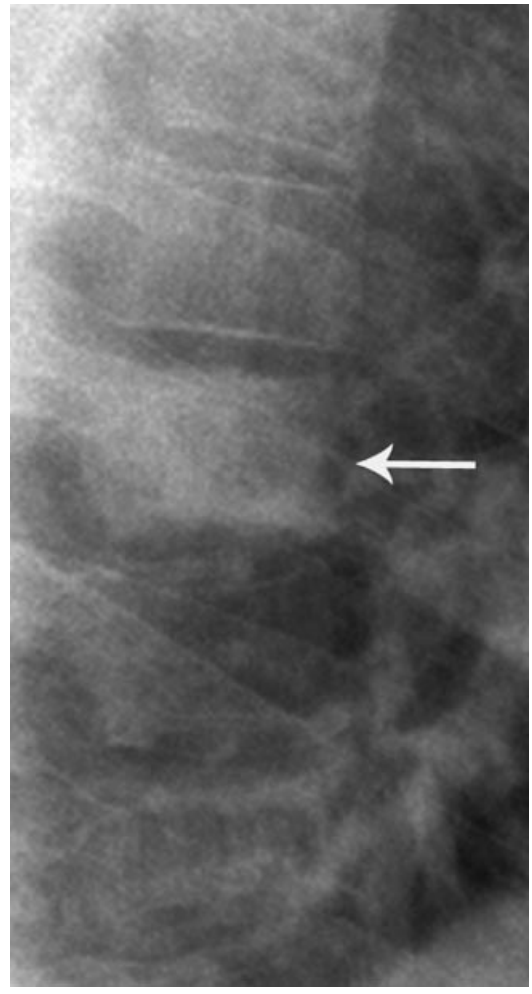

Figure 15.1 Lateral spine X-ray of a 43-year-old man with Paget disease and myopathy shows sclerotic changes of the vertebral body at the level of T7 thoracic vertebral body.

Frontotemporal dementia in IBMPFD

Frontotemporal dementia (FTD) is a clinicopathological entity comprising about 3% of all dementias

of the elderly [18–20]. Symptoms typically involve personality or mood changes such as depression and withdrawal, and language difficulties. Patients may become disinhibited or exhibit antisocial behavior. Some individuals with asymmetric involvement of the left hemisphere may develop extraordinary visual or musical creativity while experiencing language impairment. In contrast to Alzheimer disease patients who typically develop early symptoms of episodic memory loss, FTD patients exhibit altered behavior or loss of speech or language as initial manifestations. Episodic memory in FTD is relatively preserved. In later stages of FTD, patients may develop Parkinsonism or amyotrophic lateral sclerosis (ALS)-like features.

In the disinhibition-dementia-Parkinsonism-amyotrophy complex, mapping to chromosome 17q21–q22, mutations disrupt the *tau* (microtubule-associated protein tau; *MAPT*) gene. The majority of FTD families, however, have no demonstrable *tau* mutations [21–24]. Recently FTD has been associated with mutations in progranulin [25], which maps very close to the *MAPT* gene on chromosome 17, accounting for early confusion in the designation of FTD-17 families. Progranulin mutations are associated pathologically with ubiquinated neuronal cytoplasmic inclusions positive for TDP-43. In contrast, FTD associated with mutations in the *CHMP2B* gene have ubiquitin-positive but TDP-43-negative inclusions.

In patients with IBMPFD, onset of dementia in affected individuals occurred on average at 54 years (range 39–62 years) with an overall frequency of 33% [26]. The diagnosis of FTD is based on comprehensive neuropsychological assessments that reveal behavioral alteration (e.g. personal/social unawareness, or disinhibition), early expressive language dysfunction or semantic loss, and preservation of memory, orientation, and ideomotor praxis [27].

We performed a systematic analysis of the brain neuropathologic changes in eight patients with *VCP* mutations and identified ubiquitin-positive neuronal intranuclear inclusions and dystrophic neurites [28] (see Plate 15.12), making VCP disease another example of familial frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Neumann et al. [29] found that a hyperphosphorylated, ubiquitinated, and cleaved form of TDP-43, known as pathologic TDP-43, is the major disease protein in ubiquitin-positive tau- and α-synuclein-negative FTD (FTLD-U), and in ALS. Accumulations of TDP-43 colocalized with ubiquitin pathology in eight of our patient IBMPFD brains, including both intranuclear inclusions and dystrophic neurites [30]. FTD associated with VCP is now classified under the rubrick of FTLD-U along with disorders such as ALS [31, 32]. Thus our work on the FTD associated with IBMPFD has lent new insights into the common pathogenesis of a spectrum of ubiquitin-related disorders that include FTD alone (progranulin-associated), FTD plus muscle and bone disease (IBMPFD), familial FTD with ALS, and motor-system degeneration without FTD (ALS).

Because of the variable phenotype in inclusion-body myopathy, PDB and FTD modifier genes were evaluated. From a database of 231 members of 15 families, 174 had an apolipoprotein E (*APOE*) genotype available for regression analysis. Analysis of the data suggested a potential link between the *APOE* 4 genotype and the FTD found in IBMPFD. In contrast we observed no association between FTD and the *MAPT* H2 haplotype [33].

Molecular studies of IBMPFD

A genome scan, performed at the Marshfield mammalian genotyping center, revealed linkage to chromosome 9p13.3–p12 in the original family reported [5] and three other families [6]. IBMPFD was subsequently attributed to being caused by mutations in the gene encoding VCP by Watts et al. [26], who identified six missense mutations in *VCP* in 13 families. VCP is highly conserved in evolution, belonging to the family of AAA proteins (ATPases associated with a variety of cellular activities) and has two ATPase domains (D1 and D2) [34–38] and two linker domains (L1 and L2), as well as the N-terminal- and C-terminal domains (Figure 15.2). VCP forms homohexamers and binds to multiple cofactors at both its N-terminal and C-terminal domains. Through binding cofactor molecules, VCP can adapt its function to suit many homeostatic processes important for the cell's life cycle. It has

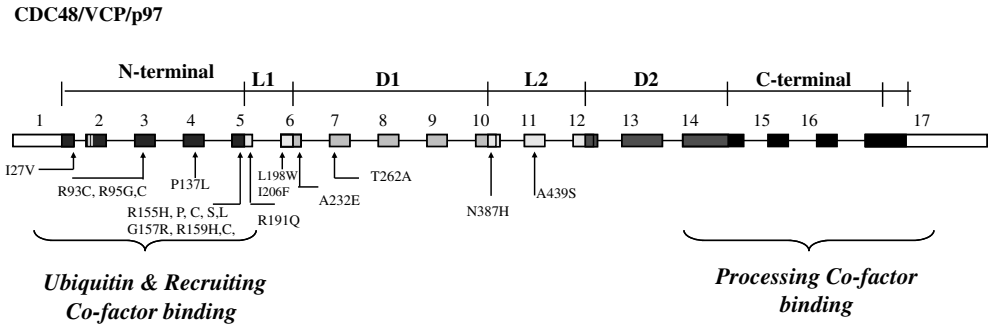

Figure 15.2 Functional domains and disease mutations in VCP. The domains of VCP include the ubiquitin-binding N-terminal domain (CDC48), flexible linker (L1), first AAA ATPase domain (D1), linker region (L2), second AAA ATPase domain (D2), and the C-terminal domains. There are 17 exons and arrows indicate the locations of all 20 mutations. The majority of mutations occur in the ubiquitin-binding N terminal domain.

been reported to be involved in several cellular activities including endoplasmic reticulum (ER)-associated degradation (ERAD) of proteins, homotypic membrane fusion, transcription activation, nuclear envelope reconstruction, postmitotic organelle reassembly, cell-cycle control, and apoptosis [39–41].

BMPFD is increasingly recognized as a distinct disorder although it is still underdiagnosed because of its variable phenotype, which leads to misdiagnoses. Kimonis et al. [8] reviewed data on 49 affected individuals in nine IBMPFD families and identified myopathy among 42 (87%) individuals, diagnoses including limb girdle muscular dystrophy (LGMD), facioscapular humeral muscular dystrophy, scapuloperoneal muscular dystrophy, and ALS, among others. Kimonis and Watts [42, 43] have reviewed clinical results in IBMPFD and summarized findings in 20 families harboring 10 missense mutations [44].

As a result of studies in patients from our North American families with *VCP* mutations [26], families are now being reported from several parts of the world with unique phenotypes: Germany [45, 46], France [47], Austria [48], Italy [49, 50], the UK [51], and other families from the USA [52] and by our group [53]. As a result of increased awareness of VCP disease, and hence accurate reporting of VCP disease, the phenotypic range associated with *VCP* mutations has significantly expanded. Dilated cardiomyopathy, cataracts, sphincter disturbance, hepatic fibrosis, and features of ALS and Parkinson disease are now a part of the spectrum of IBMPFD manifestations.

At the present time 20 disease mutations have been reported (Figure 15.2, Table 15.1) with many more mutations expected to be identified as recognition of this disorder increases. The majority of the mutations have been found to cluster in the N-terminus of VCP which encompasses a domain that can bind ubiquitin and other substrate-recruiting proteins [54, 55]. In particular, we have identified a mutation hotspot at amino acid residue 155 (R155H/P/C/S/L). Additionally, most of the mutated residues causing IBMPFD are adjacent and potentially interact with each other, suggesting that these residues may have a similar and specific function within the VCP homohexamer [53].

We reviewed clinical features of families with VCP disease in order to perform a genotype/phenotype analysis. Because of the enormous intrafamilial variation, genotype/phenotype analysis was difficult between families. Notable associations, however, included a more severe and early-onset myopathy and dementia in family 6 that had the A232E mutation. Families with the R159C mutation did not develop PDB [57]. Although, none of the mutations had a significant effect on the age of onset for FTD (which was relatively consistent between families with VCP mutations), there was an increase in the incidence of FTD among females.

Table 15.1 List of VCP disease mutations

	Amino acid	c. DNA Base change	Exon	Domain	Number of families	References
1	I27V	79A → G	2	N-terminus	1	[80]
2	R93C	277C → T	3	N-terminus	4	[10, 47, 81]
3	R95G	283C → G	3	N-terminus	2	[26]
4	R95C	283C → T	3	N-terminus	1	[44]
5	P137L	410C → T	4	N-terminus	1	[82]
6	R155C	463C → T	5	N-terminus	5	[12, 26, 45, 47, 83]
7	R155H	464G → A	5	N-terminus	8	[10, 26]
8	R155P	464G → C	5	N-terminus	1	[26]
9	R155S	463C → A	5	N-terminus	1	[69]
10	R155L	N/A	5	N-terminus	1	[84]
11	G157R	469 G → C	5	N-terminus	1	[46]
12	R159H	476G → A	5	N-terminus	2	[48]
13	R159C	476G → A	5	N-terminus	2	[49, 52]
14	R191Q	572G → A	5	Linker 1	1	[26, 52]
15	L198W	593T → G	6	Linker 1	1	[53, 84]
16	I206F	828A → T	6	Linker 1	1	[82]
17	A232E	695C → A	6	Junction (L1–D1)	1	[26]
18	T262A	N/A	7	AAA D1	1	[52]
19	N387H	1159A → C	10	AAA D1	1	[53]
20	A439S	N/A	11	Linker 2	1	[85]

VCP is at the intersection of the ubiquitin-proteasome system and autophagy

The ubiquitin-proteasome system (UPS) is the major extralysosomal pathway responsible for degradation of both structural and regulatory proteins during muscle remodeling in eukaryotes. The UPS comprises a ubiquitin-conjugating system and the 26S proteasome. The ubiquitin-proteasome protein degradation system (UPD) has been shown to involve VCP via its cooperation with a binary Ufd1/Npl4 cofactor, enabling VCP targeting of specific substrates for degradation [54, 56–58]. Protein degradation mediated by the UPS is essential for the elimination of misfolded proteins from the ER in response to ER stress. It has been reported that the AAA ATPase p97/VCP/CDC48 dislocates proteins across the ER membrane allowing subsequent ubiquitin-dependent degradation by the 26S proteasome in the cytosol. Degradation of a prototypical misfolded ERAD substrate, ΔF508 CFTR, is slowed in IBMPFD mutant-expressing cells. Consistent with this, the undegraded ΔF508CFTR colocalized with IBMPFD mutant p97/VCP in ubiquitinated inclusions. [59]. Hubbers et al. [10] found that transient and stable expression of IBMPFD mutants p97/VCP R93C, R155C, and R155H in HEK293 and C2F3 myoblasts did not result in an increase in ubiquitinated proteins. Genetic studies in *Caenorhabditis elegans* revealed that IBMPFD mutations selectively impair the proteasomal degradation of the myosin chaperone, Unc-45, lending support for the dysregulation of the UPS [60–62].

Alterations in UPS function have been implicated in the pathogenesis of a variety of sporadic and familial neurodegenerative diseases including Parkinson disease, Alzheimer disease, polyglutamine repeat diseases, and ALS [63–65]. Mizuno et al. [66] called VCP "vacuole-creating protein" and demonstrated that VCP was observed in ubiquitin-positive intraneuronal inclusions in both motor neuron disease with dementia, and ballooned neurons in Creutzfeldt–Jakob disease. In Alzheimer disease, VCP has been found in dystrophic neurites while

granules of granulovacuolar degeneration and neurofibrillary tangles were not positively stained for VCP. In Parkinson's disease, Lewy and Marinesco bodies and Lewy neurites have been found to stain positive for VCP as well. These results indicate that VCP reacts with abnormal or misfolded proteins and plays a role in accelerating the process of degeneration and cell death.

A gain-of-function concept explains much of the phenotype seen in this disease as indicated by the work by other researchers [67, 68]. The work of our laboratory and that of other researchers suggest that *VCP*-mutation-induced neurodegeneration is mediated by several mechanisms including ERAD ubiquitin-proteasome and autophagy pathways. IBMPFD thereby joins familial forms of Alzheimer disease, Parkinson disease, Marinesco–Sjögren syndrome, and other neurodegenerative diseases in which intracellular protein accumulation results from perturbation of ER chaperone function.

Autophagy is a process that degrades long-lived proteins and cytoplasmic components within autophagosomes. Proteins and cytoplasmic components destined for degradation are sequestered and enveloped into vesicles that later mature through a series of steps including membrane fusion with lysosomes. Upon activation of autophagy, the 18 kDa LC3 (LC3-I) protein undergoes proteolytic cleavage followed by lipid modification converting the 18 kDa form into the 16 kDa membrane-bound form (LC3-II). LC3-II is specifically localized to the autophagosomal membranes whereas LC3-I is primarily cytosolic. The conversion from LC3-I to LC3-II is used as a marker for autophagic processing in mammalian cells. A buildup of either molecule suggests a disruption in the normal maturation of autophagosomes. Western-blotting analysis has demonstrated that protein lysates extracted from mutant cells have significantly increased amounts of LC3-II when compared to wild-type cell lines [69]. Related research [69] found accumulation of enlarged vacuoles in myoblasts from patients with VCP-associated inclusion-body myopathy. These findings suggest an impairment of autophagosome maturation and hence accumulation of autophagosomes at an immature state which are seen as vacuoles. Further analysis of the enlarged vacuoles via immunological staining revealed positivity for LAMP-1 and LAMP-2 antibodies. LAMP proteins are lysosomal-associated membrane proteins suggesting that vacuoles are able to fuse with the endosomal or lysosomal compartments (Figure 15.3). Lysosomal membrane proteins LAMP-1 and LAMP-2, however, showed increased molecular weights in patients' myoblasts due to differential *N*-glycosylation [69].

Ju et al. [70, 71] also identified impaired autophagy in cells transfected with *VCP* mutations, and in an overexpressing transgenic mouse model, by demonstrating increased ubiquitinated p62/sequestosome, a marker for autophagy. Sequestosome is a multimeric protein complex that serves as a depot for proteins destined for degradation. p62 has an LIR domain (LC3-interacting region) that recognizes and binds LC3, thereby initiating the first steps in autophagy. It is already known that mutations in the p62/sequestosome is a cause of PDB, seen in approximately 50% of familial and 30% of simplex cases of Paget disease. Similarly, p62 is found to be associated with a number of other diseases associated with cytoplasmic inclusion bodies. In particular, p62 has been identified in neuronal and glial inclusions associated with FTD [72] and mutations have been identified in ALS.

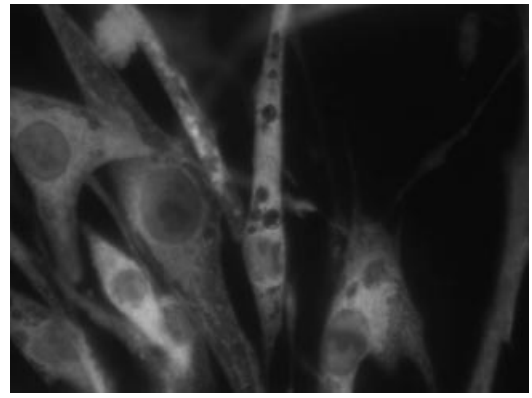

Figure 15.3 Accumulation of LAMP-1-positive vacuoles in cultured myoblasts from an IBMPFD patients with the R155H mutation. Mutant cells are also defective in myotube formation.

Autophagy has also been implicated in the other type of h-IBM, autosomal recessive distal myopathy with rimmed vacuoles (DMRV) or h-IBM. h-IBM is an early adult-onset distal myopathy caused by mutations in the *GNE* gene which encodes a bifunctional enzyme involved in sialic acid biosynthesis. It is pathologically characterized by the presence of rimmed vacuoles, especially in atrophic muscle fibers, which also occasionally contain congophilic materials that are immunoreactive to β-amyloid, lysosomal proteins, ubiquitin, and tau proteins. Hyposialylation plays an important role in the pathogenesis of DMRV/h-IBM. It is uncertain if a similar mechanism may be involved in VCP h-IBM [73]. Disruption of the ER/autophagy pathway thus holds potential for revealing insights into the pathogenesis of VCP muscle, bone, and brain disease.

VCP mouse models

Human and mouse VCP proteins differ by only one amino acid residue at position 684. The targeted homozygous deletion of VCP by Cre-loxP technology was reported to result in early embryonic lethality [74]. In contrast, heterozygous mice lacking one *VCP* allele and having one wild-type allele were apparently indistinguishable from their wild-type littermates. Weihl et al. [75] found that transgenic mice overexpressing the most common human IBMPFD mutation (R155H) under the regulation of a muscle creatine kinase promoter became progressively weaker in a dose-dependent manner starting at 6 months of age. These mutant mice showed muscle pathology including coarse internal architecture, and disorganized membrane morphology and vacuole-like clefts with reduced caveolin-3 expression at the sarcolemma. Even before animals displayed measurable weakness there was an increase in ubiquitin-containing protein inclusions and high-molecular-weight ubiquitinated proteins.

Recently Custer et al. [76] reported a transgenic mouse overexpressing mutant forms of VCP. The mice expressed muscle weakness, and pathology characteristic of inclusion-body myopathy including blue rimmed vacuoles, and TDP-43 pathology. Radiological examination of the skeleton revealed focal lytic and sclerotic regions in the vertebrae and femur. Additionally the brain revealed widespread TDP-43 lesions and the mice also exhibited abnormalities in behavioral testing. To replicate the human disease associated with VCP mutations our laboratory [77] has generated a knock-in mouse model of the common VCP R155H mutation. Mice demonstrated progressive muscle weakness, vacuolization of myofibrils, and centrally located myonuclei, in addition to TDP-43- and ubiquitin-positive inclusion bodies in quadriceps myofibrils and brain. Additionally, muscle sections showed increased numbers of autophagosomes, elevated caspase-3 activity, and an increased number of TUNEL-positive nuclei supporting involvement of autophagy and apoptosis in the pathogenesis of the disease. Bone histology showed increased osteoclastogenesis suggestive of PDB. The Custer overexpressed mutant VCP transgenic mice and our knock-in mice thus replicate the human disease and represent useful models for trials of novel therapies for diseases with similar pathogenesis.

Treatment

Currently there are no known treatments for the muscle component of VCP disease or the dementia however treatment trials are needed in this disease. PDB, however, is well treated with bisphosphonates and it is hypothesized that progressive disease can be prevented if treated at an early stage of the disease. Autophagy is negatively regulated by the mammalian target of rapamycin (mTOR) and can be induced in all mammalian cell types by mTOR inhibitors such as rapamycin. A number of investigators have reported dramatic effects of rapamycin on the size of renal angiomyolipomas and sub-ependymal giant cell astrocytomas in tuberous sclerosis patients [78, 79], neurofibromatosis, and polycystic kidney disease. Autophagy is a major clearance pathway for the removal of mutant huntington protein associated with Huntington disease, and many other disease-causing, cytoplasmic, aggregate-prone proteins. Research in IBMPFD will likely address important pathophysiologic principles underlying many other

common related disorders. Pharmacologic strategies to modify autophagy and other pathways such as proteasomal inhibition, and ER stress modifiers, may hold potential not only in VCP disease but also other disorders such as the vacuolar myopathies including GNE-associated h-IBM, sporadic inclusion-body myositis (s-IBM), oculopharyngeal muscular dystrophy (OPMD), and other proteinopathies such as FTD and ALS. Potential therapeutic strategies can be explored using the available cell and mouse models for preclinical studies.

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Electronic database information

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Online Mendelian Inheritance in Man (OMIM) http://www3.ncbi.nlm.nih.gov/omim (for inclusion body myopathy, early onset Paget disease of bone, and frontotemporal dementia; IBMPFD, MIM 167320).

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