

The Multiple Faces of Valosin-Containing Protein-Associated Diseases: Inclusion Body Myopathy with Paget's Disease of Bone, Frontotemporal Dementia, and Amyotrophic Lateral Sclerosis

Angèle Nalbandian · Sandra Donkervoort · Eric Dec · Mallikarjun Badadani · Veeral Katheria · Prachi Rana · Christopher Nguyen · Jogeshwar Mukherjee · Vincent Caiozzo · Barbara Martin · Giles D. Watts · Jouni Vesa · Charles Smith · Virginia E. Kimonis

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Abstract Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) is a progressive, fatal genetic disorder with variable penetrance, predominantly affecting three main tissue types: muscle (IBM), bone (PDB), and brain (FTD). IBMPFD is caused by mutations in the ubiquitously expressed valosin-containing protein (VCP) gene, a member of the AAA-ATPase superfamily. The majority of individuals who develop IBM have

progressive proximal muscle weakness. Muscle biopsies reveal rimmed vacuoles and inclusions that are ubiquitin- and TAR DNA binding protein-43 (TDP-43)-positive using immunohistochemistry. PDB, seen in half the individuals, is caused by overactive osteoclasts and is associated clinically with pain, elevated serum alkaline phosphatase, and X-ray findings of coarse trabeculation and sclerotic lesions. FTD diagnosed at a mean age of 55 years in a third of individuals is characterized clinically by comprehension deficits, dysnomia, dyscalculia, and social unawareness. Ubiquitin- and TDP-43-positive neuronal inclusions are also found in the brain. Genotype–phenotype correlations are difficult with marked intra-familial and inter-familial variations being seen. Varied phenotypes within families include frontotemporal dementia, amyotrophic lateral sclerosis, Parkinsonism, myotonia, cataracts, and anal incompetence, among others. Cellular and animal models indicate pathogenetic disturbances in IBMPFD tissues including altered protein degradation, autophagy pathway alterations, apoptosis, and mitochondrial dysfunction. Currently, mouse and drosophila models carrying VCP mutations provide insights into the human IBMPFD pathology and are useful as tools for preclinical studies and testing of therapeutic strategies. In this review, we will explore the pathogenesis and clinical phenotype of IBMPFD caused by VCP mutations.

A. Nalbandian · S. Donkervoort · E. Dec · M. Badadani · V. Katheria · P. Rana · C. Nguyen · J. Vesa · V. E. Kimonis (✉)
Department of Pediatrics, Division of Genetics and Metabolism,
2501 Hewitt Hall, University of California–Irvine,
2501 Hewitt Hall,
Irvine, CA 92696, USA
e-mail: vkimonis@uci.edu

J. Mukherjee
Preclinical Imaging Center, Department of Psychiatry & Human
Behavior, 162 Irvine Hall, University of California–Irvine,
Irvine, CA 92697, USA

V. Caiozzo
Department of Physiology and Biophysics, and Department of
Orthopedics, College of Health Sciences, University of California,
Irvine, CA 92697, USA

B. Martin · C. Smith
Sanders-Brown Center on Aging, University of Kentucky,
Lexington, KY, USA

G. D. Watts
Cell Biology and Biochemistry, School of Medicine, Health
Policy and Practice, Biomedical Research Centre,
University of East Anglia,
Norwich, Norfolk, UK

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Introduction

Clinical Presentation: VCP and IBMPFD

Inclusion body myopathy associated with Paget's disease and frontotemporal dementia (IBMPFD; OMIM 167320) is a complex, autosomal dominant disorder identified by Kimonis et al. in 2000 (Kimonis et al. 2000; Watts et al. 2004), attributable to mutations in the valosin-containing protein (VCP) gene (Kimonis et al. 2000; Watts et al. 2004). This rare and progressive disease is characterized by three main clinical features found alone or in combination in affected individuals: myopathy, Paget's disease of bone, and frontotemporal dementia. Myopathy is the most common feature present in 80–90% of affected individuals with an age of onset in the 30s to 40s. Patients typically demonstrate progressive weakness and atrophy of the skeletal muscles of the pelvic and shoulder girdles (Fig. 1a, b). Muscle weakness progresses to involve other limb and, importantly, respiratory muscles and heart, with death from respiratory failure or cardiomyopathy and cardiac failure typically in their 40s to 60s.

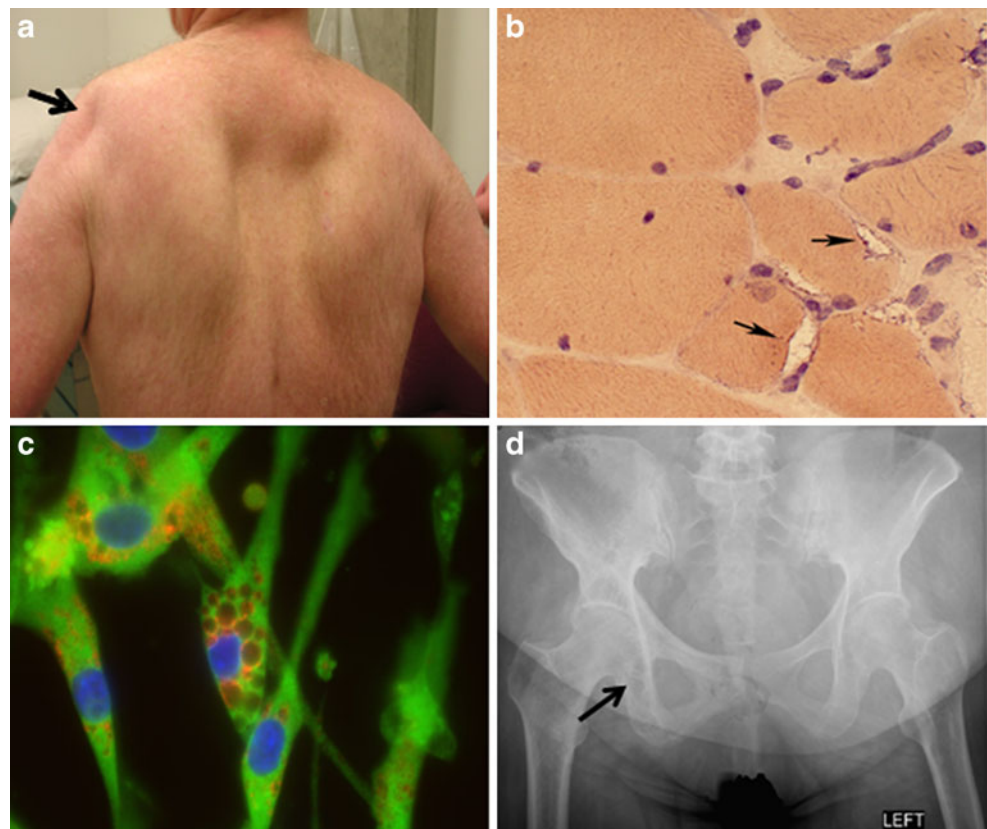
Serum creatinine kinase concentration is usually normal to mildly elevated and electromyography shows myopathic changes and, in some individuals, neuropathic changes typical of amyotrophic lateral sclerosis (ALS). Histology of the affected muscle demonstrates rimmed vacuoles, and both

myonuclear and sarcoplasmic inclusion bodies are immunoreactive for ubiquitin and TAR DNA binding protein-43 (TDP-43) (Kimonis et al. 2008a; Watts et al. 2004).

Paget's Disease of Bone

Paget's disease of bone (PDB) is observed in half the patients (Kimonis et al. 2000; Watts et al. 2003), typically with an early onset in the 30s to 40s. PDB is caused by excessive osteoclastic activity and increased bone turnover, resulting in susceptibility to long bone deformities (such as bowing) and fractures. PDB is often focal, and the bones most commonly involved are the skull, vertebrae, and pelvis (Fig. 1d). Pagetoid osteoclasts contain ubiquitinated nuclear and cytoplasmic inclusions. The diagnosis of PDB is based on elevated serum alkaline phosphatase, diagnostic changes of coarse trabeculation, cortical thickening, and spotty sclerosis in the skull, pelvis, hips, spine, and/or scapulae or typical findings of radionuclide scans. These findings may be present 10–15 years before the diagnosis of PDB can be made in pre-symptomatic individuals (Farpour et al. 2011; Kimonis et al. 2008b). Oral and intravenous bisphosphonates such as Zoledronic acid, known for their potent ability to suppress osteoclastic activity (Reid et al. 2005; Siris et al. 1996), are effective and potentially may be useful in preventing PDB in individuals at risk of VCP-associated PDB.

Fig. 1 Characteristics of IBMPFD. **a** Scapular winging and deltoid wasting (*arrow*) in a male with proximal limb girdle weakness. **b** Muscle biopsy from a patient with VCP myopathy showing two rimmed vacuoles (*arrows*). **c** Human patient mutant myoblasts were stained with VCP and LAMP-1 antibodies. Nuclei were stained with DAPI (magnification, $\times 63$). IBMPFD patient myoblasts show marked vacuolization and LAMP staining, suggesting that the vacuoles are autophagosomic in origin. **d** Bone X-ray image of a 59-year-old female depicting coarse trabecular pattern and lytic changes of the right ileum (*arrow*), suggestive of Paget's disease of bone



Frontotemporal Dementia

Frontotemporal dementia (FTD) is a degenerative condition of the brain that primarily affects the frontal and anterior temporal lobes (Arnold et al. 2000; Turner et al. 1996; Zhukareva et al. 2001). FTD is associated with alterations in complex reasoning, mental flexibility and set shifting, working memory, judgment, expressive speech, and social awareness and regulation with relative preservation of memory. Common neuropathological findings in FTD are atrophy and neuronal loss in the frontal and temporal lobes. Approximately 55% of patients have ubiquitin-positive inclusions and 45% have tau-positive inclusions (Leyton and Hodges 2010). TDP-43 is a major component of inclusions characteristic of VCP-associated FTD and ALS pathology, placing VCP disease in a novel category of neurodegenerative diseases termed TDP-43 proteinopathies (Cairns et al. 2007; van der Zee et al. 2009).

In VCP disease, clinical FTD is observed in a third of patients with an average age of onset in the mid-50s and is characterized by dysnomia, dyscalculia, comprehension deficit, paraphasic errors, and relative preservation of memory. In later stages, auditory comprehension deficits for simpler one-step commands, alexia, and agraphia may be seen (Kimonis et al. 2000; Watts et al. 2003). The diagnosis of FTD requires a thorough understanding of family history of dementia, with assessment of behavioral and personality changes, in addition to neuropsychological testing. The frontal assessment battery is useful at the bedside to supplement the neurologic examination and the Frontal Behavioral Inventory checklist given to the caregiver to cover common behavioral symptoms. Currently, there are no curative treatments for any variant of FTD. However, selective serotonin reuptake inhibitors are sometimes used in patients to control obsessive symptoms and mood (Roberson 2011). Thus, there is an urgent need to develop therapeutic strategies and novel agents for the disease.

Less common phenotypic features that have been reported in VCP disease include cardiomyopathy, hepatic steatosis, cataracts, sensory motor axonal neuropathy, pyramidal tract dysfunction, sphincter disturbance, sensorineural hearing loss, and amyotrophic lateral sclerosis-like features (Djamshidian et al. 2009; Guyant-Marechal et al. 2006; Haubenberger et al. 2005; Hubbers et al. 2007; Kumar et al. 2010; Miller et al. 2009).

VCP and ALS

ALS is an adult-onset progressive neurodegenerative disease involving both upper and lower motor neuron degeneration (Leigh and Wijesekera 2010). Patients typi-

cally present asymmetric focal weakness of the extremities or bulbar findings (dysarthria, dysphagia). Muscle atrophy and weakness progress to affect other muscles, ultimately leading to death from respiratory failure 3–5 years after symptom onset. ALS affects all populations, having a lifetime risk of 1 in 350 to 1 in 600 for males and 1 in 450 to 1 in 800 for females, with a reported population incidence of 1.5–2.5 per 100,000 per year (Alonso et al. 2009; Logroscino et al. 2008; McGuire et al. 1996; Traynor et al. 1999).

About 5% of individuals with ALS meet the clinical criteria of Neary et al. (1998) for a diagnosis of FTD. Impairment in executive functioning is seen in 30% to 50% of individuals with ALS, but do not meet these criteria (Lomen-Hoerth et al. 2003; Strong et al. 2003). Approximately 5–10% of ALS patients have familial amyotrophic lateral sclerosis (fALS). The remainder of patients has simplex or sporadic disease, the etiology of which remains unknown. In familial fALS, 15% of cases are attributable to mutations in the superoxide dismutase (SOD1) gene, and 3% of cases are due to pathogenic variants in the TDP-43 or FUS/TLS genes (Lagier-Tourenne et al. 2010). TDP-43 has been identified as a major disease protein in ubiquitinated inclusions, and recently, mutations have been found in ALS (Ling et al. 2010). Pathological TDP-43 is hyperphosphorylated and ubiquitinated from the affected central nervous system regions in sporadic and familial ALS (Sreedharan et al. 2008) and neurotoxic *in vivo*. FUS is a functional homolog of TDP-43 and plays an important role in RNA processing and micro-RNA biogenesis. Patients with ALS with FUS mutations develop large cytoplasmic aggregates within the spinal motor neurons (Kwiatkowski et al. 2009; Vance et al. 2009).

Until recently, work in fALS has focused on mutations in the FUS, TDP-43, and SOD1 genes in providing insights into motor neuron pathogenesis. Johnson et al. (2010) performed exomic sequencing in an Italian family with ALS and identified the R191Q mutation. Screening of VCP in 78 familial ALS and 210 isolated cases identified VCP mutations including p.R159G, p.R159C, p.R191Q, p.D592N, and p.R155H, suggesting that VCP mutations account for 2% of fALS. The frequency in different populations needs to be established by screening larger fALS and sALS cohorts.

The molecular mechanisms and by which VCP mutations cause motor neuron degeneration needs further investigation. However, the involvement of VCP in ALS lends support to the importance of disrupted autophagy and ubiquitinated proteasomal degradation in its pathogenesis. By further deepening our understanding of the underlying causes and signaling mechanisms of the ALS phenotype, we hope to find genetic therapeutic strategies aimed to ameliorate the disease (Shaw 2010).

VCP Disease Has a Worldwide Distribution

To date, there are approximately 20 missense mutations discovered in the VCP gene (Ju and Weihl 2010), and IBMPFD has been reported in more than 39 families worldwide (Table 1). IBMPFD cases have been reported in German (Djamshidian et al. 2009; Kimonis et al. 2008b; Schroder et al. 2005), Italian (Bersano et al. 2009; Gidaro et al. 2008), French, Spanish (Stojkovic et al. 2009), Austrian (Haubenberger et al. 2005), Belgian (van der Zee et al. 2009), Brazilian (Fanganiello et al. 2011), British (Miller et al. 2009; Rohrer et al. 2011), Australian (Kumar et al. 2010), and Korean (Kim et al. 2011) patients. Recently, Rohrer et al. (2011) identified a novel exon 2 I27V VCP variant in a man with frontotemporal dementia, elevated alkaline phosphatase, and a family history of progressive muscle weakness and

personality changes in his father, and also in a woman with isolated progressive dysarthria; this mutation was not identified in healthy control populations (Rohrer et al. 2011). Interestingly, Kaleem et al. (2007) sequenced 188 individuals with Alzheimer's disease and from the set of sibling pairs that suggested linkage to chromosome 9 and found the R95H in one individual with pathologically confirmed AD (Kaleem et al. 2007). Although these clinical cases provide the pathogenic and phenotypic spectrum of VCP-associated IBMPFD, further analyses are needed to define the genotype–phenotype relationships.

Characterization of VCP

Linkage studies have localized the IBMPFD gene mutation to VCP on chromosome 9p21.1-p12 (Kovach et al. 2001).

Table 1 List of VCP missense mutations

	Amino acid mutation	c.DNA Base change (ORF)	Exon location	Domain location	No. of affected families	References
1	I27V	79A>G	2	N terminus	1	Rohrer et al. (2011)
2	R95H	284G>A	3	N terminus	1	Kaleem et al. (2007)
3	R93C	277C>T	3	N terminus	4	Hubbers et al. (2007); Guyant-Marechal et al. (2006); Krause et al. (2007)
4	R95G	283C>G	3	N terminus	2	Watts et al. (2004)
5	R95C	283C>T	3	N terminus	1	Kimonis et al. (2008a)
6	P137L	410C>T	4	N terminus	1	Gidaro et al. (2008)
7	R155C	463C>T	5	N terminus	5	Hubbers et al. (2007); Watts et al. (2004); Schroder et al. (2005); Guyant-Marechal et al. (2006); Gidaro et al. (2008)
8	R155H	464G>A	5	N terminus	8	Hubbers et al. (2007); Watts et al. (2004)
9	R155P	464G>C	5	N terminus	1	Watts et al. (2004)
10	R155S	463C>A	5	N terminus	1	Vesa et al. (2009)
11	R155L	N/A	5	N terminus	1	Gidaro et al. (2008)
12	G157R	469G>C	5	N terminus	1	Djamshidian et al. (2009)
13	R159H	476G>A	5	N terminus	2	Haubenberger et al. (2005)
14	R159C	476G>A	5	N terminus	2	Bersano et al. (2009); Spina et al. (2008)
15	R191Q	572G>A	5	Linker 1	1	Watts et al. (2004); Spina et al., (2008)
16	L198W	593T>G	6	Linker 1	1	Watts et al. (2007)
17	A232E	695C>A	6	Junction (L1-D1)	1	Watts et al. (2004)
18	T262A	N/A	7	AAA D1	1	Spina et al. (2008)
19	N387H	1159A>C	10	AAA D1	1	Watts et al. (2007)
20	A439S	N/A	11	Linker 2	1	Gidaro et al. (2008)

Later, disease mutations were identified within the VCP gene (Watts et al. 2004). VCP and its homologs TER94, CDC48, and VCP-like ATPase are highly conserved in evolution. VCP belongs to the type II AAA-ATPase gene superfamily characterized by two ATPase domains (D1 and D2; Confalonieri and Duguet 1995; Wang et al. 2004), two linker domains (L1 and L2), as well as the amino terminal and carboxyl terminal domains. Mutations are mainly located in three different domains—R95H, R93C, R95C/G, P137L, R155H/P/C/S/L, G157R, and R159H/C within the N domain; R191Q and L198W within the linker domains; and A232E, T262A, N387H, and A439S within the D1 ATPase domain (Kumar et al. 2010; Stojkovic et al. 2009; Wehl et al. 2009; Table 1).

It has been reported that VCP is involved in a plethora of distinct cellular processes including homotypic membrane fusion, transcription activation, nuclear envelope reconstruction, DNA repair, post-mitotic organelle reassembly, cell cycle control, apoptosis (Jarosch et al. 2002; Rabinovich et al. 2002; Rabouille et al. 1998), and endoplasmic reticulum-associated degradation of proteins (ERAD) capable of destroying both integral membrane and luminal proteins. This latter activity functions as a quality control for newly synthesized polypeptides, which selectively eliminates aberrant proteins in the secretory pathway (Jarosch et al. 2002).

VCP forms homohexamers and binds to several different adapter proteins, enabling VCP to target specific substrates for degradation (Kondo et al. 1997; Meyer et al. 2000). Disease mutations of VCP cluster in the CDC48 domain located in the N terminus of the protein, which is involved in ubiquitin binding and protein–protein interactions (Dai and Li 2001; Rape et al. 2001). CDC48 interacts with a multitude of different cofactors transporting proteins from the ER into the cytosol and primarily ubiquitinated substrates of proteasomal degradation and ubiquitin system whose cellular functions remain unknown (Ye et al. 2001). The passage of ubiquitinated substrates to the 26S proteasome is in flux with the alternative route of degradation by autophagy (Korolchuk et al. 2009). VCP is important for the retro-translocation of misfolded ER proteins, and failure in this activity results in defective ERAD and ER stress responses (DeLaBarre et al. 2006). Wehl et al. (2006) have previously shown that mutant VCP fails to degrade prototypical ERAD substrates. Degradation of specific substrates including I κ B, Unc-45b, and hypoxia-inducible factor-1 α is mediated by VCP (Alexandru et al. 2008; Dai et al. 1998; Janiesch et al. 2007).

The most common R155H mutation has been shown to have a normal hexameric structure (Wehl et al. 2006), and elevated ATPase activity was identified in cells transfected with IBMPFD mutants (Kakizuka 2008). Fernandez-Saiz and Buchberger (2010) have demonstrated that mutant VCP induces conformational changes in the p97 N domain, the

main binding site for regulatory cofactors, and exhibits strongly altered cofactor interactions, e.g. for binding of the ubiquitin ligase E4B was reduced whereas binding of ataxin 3 was enhanced. These results suggest that imbalanced cofactor binding to p97 is a key pathological feature of IBMPFD and potentially of other proteinopathies involving VCP/p97 (Kaiser et al., personal communication).

VCP and Autophagy

Recent studies have implicated VCP at the intersection of the autophagy and ubiquitin–proteasome signaling pathways, facilitating intracellular protein degradation (Balch et al. 2008). Autophagy has been postulated as a pathogenetic event occurring in Alzheimer's disease, Huntington's disease, and ALS, among other neurodegenerative and inflammatory disorders (Wong and Cuervo 2010). Autophagy is responsible for the degradation of defective organelles and the bulk degradation of the cytoplasm during starvation. On induction of autophagy, cytoplasmic constituents are first sequestered by a phagophore and subsequently form a double-membrane structure known as the autophagosome. Autophagosome-associated proteins including Light Chain 3 (LC3), γ -aminobutyric acid type A receptor-associated protein, and Golgi-associated ATPase enhancer of 16 kDa (GATE-16) are speculated in mediating the autophagosome–lysosome fusion. Autophagosomes fuse with lysosomal vesicles and form digestive autolysosomes (Rusten et al. 2008) in the cascade (Fig. 2).

Evidence suggests that the autophagic process is related to cell cycle regulation, starvation adaptation, aging, and cancer development. P62 plays a key role in the autophagic process and can interact with effector protein LC3 and mediate the uptake of aggregated proteins. One of the neuropathological hallmarks of ALS is the misfolding of proteins that may perturb a number of intracellular signaling and degradation pathways. The role of autophagy in sIBM and ALS pathogenesis has led to clinical trials such as lithium treatments (Crippa et al. 2010a, b; Pasquali et al. 2010; Siciliano et al. 2010).

Involvement of the NF κ B Pathway in VCP Disease

In Paget's disease of bone, NF κ B (NF κ B) is known to be upregulated and stimulates osteoclastic activity (Daroszewska and Ralston 2006). NF κ B is a major nuclear transcription factor known to regulate the expression of many genes involved in inflammation, cellular proliferation, anti-apoptosis, and immune system development (Aggarwal 2004; Hayden and Ghosh 2004). The NF κ B family consists of five members retained in the cytoplasm, including p50, p52, p65 (RelA), c-Rel, and RelB, forming various homo- and heterodimers. These dimers bind to specific inhibitors of

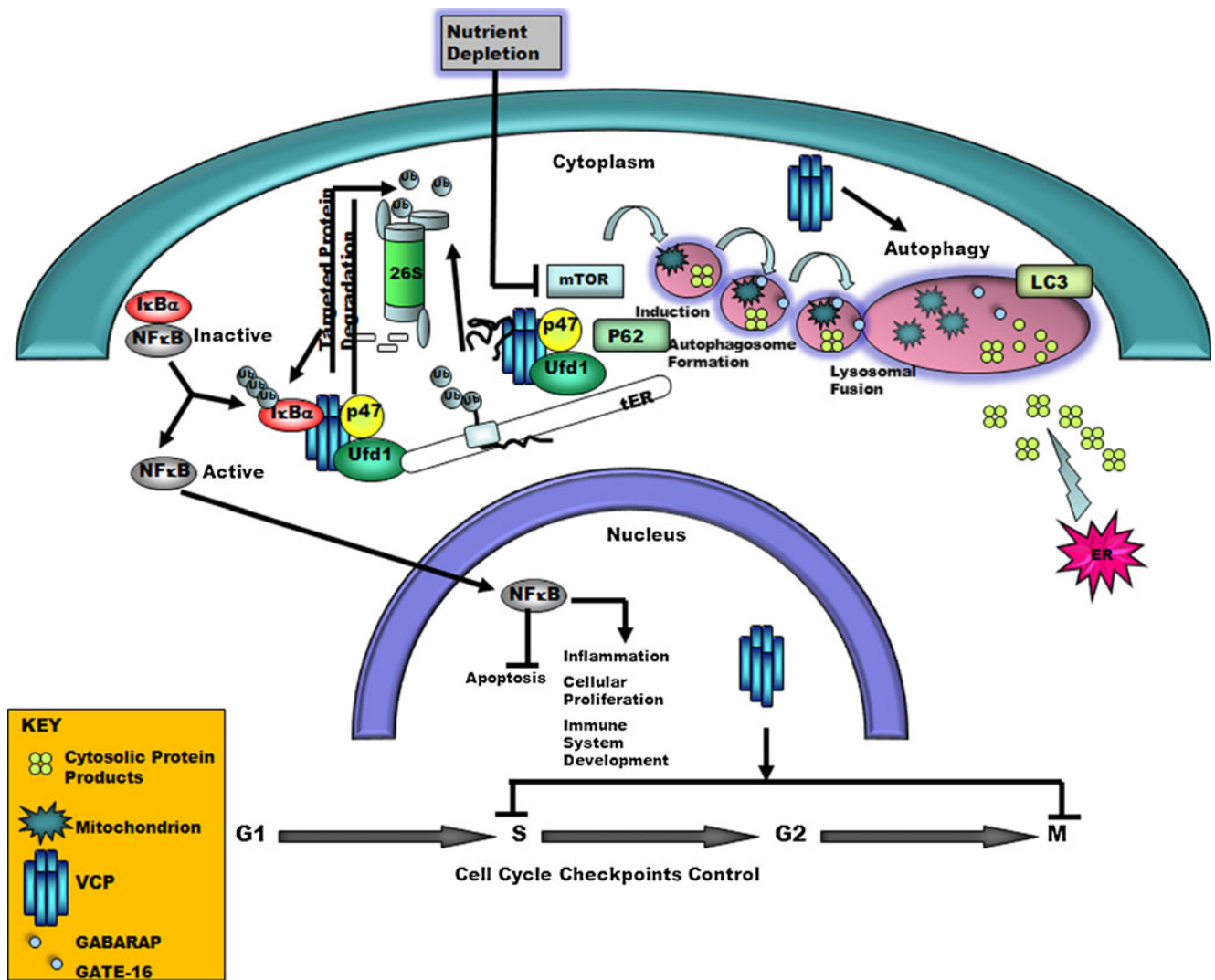


Fig. 2 Proposed functions and mechanisms of VCP. VCP is involved in a plethora of cellular functions including targeted protein degradation by ubiquitin/proteasome system, surveillance of misfolded proteins, nuclear membrane condensation, membrane fusion, apoptosis, and cell cycle control. Suggested mechanisms of VCP include signaling transduction cascades such as the ubiquitin/proteasome, NFκB, lysosomal, and mTOR/autophagy. Nutrient depletion,

endoplasmic stress, and normal VCP lead to mTOR inhibition, subsequently leading to induction, autophagosome formation, and lysosomal fusion, ultimately concluding in the autophagy signaling cascade. The activation of the NFκB pathway by lack of inhibition by IκB leads to the translocation of NFκB into the nucleus, subsequently leading to inflammation, cellular proliferation, development of the immune system, and inhibition of apoptosis

NFκB (IκBs), masking the nuclear localization sequence in the complex and thereby maintaining it in an inactive state (Hayden and Ghosh 2004).

There are now data to implicate increased NFκB activity in the pathogenesis of IBMPFD (Custer et al. 2010). Mechanistic considerations regarding how VCP mutations might contribute to NFκB activity begin with understanding how VCP affects NFκB’s repressor, IκBα. In 1998, Dai et al. (Dai et al. 1998) reported evidence suggesting that VCP co-precipitates with IκBα and that this interaction is important for IκBα’s degradation. The IBMPFD/myogenic transcriptome consisting of differentially expressed genes broadly matches the NFκB transcriptome known to be

involved in myogenesis, prompting investigation of the terminal stages of myogenesis. Specifically, intracellular adhesion molecule 1, myogenin, and m-cadherin are all direct or indirect targets of NFκB, and most are involved in myogenesis and myotube formation. VCP interacts with IκB alpha and by implication is a regulator of NFκB activity. Upregulation of the NFκB pathway is also seen in Duchenne muscular dystrophy and limb girdle muscular dystrophy type 2A (Acharyya et al. 2007; Graham et al. 2010; Haslbeck et al. 2005). The NFκB pathway impacts skeletal muscle atrophy in one of three ways, either by upregulation of proteins involved in the ubiquitin/proteasome system, inflammatory cytokines, or via the

inhibition of myogenesis. Studies are currently dissecting the molecular mediators in skeletal muscle causing hypertrophy and atrophy in hopes of finding therapeutic targets (Bhatnagar and Kumar 2010). Therapies using pharmacological and genetic approaches that normalize NF κ B activity via restoration of native levels of its inhibitor may allow for more physiologic myotube formation and potentially ameliorate the weakness in both skeletal and respiratory muscles in VCP disease.

IBMPFD: VCP *In Vitro* and Animal Models

IBMPFD Pathogenesis in Myoblasts

Cellular and animal models have been helpful in clarifying the role of ERAD, autophagy, apoptosis, and mitochondrial dysfunction in the pathogenesis of IBMPFD. Myoblasts from patients identified enlarged ubiquitin-containing vacuoles that accumulate membrane-bound Light Chain 3 (LC3-II), a marker of autophagy (Vesa et al. 2009). Upon activation of autophagy, cytosolic LC3-I undergoes proteolytic cleavage and is converted to the membrane-bound form, LC3II, which is localized to the autophagosomal membranes (Mizushima et al. 2004). Lysosomal-associated membrane protein 1 (LAMP-1) and LAMP-2 proteins demonstrated differential *N*-glycosylation in mutant myoblasts by Western blotting (Vesa et al. 2009) and immunohistochemical analyses (Fig. 1c). Patients' myoblasts thus serve as a useful *in vitro* model to recapitulate human disease and may further be used for dissecting molecular signaling cascades and for the development of therapeutic strategies and advances.

Mouse Models

Mouse models have been created in order to investigate the *in vivo* effects of VCP mutations as well as to further our understanding of the pathogenesis and mechanisms of IBMPFD. Both human and mouse VCP proteins consist of 806 amino acids, and the mouse protein differs by only one amino acid residue at position 684 when compared with the human protein.

Muller et al. (2007) reported that the deletion of p97 (VCP/CDC48) results in early embryonic lethality in the mouse, demonstrating the importance of p97 as an essential gene in mouse development. Homozygous p97^{-/-} mice died at pre-implantation stages, thus indicating a significant role for p97 in the coordination of cellular events in embryonic development. Heterozygous mice lacking one p97 allele and having one wild-type allele were indistinguishable from their wild-type littermates. Weihl et al. (2007) reported VCP transgenic mice with p97/VCP-WT or p97/VCP mutations under a muscle-specific promoter. These mice became

weaker at 6 months of age and depicted abnormal muscle pathology including disorganized membrane morphology and vacuolation with reduced caveolin-3 expression as compared with wild-type mice. Custer et al. (2010) has reported R155H mutant transgenic mice under the control of the CMV-enhanced chicken beta actin promoter, recapitulating pathology in muscle, brain, and bone typical of human IBMPFD. These mice developed muscle weakness, including rimmed vacuoles and TDP-43/ubiquitin-positive myopathy.

Additionally, Badadani et al. (2010) have generated a VCP knock-in mouse model with the common R155H^{+/-} mutation, typical of human IBMPFD pathogenesis. The mouse model demonstrated progressive muscle weakness and vacuolization of myofibrils and centrally located nuclei beginning at approximately 6 months of age. MicroCT images showed radiolucency of distal femurs and proximal tibiae in knock-in mice, and CT morphometrics showed decreased trabecular pattern and increased cortical wall thickness. Bone histology and bone marrow-derived macrophage cultures in these mice revealed increased osteoclastogenesis observed by tartrate-resistant acid phosphatase staining suggestive of PDB. Immunohistochemical analyses exhibited progressive cytoplasmic accumulation of TDP-43 and ubiquitin-positive inclusion bodies in the quadriceps myofibrils and brain of these mice. Increased LC3-II staining of brain sections representing increased number of autophagosomes suggested impaired autophagy in the brain pathogenesis. Our hypothesis is that immature autophagosomes accumulate in tissues secondary to impaired lysosome–autophagosome fusion, further contributing to tissue pathology. Increased apoptosis was demonstrated by elevated caspase-3 activity and increased TUNEL-positive nuclei. Therefore, the VCP R155H^{+/-} knock-in mice exhibit tissue pathology typical of human IBMPFD disease and represent a useful model for preclinical and future therapeutic studies (Badadani et al. 2010). We can use these approaches to dissect and understand the mechanisms and signaling cascades underlying the developmental, physiological, and pathological processes in VCP disease (Fig. 2).

Drosophila Models

Drosophila contains a single VCP homolog, TER94, which interestingly shares approximately over 80% identity of protein sequences with human VCP. *Drosophila* models have recapitulated a variety of diseases including IBMPFD. In *Drosophila* VCP (TER94 in *Drosophila melanogaster*), loss of function has been identified as a dominant repressor of expanded poly-glutamine-induced neuronal degeneration. Ritson et al. (2010) developed and characterized the first *Drosophila* model to elucidate the pathogenic mecha-

nisms of IBMPFD. They demonstrated that mutations in VCP lead to TDP-43 redistribution to the cytoplasm, thereby inducing degeneration *in vivo*.

Chang et al. (2011) developed *Drosophila* IBMPFD models by introducing amino acid substitutions at three residues in TER94 (R152H, R188Q, and A229E). These mutants exhibit learning deficits, tissue degeneration in muscle, and nervous systems typical of VCP disease. Chang et al. (2011) also demonstrated that an increase in cellular ATP could suppress the TER94 mutant phenotypes and that, conversely, a decrease in ATP would enhance the phenotype, thus linking ATP levels with the pathogenesis of IBMPFD. The *Drosophila* model of IBMPFD may thus aid in the development of novel therapeutic strategies/agents for this disease.

Therapeutic Approaches

There are no disease-modifying treatments to prevent the progression of the VCP-associated myopathy or FTD. PDB can be treated with bisphosphonates. We are improving our understanding of how disease mutations in VCP affect its function, but the pathogenic mechanisms for the progressive proximal muscle weakness, as well as for the inclusion and vacuole formation in IBMPFD muscle fibers, remain unknown. To be able to develop treatments, it is necessary to understand and elucidate the pathological molecular cascades resulting in the clinical manifestation of the disease. Mouse and *Drosophila* models recapitulating the IBMPFD phenotype in addition to *in vitro* systems will be useful for preclinical studies and in developing therapeutic strategies and novel drugs for the treatment of this disease as well as for several muscular and neurodegenerative diseases.

Future Directions

IBMPFD is a multisystem disorder caused by disease mutations in VCP, and pathology includes the degeneration of tissue and accumulation of ubiquitin and TDP-43-positive protein aggregates in the affected tissue. IBMPFD is an under-diagnosed disease, and VCP mutation testing should be considered in an individual with two or more of the associated characteristics. Increased cofactor binding of VCP with the multiple adaptor proteins appears to be a key pathogenic mechanism in VCP-associated disease. The specific mechanism(s) whereby VCP mutations result in IBMPFD remain unknown. Various signaling networks are currently being connected to mutations in the multifunctional chaperone VCP, including the NF κ B, autophagy, and apoptotic pathways (Fig. 2). Elucidating the basic molecular signaling cascades involved in IBMPFD disease will

ultimately lead to a better understanding of IBMPFD. The animal models recapitulate the IBMPFD pathology and offer the prospect of novel therapeutic interventions.

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Conflict of Interest Statement None declared.

References

- Acharyya S et al (2007) Interplay of IKK/NF-kappaB signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy. *J Clin Invest* 117:889–901
- Aggarwal BB (2004) Nuclear factor-kappaB: the enemy within. *Cancer Cell* 6:203–208
- Alexandru G et al (2008) UBXD7 binds multiple ubiquitin ligases and implicates p97 in HIF1alpha turnover. *Cell* 134:804–816
- Alonso A et al (2009) Incidence and lifetime risk of motor neuron disease in the United Kingdom: a population-based study. *Eur J Neurol* 16:745–751
- Arnold SE et al (2000) Quantitative neurohistological features of frontotemporal degeneration. *Neurobiol Aging* 21:913–919
- Badarani M et al (2010) VCP associated inclusion body myopathy and Paget disease of bone knock-in mouse model exhibits tissue pathology typical of human disease. *PLoS One* 5:1–15
- Balch WE et al (2008) Adapting proteostasis for disease intervention. *Science* 319:916–919
- Bersano A et al (2009) Inclusion body myopathy and frontotemporal dementia caused by a novel VCP mutation. *Neurobiol Aging* 30:752–758
- Bhatnagar S, Kumar A (2010) Therapeutic targeting of signaling pathways in muscular dystrophy. *J Mol Med* 88:155–166
- Cairns NJ et al (2007) TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 171:227–240
- Chang YC et al (2011) Pathogenic VCP/TER94 alleles are dominant actives and contribute to neurodegeneration by altering cellular ATP level in a *Drosophila* IBMPFD model. *PLoS Genet* 7:e1001288
- Confalonieri F, Duguet M (1995) A 200-amino acid ATPase module in search of a basic function. *Bioessays* 17:639–650
- Crippa V et al (2010a) A role of small heat shock protein B8 (HspB8) in the autophagic removal of misfolded proteins responsible for neurodegenerative diseases. *Autophagy* 6:958–960
- Crippa V et al (2010b) The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum Mol Genet* 19:3440–3456
- Custer SK et al (2010) Transgenic mice expressing mutant forms VCP/p97 recapitulate the full spectrum of IBMPFD including degeneration in muscle, brain and bone. *Hum Mol Genet* 19:1741–1755
- Dai RM et al (1998) Involvement of valosin-containing protein, an ATPase Co-purified with IkappaBalpha and 26 S proteasome, in ubiquitin-proteasome-mediated degradation of IkappaBalpha. *J Biol Chem* 273:3562–3573
- Dai RM, Li CC (2001) Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. *Nat Cell Biol* 3:740–744

- Daroszewska A, Ralston SH (2006) Mechanisms of disease: genetics of Paget's disease of bone and related disorders. *Nat Clin Pract Rheumatol* 2:270–277
- DeLaBarre B et al (2006) Central pore residues mediate the p97/VCP activity required for ERAD. *Mol Cell* 22:451–462
- Djamshidian A et al (2009) A novel mutation in the VCP gene (G157R) in a German family with inclusion-body myopathy with Paget disease of bone and frontotemporal dementia. *Muscle Nerve* 39:389–391
- Fanganiello RD et al (2011) A Brazilian family with hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. *Braz J Med Biol Res* 44:374–380
- Farpour F et al (2011) Radiological features of Paget disease of bone associated with VCP myopathy. PMID: 21643886
- Fernandez-Saiz V, Buchberger A (2010) Imbalances in p97 co-factor interactions in human proteinopathy. *EMBO Rep* 11:479–485
- Gidaro T et al (2008) An Italian family with inclusion-body myopathy and frontotemporal dementia due to mutation in the VCP gene. *Muscle Nerve* 37:111–114
- Graham KM et al (2010) Excessive collagen accumulation in dystrophic (mdx) respiratory musculature is independent of enhanced activation of the NF-kappaB pathway. *J Neurol Sci* 294:43–50
- Guyant-Marechal L et al (2006) Valosin-containing protein gene mutations: clinical and neuropathologic features. *Neurology* 67:644–651
- Haslbeck KM et al (2005) The RAGE pathway in inflammatory myopathies and limb girdle muscular dystrophy. *Acta Neuropathol* 110:247–254
- Haubenberger D et al (2005) Inclusion body myopathy and Paget disease is linked to a novel mutation in the VCP gene. *Neurology* 65:1304–1305
- Hayden MS, Ghosh S (2004) Signaling to NF-kappaB. *Genes Dev* 18:2195–2224
- Hubbers CU et al (2007) Pathological consequences of VCP mutations on human striated muscle. *Brain* 130:381–393
- Janiesch PC et al (2007) The ubiquitin-selective chaperone CDC-48/p97 links myosin assembly to human myopathy. *Nat Cell Biol* 9:379–390
- Jarosch E et al (2002) Protein dislocation from the endoplasmic reticulum—pulling out the suspect. *Traffic* 3:530–536
- Johnson JO et al (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 68:857–864
- Ju JS, Weihl CC (2010) Inclusion body myopathy, Paget's disease of the bone and fronto-temporal dementia: a disorder of autophagy. *Hum Mol Genet* 19:R38–R45
- Kakizuka A (2008) Roles of VCP in human neurodegenerative disorders. *Biochem Soc Trans* 36:105–108
- Kaleem M et al (2007) Identification of a novel valosin-containing protein polymorphism in late-onset Alzheimer's disease. *Neurodegener Dis* 4:376–381
- Kim EJ, Park YE, Kim DS et al (2011) Inclusion body myopathy with Paget disease of bone and frontotemporal dementia linked to VCP p.Arg155Cys in a Korean family. *Arch Neurol* 68:787–796
- Kimonis VE et al (2008a) VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: review of a unique disorder. *Biochim Biophys Acta* 1782:744–748
- Kimonis VE et al (2000) Clinical and molecular studies in a unique family with autosomal dominant limb-girdle muscular dystrophy and Paget disease of bone. *Genet Med* 2:232–241
- Kimonis VE et al (2008b) Clinical studies in familial VCP myopathy associated with Paget disease of bone and frontotemporal dementia. *Am J Med Genet A* 146A:745–757
- Kondo H et al (1997) p47 is a cofactor for p97-mediated membrane fusion. *Nature* 388:75–78
- Korolchuk VI et al (2009) Autophagy inhibition compromises degradation of ubiquitin–proteasome pathway substrates. *Mol Cell* 33:517–527
- Kovach MJ et al (2001) Clinical delineation and localization to chromosome 9p13.3-p12 of a unique dominant disorder in four families: hereditary inclusion body myopathy, Paget disease of bone, and frontotemporal dementia. *Mol Genet Metab* 74:458–475
- Krause S et al (2007) Brain imaging and neuropsychology in late-onset dementia due to a novel mutation (R93C) of valosin-containing protein. *Clin Neuropathol* 26:232–240
- Kumar KR et al (2010) Two Australian families with inclusion-body myopathy, Paget's disease of bone and frontotemporal dementia: novel clinical and genetic findings. *Neuromuscul Disord* 20:330–334
- Kwiatkowski TJ Jr et al (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323:1205–1208
- Lagier-Tourenne C et al (2010) TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum Mol Genet* 19:R46–R64
- Leigh PN, Wijesekera LC (2010) Motor neuron disease: focusing the mind on ALS: updated practice parameters. *Nat Rev Neurol* 6:191–192
- Leyton CE, Hodges JR (2010) Frontotemporal dementias: recent advances and current controversies. *Ann Indian Acad Neurol* 13: S74–S80
- Ling SC et al (2010) ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. *Proc Natl Acad Sci U S A* 107:13318–13323
- Logroscino G et al (2008) Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. *J Neurol Neurosurg Psychiatry* 79:6–11
- Lomen-Hoerth C et al (2003) Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology* 60:1094–1097
- McGuire V et al (1996) Incidence of amyotrophic lateral sclerosis in three counties in western Washington state. *Neurology* 47:571–573
- Meyer HH et al (2000) A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J* 19:2181–2192
- Miller TD et al (2009) Inclusion body myopathy with Paget disease and frontotemporal dementia (IBMPFD): clinical features including sphincter disturbance in a large pedigree. *J Neurol Neurosurg Psychiatry* 80:583–584
- Mizushima N et al (2004) In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell* 15:1101–1111
- Muller JM et al (2007) Targeted deletion of p97 (VCP/CDC48) in mouse results in early embryonic lethality. *Biochem Biophys Res Commun* 354:459–465
- Neary D et al (1998) Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 51:1546–1554
- Pasquali L et al (2010) The role of autophagy: what can be learned from the genetic forms of amyotrophic lateral sclerosis. *CNS Neurol Disord Drug Targets* 9:268–278
- Rabinovich E et al (2002) AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol Cell Biol* 22:626–634
- Rabouille C et al (1998) Syntaxin 5 is a common component of the NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments *in vitro*. *Cell* 92:603–610
- Rape M et al (2001) Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone. *Cell* 107:667–677
- Reid IR et al (2005) Comparison of a single infusion of zoledronic acid with risedronate for Paget's disease. *N Engl J Med* 353:898–908

- Ritson GP et al (2010) TDP-43 mediates degeneration in a novel *Drosophila* model of disease caused by mutations in VCP/p97. *J Neurosci* 30:7729–7739
- Roberson ED (2011) Contemporary approaches to Alzheimer's disease and frontotemporal dementia. *Methods Mol Biol* 670:1–9
- Rohrer JD, Warren JD, Reiman D et al (2011) A novel exon 2 I27V VCP variant is associated with dissimilar clinical syndromes. *J Neurol* 258:1494–1496
- Rusten TE, Filimonenko M, Rodahl LM et al (2008) ESCRTing autophagic clearance of aggregating proteins. *Autophagy* 4:233–236
- Schroder R et al (2005) Mutant valosin-containing protein causes a novel type of frontotemporal dementia. *Ann Neurol* 57:457–461
- Shaw CE (2010) Capturing VCP: another molecular piece in the ALS jigsaw puzzle. *Neuron* 68:812–814
- Siciliano G et al (2010) Clinical trials for neuroprotection in ALS. *CNS Neurol Disord Drug Targets* 9:305–313
- Siris E et al (1996) Comparative study of alendronate versus etidronate for the treatment of Paget's disease of bone. *J Clin Endocrinol Metab* 81:961–967
- Spina et al (2008) Frontotemporal dementia associated with a valosin-containing protein mutation: report of three families. *The FASEB Journal* 22:58.4 (Abstract)
- Sreedharan J et al (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319:1668–1672
- Stojkovic T et al (2009) Clinical outcome in 19 French and Spanish patients with valosin-containing protein myopathy associated with Paget's disease of bone and frontotemporal dementia. *Neuromuscul Disord* 19:316–323
- Strong MJ et al (2003) Cognitive impairment, frontotemporal dementia, and the motor neuron diseases. *Ann Neurol* 54(Suppl 5):S20–S23
- Traynor BJ et al (1999) Incidence and prevalence of ALS in Ireland, 1995–1997: a population-based study. *Neurology* 52:504–509
- Turner RS et al (1996) Clinical, neuroimaging, and pathologic features of progressive nonfluent aphasia. *Ann Neurol* 39:166–173
- van der Zee J et al (2009) Clinical heterogeneity in 3 unrelated families linked to VCP p.Arg159His. *Neurology* 73:626–632
- Vance C et al (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323:1208–1211
- Vesa J et al (2009) Valosin containing protein associated inclusion body myopathy: abnormal vacuolization, autophagy and cell fusion in myoblasts. *Neuromuscul Disord* 19:766–772
- Wang Q et al (2004) Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. *J Struct Biol* 146:44–57
- Watts GD et al (2003) Clinical and genetic heterogeneity in chromosome 9p associated hereditary inclusion body myopathy: exclusion of GNE and three other candidate genes. *Neuromuscul Disord* 13:559–567
- Watts GD et al (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat Genet* 36:377–381
- Watts GD et al (2007) Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. *Clin Genet* 72:420–426
- Weihl CC et al (2006) Inclusion body myopathy-associated mutations in p97/VCP impair endoplasmic reticulum-associated degradation. *Hum Mol Genet* 15:189–199
- Weihl CC et al (2007) Transgenic expression of inclusion body myopathy associated mutant p97/VCP causes weakness and ubiquitinated protein inclusions in mice. *Hum Mol Genet* 16:919–928
- Weihl CC et al (2009) Valosin-containing protein disease: inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia. *Neuromuscul Disord* 19:308–315
- Wong E, Cuervo AM (2010) Autophagy gone awry in neurodegenerative diseases. *Nat Neurosci* 13:805–811
- Ye Y et al (2001) The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414:652–656
- Zhukareva V et al (2001) Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. *Ann Neurol* 49:165–175