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Genotype-Phenotype studies of VCP-associated Inclusion Body Myopathy with Paget Disease of Bone and/or Frontotemporal Dementia

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ABSTRACT

VCP disease associated with Inclusion body myopathy, Paget disease of the bone and frontotemporal dementia is a progressive autosomal dominant disorder caused by mutations in Valosin containing protein gene. To establish genotype-phenotype correlations we analyzed clinical and biochemical markers from a database of 190 members in 27 families harboring ten missense mutations. Individuals were grouped into three categories: symptomatic, presymptomatic carriers and non-carriers. The symptomatic families were further divided into ten groups based on their VCP mutations. There was marked intra and inter-familial variation; and significant genotype-phenotype correlations were difficult because of small numbers. Nevertheless when comparing the two most common mutations, R155C mutation was found to be more severe, with earlier onset of myopathy and Paget ($p=0.03$).

Survival analysis of all subjects revealed an average life span after diagnosis of myopathy and Paget of 18 and 19 years respectively, and after dementia only 6 years. R155C had a reduced survival compared to the R155H mutation ($p=0.03$). We identified amyotrophic lateral sclerosis (ALS) in thirteen individuals (8.9%) and Parkinson's disease in five individuals (3%); however there was no genotypic correlation. This study represents the largest dataset of patients with VCP disease and expands our understanding of natural history and provides genotype-phenotype correlations in this unique disease.

Key Words: amyotrophic lateral sclerosis, frontotemporal dementia, genotype-phenotype, inclusion body myopathy, Paget's disease of bone, valosin containing protein.

INTRODUCTION

Inclusion Body Myopathy associated with Paget's disease of the bone and Frontotemporal Dementia, (IBMPFD, OMIM 167320), first reported by Kimonis *et al.* (2000) is an autosomal dominant, progressive, and ultimately lethal condition with onset typically in 20s to 40s¹⁻⁴.

Myopathy is the most common feature (90%) and is characterized by progressive weakness and atrophy of skeletal muscles of pelvic and shoulder girdle muscles. Muscle weakness progressively involves the other limb, trunk and respiratory muscles with death from respiratory failure, cardiomyopathy and cardiac failure⁵. Electromyography is characterized by myopathic and/or neuropathic changes and histologically patients reveal presence of rimmed vacuoles and TDP positive inclusion bodies in the muscles^{3, 6-8}.

VCP disease is associated with amyotrophic lateral sclerosis (ALS), and VCP mutations have been seen in both isolated familial⁹, and sporadic¹⁰ ALS. Features of ALS include bulbar signs, spasticity, hyperreflexia, fibrillations, fasciculations, and electrophysiological evidence of motor neuron involvement such as denervation, and reinnervation¹¹. EMG in ALS is characterized by neurogenic features which include moderate fibrillation potentials, increased muscle fiber irritability and small amplitude motor potentials. Tucker *et al.*¹² described the first family with combined lower motor neuron degeneration and skeletal disorganization. This family was subsequently reported to have a VCP mutation by Watts *et al.*⁷. Kumar *et al.* reported the first Australian families with VCP disease and pyramidal tract dysfunction in one family member¹³.

PDB is characterized by excessive osteoblastic and osteoclastic activity, resulting in the enhancement of bone remodeling with focal areas of increased bone turnover that leads to bone pain, deformities like enlargement or bowing and pathological fractures. The bones most commonly involved in VCP-associated PDB are spine, pelvis, scapulae and skull. These bones may manifest the findings suggestive of PDB like cortical thickening, coarse trabeculation and spotty sclerosis in presymptomatic individuals 10-15 years before the diagnosis of PDB can be made¹⁴. VCP mutations disrupt the normal structure of the bone by blocking the signaling in bone remodeling. Paget disease of bone is responsive to treatment with bisphosphonates¹⁵, and this treatment may be potentially useful in preventing PDB in individuals at risk of VCP-associated PDB.

Frontotemporal Dementia (FTD) is a degenerative condition of the frontal and anterior temporal lobes of brain which accounts for a substantial proportion of primary degenerative dementia that occurs earlier than the typical age of 65 years seen in the general population¹⁶. Disproportionately impaired executive or other frontal lobe functions, associated with changes in behavior and conduct, early in the course of the illness with relative sparing of memory and visuospatial abilities provide strong support for the diagnosis of FTD. Common neuropathological findings in FTD are atrophy and neuronal loss in the frontal and temporal lobes with TDP-43 positive inclusions which co-localize with ubiquitin¹⁷. TDP-43 is a major component of inclusions that characterize VCP-associated FTD and ALS pathology, thereby placing VCP disease in a unique category of neurodegenerative diseases termed TDP-43 proteinopathies

¹⁸⁻¹⁹. Forman *et al.* (2006) evaluated morphology, neuroanatomic distribution, and density of ubiquitin pathology in several different VCP disease families. Immunohistochemical analysis in their study revealed extensive ubiquitin-positive intranuclear inclusions and dystrophic neurites throughout the neocortex that was most severe in the temporal lobe. The inclusions were negative for tau, alpha-synuclein, and expanded polyglutamine repeats. The presence of similar but less robust pathology in preclinical stage of disease suggests that the relatively low frequency of clinical FTD in VCP disease may be a reflection of the early age at death in most patients that occurs before the clinical manifestation of brain pathology ²⁰.

Mutations in the gene encoding Valosin-Containing Protein (VCP) ^{2-3, 7}, which maps to chromosome 9p13.3-12, lead to this unique disease. The 97-kDa VCP is a member of the type II AAA (ATPases Associated with a variety of Activities) ATPases and is characterized by the presence of the N-terminal domain involved in ubiquitin binding ²¹, and conserved ATPase domains, also called AAA domains ²². VCP is ubiquitous, essential, and highly abundant in cells, and is involved in an unusually wide variety of functions like nuclear envelope reconstruction; post mitotic Golgi reassembly, and endoplasmic reticulum ER-associated degradation (ERAD) pathway during the “quality control process” and apoptosis ²¹. Vesa *et al.* have shown in myoblast cultures that myoblasts with mutant VCP accumulate large vacuoles. The mutant myoblasts show increased autophagy when cultured in the absence of nutrients, as well as defective cell fusion and increased autophagic protein degradation ²³.

VCP is a complex homohexamer that has various functional domains. We explored whether the location of ten different mutations in the VCP gene in 190 individuals from 27 families recruited for genotype- phenotype analyses was related to the progression and severity of symptoms and association with unique phenotypes. We also reviewed the clinical features of patients with fifteen additional mutations reported in the literature.

METHODS

Clinical Evaluation and Diagnosis

Informed consent was obtained from each subject prior to participation. Research studies were approved by the Institutional Review Boards of Southern Illinois School of Medicine, Children’s Hospital Boston and University of California, Irvine. Individuals who participated in clinical, biochemical, and molecular studies were over age 18 years. Subjects were grouped into three categories based upon their mutation status; symptomatic, carriers and non-carrier first degree at risk relatives. Affected individuals were grouped into ten groups based on their VCP mutation R155H, R155C, R155P, R191Q, R159C, R95G, R93C, A232E, L198W and N387H (**Table 1**).

A diagnosis of myopathy was based on the presence of muscular weakness, elevated creatinine kinase in some individuals, and in several patients by EMG and muscle biopsy findings. Clinical findings included an inability to raise their arms and walk up stairs, a lordotic gait from the proximal weakness and in some mild weakness of the hands. Tendon reflexes were absent or reduced. Electrodiagnostic studies, EMG and nerve conduction studies were performed to look for myopathic/neuropathic changes. Sections of unfixed muscle were stained by standard procedures.

Diagnosis of PDB included clinical features like spine or hip pain, pathologic fractures and long bone or cranial bone deformity. Routine measurements of serum alkaline phosphatase (ALP) and collagen degradation markers like urine pyridinoline (PYD) and deoxypyridinoline (DPD), reflecting increased bone turnover in PDB, were made in all the individuals. Whenever possible, a clinical diagnosis of PDB was confirmed by skeletal radiologic surveys including views of the skull, spine, hips, long bones, hands and feet, typical findings of PDB including coarse trabeculation, cortical thickening and spotty sclerosis. Radionucleotide scans show focally increased bony uptake and are considered sensitive indicators of PDB than plain survey films.

The diagnosis of frontotemporal dementia was made by comprehensive neuropsychological assessments and imaging studies when available, together with assessment of behavioral and personality changes, like personal/social unawareness, perseveration, abulia, and disinhibition. Patients were tested with a standard battery targeted to assess FTD including MMSE, Trails A & B, short version of the Stroop test, digit span, letter and category fluency and Boston naming, plus the neuropsychiatric inventory (NPI- short version) and Beck Depression Inventory for behavioral symptoms.

Statistical analysis

Tables of key clinical variables for statistical analysis were generated by cross-tabulation of all the available clinical and laboratory data for members of the 27 families. All the analysis was performed using SPSS® statistical package (v.15.0). Survival time was analyzed using the Kaplan-Meier survival method. Comparisons were made between normal controls, presymptomatic and symptomatic patients. For each marker, the least squares means were calculated for each of the 3 diagnosis groups (adjusted for age and gender). Possible interactions of age and gender were analyzed. A p-value of ≤ 0.05 was considered significant.

Molecular Studies

Mutation analysis of the VCP gene (NM_007126) was carried out as previously described ⁷. Sequences longer than 1000 bp were divided into multiple, overlapping segments for amplification. PCR products were gel purified using the Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced with an ABI 377 sequencer, using a dRhodamine terminator cycle sequencing kit (Applied BioSystems Inc., Foster City, CA).

RESULTS

The clinical data from the 27 families representing 190 individuals carrying VCP mutations (94 males, 96 females) were analyzed. Of these individuals, 145 (73 males, 72 females) were clinically symptomatic, and 45 (21males, 24 females) were presymptomatic carriers.

Myopathy

Of the 145 symptomatic individuals with VCP mutations 132 (91.03%) had myopathy with onset of weakness typically in adulthood, at a mean age 43.3 years. Higher CPK levels were seen in gene

carriers (169.91 ± 25.73 IU/l) and symptomatic individuals expressing inclusion body myopathy (165.25 ± 33.44 IU/l, $p=0.04$), compared to unaffected normal subjects (117.94 ± 17.98 IU/l), however these values fell within the normal range (**Table 2**). Mean survival after onset of myopathy was 18.22 ± 1.92 years.

Electromyography and Nerve Conduction Studies

41/131 (31.2%) of symptomatic individuals revealed pure myopathic changes with brief small polyphasic action potentials on EMG, 14/131 (10.68%) of symptomatic individuals revealed neurogenic changes including denervation, fasciculations, positive sharp waves and 18/131 (13.74%) of symptomatic individuals showed mixed myopathic and neuropathic changes. In this study population 24% therefore revealed some neurogenic features on EMG. The nerve conduction studies on these patients were essentially normal.

Muscle biopsies

Histopathology of muscle biopsies revealed myopathic changes including variation in muscle fiber size and mildly increased endomysial connective tissue in some regions of the specimen. Rimmed vacuoles were present in 35% of 105 muscle biopsies studied. Nuclear and cytoplasmic inclusions were immunopositive for ubiquitin and TAR DNA-binding protein 43 (TDP-43).

Paget's disease of bone (PDB)

PDB was identified in 75 individuals (51.72%) with a mean age of onset of 40.7 years (23 to 65 years). Mean alkaline phosphatase (ALP) levels across all symptomatic individuals was 310.10 ± 101.2 IU/l ($p < 0.001$); in those with Paget disease of the bone was 430.05 ± 162.6 IU/l ($p < 0.001$) and in presymptomatic gene carriers 87.7 ± 5.5 IU/l the latter being comparable with non carriers (91.12 ± 10.34 IU/l). Urinary PYD and DPD levels were significantly increased in the presymptomatic ($p < 0.05$) and the symptomatic individuals ($p < 0.001$) compared to controls, while the ratio of the DPD to PYD was similar in all groups (**Table 2**). Mean survival after onset of PDB was 19.59 ± 3.92 years.

Frontotemporal dementia (FTD)

Frontotemporal dementia was diagnosed in 44 (30.34%) symptomatic individuals with VCP mutations and occurred at a mean age of 55.3 years (Range 46 to 79 years). To evaluate the effect of gender on the prevalence of FTD we grouped all of the symptomatic individuals (alive and deceased) by sex and then by those who had survived for 40 yrs, 50 yrs, 60 yrs, and beyond. We identified a ratio of approximately 2:1 ($p=0.035$) (**Table 3**) however, there was no significant difference in the mean age of onset of FTD between males and females. Mean survival after onset of FTD was 6.54 ± 1.48 years.

The overall mean survival time, for individuals with VCP mutations with any aspect of the disease was 62.53 ± 1.88 . Mean survival after onset of myopathy, Paget and dementia was 18.22 ± 1.92 years, 19.59 ± 3.92 years and 6.54 ± 1.48 years respectively.

Genotype / Phenotype Studies

To investigate the phenotypic variations of the different mutations, the families were divided into ten groups depending on the VCP mutation identified in the gene (**Table 1**). The mean age of onset for IBM, PDB, FTD and biological markers for affected individuals within each mutation group (**Table 4**) were analyzed, however, some of the groups were very small and therefore, statistically significant differences could not be identified except for Group 2 with mutation R155C associated with a significantly earlier onset for symptoms of myopathy and PDB when compared with group 1 with mutation R155H ($p=0.033$ and 0.023 respectively). This group showed significantly reduced mean survival ($p=0.029$) when compared to other groups. There were no significant differences in the other markers for muscle and bone turnover like ALP, PYD, DPD or DPD/PYD between the groups. The clinical and biochemical markers were also analyzed by grouping mutations in different domains (CDC ubiquitin binding, Linker L1 and ATPase domains), but statistically significant differences were not appreciated.

Survival with different mutation groups was also analyzed. Group 2 with mutation R155C had a reduced mean survival of 58.518 ± 2.78 years compared to group 1 with mutation R155H with a mean survival of 63.195 ± 2.62 years ($p=0.029$) (**Figure 1**). The majority of the groups were too small for intergroup analysis.

Amyotrophic lateral sclerosis (ALS)

ALS was reported in at least 13 individuals in these 27 families; 10 individuals with mutation R155H (three from family 4, three from family 7 and two from family 3), one with mutation R155C (family 2), one with mutation R95G (family 9) and one with mutation A232E (family 6). This accounts for 8.9% of individuals affected with VCP disease, this being significantly higher than would be expected 'by chance' given the incidence rate of ALS in general population of 2 per 100,000 per year. Interestingly in family 6 with mutation A232E, previously reported as having a more severe phenotype with earlier age of onset and more aggressive disease⁷, one of the three individuals was diagnosed with ALS.

Parkinson's disease

The proband of family 24 with an R159C mutation had classic Parkinson's disease (PD)²⁴, and recently his sister has also been diagnosed with PD. Several individuals in family 16 were diagnosed with PD, however these individuals are deceased and PD could not be conclusively confirmed in them. Interestingly, none of the 13 individuals in family 24 had PDB suggesting that mutation R159C may be protective for PDB. Further studies in larger groups of patients with this mutation are necessary to evaluate these findings.

Alzheimer's disease

Some individuals were diagnosed with Alzheimer's disease, this diagnosis having been confirmed by neuropathology in one patient from family 3 with mutation R155H and two APOE4 alleles, which has been previously shown to increase the risk for FTD, but not PDB or IBM²⁵.

Genotype phenotype analysis thus revealed Group 2 with mutation R155C to be severe compared to Group 1 with R155H mutation with an earlier age of onset for myopathy and Paget's disease and a reduced mean survival. Symptomatic individuals demonstrated increased levels for all biochemical markers used in this study. Mean survival after onset of myopathy, Paget and dementia was 18.22 ± 1.92 years, 19.59 ± 3.92 years and 6.54 ± 1.48 years respectively.

DISCUSSION

Since the first family reported by Kimonis *et al.*³, there has been an increasing awareness of this disorder which may present as limb girdle muscular dystrophy (LGMD), facioscapulo humeral muscular dystrophy (FSHD), or amyotrophic lateral sclerosis (ALS) in addition to Paget disease and/or FTD. The TDP-43 pathology links this disease to frontotemporal lobar degeneration +/- ALS in addition to a broad array of sporadic and inherited diseases.

Symptomatic individuals demonstrated increased levels for all biochemical markers. Presymptomatic individuals showed an increase of DPD and PYD markers compared to their non mutation carrying relatives indicating that there are biochemical changes of bone and muscle turnover before the clinical manifestation of PDB and myopathy. Recently other biochemical markers such as serum and urinary C and N terminal telopeptides of type 1 collagen have been used as markers for bone turnover and may be used for monitoring of Pagetic activity²⁶. This has implications for the early identification and prophylactic treatment of PDB with bisphosphonates.

Phenotypic heterogeneity, both within and between different families containing the same VCP mutation of disease severity, distribution of weakness, presence or absence of Paget's disease or cognitive impairment makes genotype-phenotype correlations difficult, nevertheless we found that the R155C mutation was more severe than R155H. We previously reported a severe phenotype in a small family with A232E mutation¹¹, one member of which developed ALS. In a recent study, Niwa *et al.* (2012) analyzed the ATPase activities of ten major IBMPFD mutants and found that all have increased activity over the wild type, with the A232E and R155C mutations having the highest activities²⁷. The molecular mechanism for these observed differences may be related to its binding with its cofactors²⁸.

Review of the survival curves indicated that the average life span after diagnosis of myopathy and Paget is 18 and 19 years respectively. However, dementia heralded rapid acceleration of the progression of the disease with an average life span of 6 years. As the muscle weakness ultimately involves respiratory muscles, death is typically from respiratory failure or cardiac failure. Sex differences were observed in the incidence of FTD but not in the incidence of myopathy and Paget's disease. Although, none of the mutations had a significant effect on the age of onset for FTD, there was an increase in the incidence of FTD among females (F: M showed a 2:1 ratio).

8.9 % of the individuals from different mutation groups in our study had an initial diagnosis of ALS and 24% of the affected individuals revealed some neurogenic changes like denervation or fasciculations on the EMG. Kumar *et al.* studied axonal hyperpolarization in IBMPFD, affecting motor and sensory

axons. Their studies suggest that, in addition to any primary muscle disease, neuropathic abnormalities can occur in all patients with IBMPFD, and that large myelinated axons, motor and possibly sensory, are hyperpolarized in these patients²⁹. It is not surprising that exomic sequencing study by Johnson *et al.* identified VCP mutations in 1%-2% of familial ALS⁹, suggesting the ubiquitination/ protein degradation pathway plays a role in the pathogenesis of motor neuron degeneration in addition to FTD. In familial ALS, in addition to screening for FTD, PDB should be carefully sought for by measuring alkaline phosphatase levels and urine collagen cross linking markers and if necessary bone scan and/skeletal X-ray evaluations.

Parkinson's disease (PD) is also now recognized as a clinical manifestation of VCP related disease. We recently reported one individual with mutation R159C²⁴ whose sister has subsequently been diagnosed with PD. Two other families were reported by Spina *et al.* with PD³⁰.

Kaleem *et al.* performed several genome-wide linkage screens and identified a novel Late Onset Alzheimer's Disease (LOAD) risk factor on chromosome 9 and identified a novel R92H mutation³¹. One individual with mutation R155H and two apolipoprotein E (APOE) 4 alleles was identified in our study group who was diagnosed with true Alzheimer's disease (AD).

We also reviewed the clinical features in patients and families with IBMPFD reported from several parts of the world (**Table 5**). IBMPFD patients have been reported in a variety of ethnic backgrounds: German^{11, 32-33}, Italian³⁴⁻³⁵, Spanish³⁶, Austrian³⁷, Belgian¹⁹, Brazilian³⁸, British³⁹⁻⁴⁰, Australian¹³, Korean⁴¹ and Japanese⁴² patients. Miller's UK family with the R155H mutation had prominent sphincter disturbance involving bladder, bowel and erectile dysfunction in all five assessed members³⁹; additionally four had echocardiography features of cardiomyopathy. Rohrer *et al.* described two patients with a novel mutation I27V⁴⁰, one of whom had a symmetrical akinetic rigid syndrome with myoclonus in upper limbs and bilateral limb apraxia and the other individual exhibited an isolated progressive dysarthria. Two patients who suffered from a psychiatric disorder are described by Gidaro *et al.*³⁵. It is of interest that paranoid schizophrenia has been earlier described as a clinical feature in a patient carrying the R155C mutation⁴³.

VCP disease is an under diagnosed disease because of its variable phenotype, encompassing myopathy in 90%, PDB in 50%, FTD in 30%, cardiomyopathy, ALS, Parkinsonism, Alzheimer's disease, cataracts, sphincter disturbance and hepatic steatosis, which often leads to misdiagnosis. Identification of this disease is critical in advancing knowledge of the pathogenesis and possible novel treatments. Testing should be considered for the VCP mutations in individuals presenting two or more of the associated characteristics of VCP disease⁴. Molecular testing should include testing for familial mutation if known. If the mutation is unknown testing strategy should include exon 5 sequencing which will identify >50% of the mutations followed by sequential sequencing of the other common exons 3, 6, 7 and 10 and the remaining exons. Once the diagnosis is established a surveillance protocol is recommended (**Figure 2**) however these guidelines should be regularly reviewed in the light of future studies.

REFERENCES

1. Watts GD, Thorne M, Kovach MJ, Pestronk A, Kimonis VE. Clinical and genetic heterogeneity in chromosome 9p associated hereditary inclusion body myopathy: exclusion of GNE and three other candidate genes. *Neuromuscul Disord*. Sep 2003;13(7-8):559-567.
2. Kovach MJ, Waggoner B, Leal SM, et al. 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. *Molecular genetics and metabolism*. Dec 2001;74(4):458-475.
3. Kimonis VE, Kovach MJ, Waggoner B, et al. Clinical and molecular studies in a unique family with autosomal dominant limb-girdle muscular dystrophy and Paget disease of bone. *Genet Med*. Jul-Aug 2000;2(4):232-241.
4. Kimonis V, Donkervoort S, Watts G. Inclusion Body Myopathy Associated with Paget Disease of Bone and/or Frontotemporal Dementia Gene *GeneTests* (www.genetests.org) and *University of Washington, Seattle* [Review]. 2011; <http://www.ncbi.nlm.nih.gov/pubmed/20301649>.
5. Nalbandian A, Donkervoort S, Dec E, et al. The multiple faces of valosin-containing protein-associated diseases: inclusion body myopathy with Paget's disease of bone, frontotemporal dementia, and amyotrophic lateral sclerosis. *J Mol Neurosci*. Nov 2011;45(3):522-531.
6. Kimonis VE, Fulchiero E, Vesa J, Watts G. VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: review of a unique disorder. *Biochimica et biophysica acta*. Dec 2008;1782(12):744-748.
7. Watts GD, Wymer J, Kovach MJ, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nature genetics*. Apr 2004;36(4):377-381.
8. Weihl CC, Temiz P, Miller SE, et al. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *Journal of neurology, neurosurgery, and psychiatry*. Oct 2008;79(10):1186-1189.
9. Johnson JO, Mandrioli J, Benatar M, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron*. Dec 9 2010;68(5):857-864.
10. Abramzon Y, Johnson JO, Scholz SW, et al. Valosin-containing protein (VCP) mutations in sporadic amyotrophic lateral sclerosis. *Neurobiology of aging*. May 7 2012.
11. Kimonis VE, Mehta SG, Fulchiero EC, et al. Clinical studies in familial VCP myopathy associated with Paget disease of bone and frontotemporal dementia. *American journal of medical genetics*. Mar 15 2008;146(6):745-757.
12. Tucker WS, Jr., Hubbard WH, Stryker TD, et al. A new familial disorder of combined lower motor neuron degeneration and skeletal disorganization. *Transactions of the Association of American Physicians*. 1982;95:126-134.
13. Kumar KR, Needham M, Mina K, et al. Two Australian families with inclusion-body myopathy, Paget's disease of bone and frontotemporal dementia: novel clinical and genetic findings. *Neuromuscul Disord*. May 2010;20(5):330-334.

14. Farpour F, Tehranzadeh J, Donkervoort S, et al. Radiological features of Paget disease of bone associated with VCP myopathy. *Skeletal radiology*. Jun 4 2012;41(3):329-337.
15. Ralston SH. Pathogenesis of Paget's disease of bone. *Bone*. Nov 2008;43(5):819-825.
16. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology*. Dec 1998;51(6):1546-1554.
17. Neumann M, Mackenzie IR, Cairns NJ, et al. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *Journal of neuropathology and experimental neurology*. Feb 2007;66(2):152-157.
18. Cairns NJ, Neumann M, Bigio EH, et al. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *The American journal of pathology*. Jul 2007;171(1):227-240.
19. van der Zee J, Pirici D, Van Langenhove T, et al. Clinical heterogeneity in 3 unrelated families linked to VCP p.Arg159His. *Neurology*. Aug 25 2009;73(8):626-632.
20. Forman MS, Mackenzie IR, Cairns NJ, et al. Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. *Journal of neuropathology and experimental neurology*. Jun 2006;65(6):571-581.
21. Dai RM, Li CC. Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. *Nature cell biology*. Aug 2001;3(8):740-744.
22. Ogura T, Wilkinson AJ. AAA+ superfamily ATPases: common structure--diverse function. *Genes Cells*. Jul 2001;6(7):575-597.
23. Vesa J, Su H, Watts GD, et al. Valosin containing protein associated inclusion body myopathy: abnormal vacuolization, autophagy and cell fusion in myoblasts. *Neuromuscul Disord*. Nov 2009;19(11):766-772.
24. Chan NL LC, Shieh P, Mozaffar T, Khare M, Bronstein J, Kimonis V. Valosin-containing protein mutation and Parkinson's disease. *Parkinsonism Relat Disorders*. 3 2012;18(1):107-109.
25. Mehta SG, Watts GD, Adamson JL, et al. APOE is a potential modifier gene in an autosomal dominant form of frontotemporal dementia (IBMPFD). *Genet Med*. Jan 2007;9(1):9-13.
26. Szulc P, Delmas PD. Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. *Osteoporos Int*. Dec 2008;19(12):1683-1704.
27. Niwa H, Ewens CA, Tsang C, Yeung HO, Zhang X, Freemont PS. The role of the N-domain in the ATPase activity of the mammalian AAA ATPase p97/VCP. *The Journal of biological chemistry*. Mar 9 2012;287(11):8561-8570.
28. Tang WK, Li D, Li CC, et al. A novel ATP-dependent conformation in p97 N-D1 fragment revealed by crystal structures of disease-related mutants. *The EMBO journal*. Jul 7 2010;29(13):2217-2229.
29. Kumar KR, Liang C, Needham M, Burke D, Sue CM, Ng K. Axonal hyperpolarization in inclusion-body myopathy, Paget disease of the bone, and frontotemporal dementia (IBMPFD). *Muscle & nerve*. Aug 2011;44(2):191-196.

30. Spina S, Van Laar A, Murrell JR, et al. Frontotemporal dementia associated with a Valosin-Containing Protein mutation: report of three families. *The FASEB Journal* 2008;22: 58-54.
31. Kaleem M, Zhao A, Hamshere M, Myers AJ. Identification of a novel valosin-containing protein polymorphism in late-onset Alzheimer's disease. *Neurodegener Dis.* 2007;4(5):376-381.
32. Djamshidian A, Schaefer J, Haubenberger D, et al. 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.* Mar 2009;39(3):389-391.
33. Schroder R, Watts GD, Mehta SG, et al. Mutant valosin-containing protein causes a novel type of frontotemporal dementia. *Annals of neurology.* Mar 2005;57(3):457-461.
34. Bersano A, Del Bo R, Lamperti C, et al. Inclusion body myopathy and frontotemporal dementia caused by a novel VCP mutation. *Neurobiology of aging.* May 2009;30(5):752-758.
35. Gidaro T, Modoni A, Sabatelli M, Tasca G, Broccolini A, Mirabella M. An Italian family with inclusion-body myopathy and frontotemporal dementia due to mutation in the VCP gene. *Muscle & nerve.* Jan 2008;37(1):111-114.
36. Stojkovic T, Hammouda el H, Richard P, et al. 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.* May 2009;19(5):316-323.
37. Haubenberger D, Bittner RE, Rauch-Shorny S, et al. Inclusion body myopathy and Paget disease is linked to a novel mutation in the VCP gene. *Neurology.* Oct 25 2005;65(8):1304-1305.
38. Fanganiello RD, Kimonis VE, Corte CC, Nitrini R, Passos-Bueno MR. A Brazilian family with hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. *Braz J Med Biol Res.* Apr;44(4):374-380.
39. Miller TD, Jackson AP, Barresi R, et al. Inclusion body myopathy with Paget disease and frontotemporal dementia (IBMPFD): clinical features including sphincter disturbance in a large pedigree. *Journal of neurology, neurosurgery, and psychiatry.* May 2009;80(5):583-584.
40. Rohrer JD, Warren JD, Reiman D, et al. A novel exon 2 I27V VCP variant is associated with dissimilar clinical syndromes. *J Neurol.* Aug 2011;258(8):1494-1496.
41. Kim EJ, Park YE, Kim DS, et al. Inclusion body myopathy with Paget disease of bone and frontotemporal dementia linked to VCP p.Arg155Cys in a Korean family. *Archives of neurology.* Jun 2011;68(6):787-796.
42. Shi Z, Hayashi YK, Mitsuhashi S, et al. Characterization of the Asian myopathy patients with VCP mutations. *Eur J Neurol.* Mar 2012;19(3):501-509.
43. Guyant-Marechal L, Laquerriere A, Duyckaerts C, et al. Valosin-containing protein gene mutations: clinical and neuropathologic features. *Neurology.* Aug 22 2006;67(4):644-651.
44. DeJesus-Hernandez M, Desaro P, Johnston A, et al. Novel p.Ile151Val mutation in VCP in a patient of African American descent with sporadic ALS. *Neurology.* Sep 13 2011;77(11):1102-1103.

45. Peyer AK KJ, Frank S, Fuhr P, Fischmann A, Kneifel S, Thomann S, Camano P, Sinnreich M, Renaud S. Novel valosin containing protein mutation in a Swiss family with hereditary inclusion body myopathy, Paget's disease of the bone and dementia *World Federation of Neurology XII International Congress on Neuromuscular Diseases, Naples, Italy. 2010.*

Figure Legend:

Figure 1

[A]. Survival in symptomatic individuals: The Mean Survival for the affected individuals is 62.53 years, Standard Error 0.94, 95% CI is 60.68 – 64.39. **[B]. Survival after onset of IBM, PDB and FTD:** IBM – Mean Survival is 18.22 years, Standard Error 0.957, 95% CI is 16.35 – 20.10. PDB – Mean Survival is 19.59 years, Standard Error 1.968, 95% CI is 15.74 – 23.45. FTD – Mean Survival is 6.54 years, Standard Error 0.747, 95% CI is 5.08- 8.01. **[C]. Survival of the symptomatic individuals in mutation groups 1 and 2:** Mutation Group 1 (R155H) – Mean Survival is 63.19 years, Standard Error 1.316, 95% CI is 60.16 – 65.77. Mutation Group 2 (R155C) – Mean Survival is 58.51 years, Standard Error 1.39, 95% CI is 55.79 – 61.24. The comparison was significant ($p=0.020$) by log rank test.

Table 1 IBMPFD mutations with assigned group for analysis.

Groups	Families	VCP Base Change	Amino acid mutation	VCP Exon location	Domain location	Total individuals with VCP mutation	Affected	Carriers
1	1, 3, 4, 7, 10, 15, 16, 19b, 22, & 25	464 G>A	R155H	5	N terminus	99	78	21
2	2, 5, 14, 19a, 26, & 34	463 C>T	R155C	5	N terminus	35	28	7
3	11, 40	646 G>C	R155P	5	N terminus	11	9	2
4	13, 33	572 G>A	R191Q	5	Linker 1	9	5	4
5	24	475 C>T	R159C	5	N terminus	12	6	6
6	9	283 C>G	R95G	3	N terminus	7	5	2
7	36	277 C>T	R93C	3	N terminus	3	2	1
8	6	695 C>A	A232E	6	Junction (L1-D1)	3	3	0
9	30, 43	593 T>G	L198W	6	Linker 1	9	7	2
10	23	1159A>C	N387H	10	AAA D1	2	2	0
TOTAL	27	10	10			190	145	45

Table 2 Muscle and bone turnover marker values of the study groups

Markers	Normal		Presymptomatic		Symptomatic Individuals	
	N	Mean(SD)	N	Mean(SD)	N	Mean(SD)
ALP	82	91.1(46.8)	33	87.7(31.6)	82	***310.1(458.3) p<0.001
CPK	80	117.9(80.4)	33	*169.9(147.8) p=0.039	80	*165.2(149.6) p=0.042
PYD	65	32.4(14.1)	26	*38.5(13.5) p=0.017	45	***100.2(56.0) p<0.001
DPD	65	6.9(3.6)	26	**8.3(4.4) p=0.004	45	***25.0(31.9) p<0.001
DPD/PYD	65	0.21(0.53)	26	0.20(0.53)	45	0.25(0.14)

Key: Values with '' within a row are significantly different (*=p<0.05, **=p<0.01 and ***=p<0.001). ALP=Alkaline Phosphatase, Normal range, 30-130 IU/L, CPK=Creatine Kinase, Normal range, 20.0-222.0 U/L, PYD = Pyridinoline, Normal range, 23.2-43.2 μ mol/mol, DPD= Normal range, 6.4-11 μ mol/mol*

Table 3 Prevalence of FTD defined by sex and age.

Age (years)	Sex	
	Male	Female
≤40	0/7	0/6
41-50	1/16	3/10
51-60	7/24	11/29
≥61	7/23	13/19
Total	15/73	* 29/72

Key: Values with ‘’ within a row are significantly different (*= $p < 0.05$). The ratio of Females to males with FTD is 2:1. ($p = 0.035$).*

Table 4 Clinical and biochemical markers of symptomatic individuals in different mutation groups.

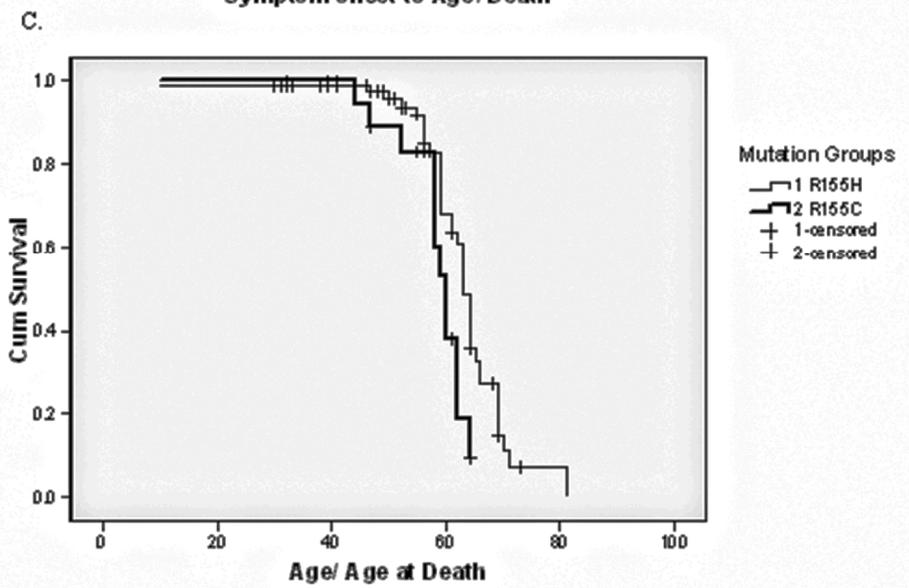
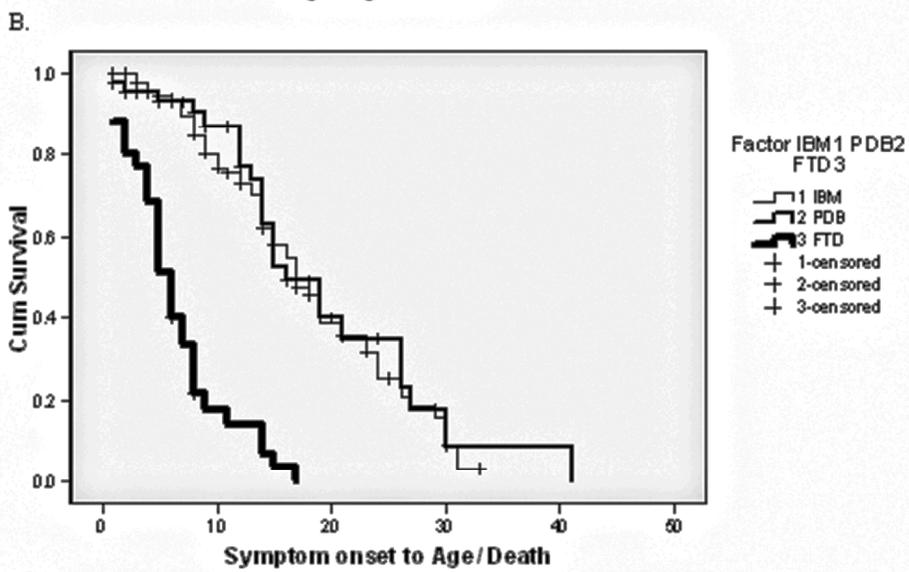
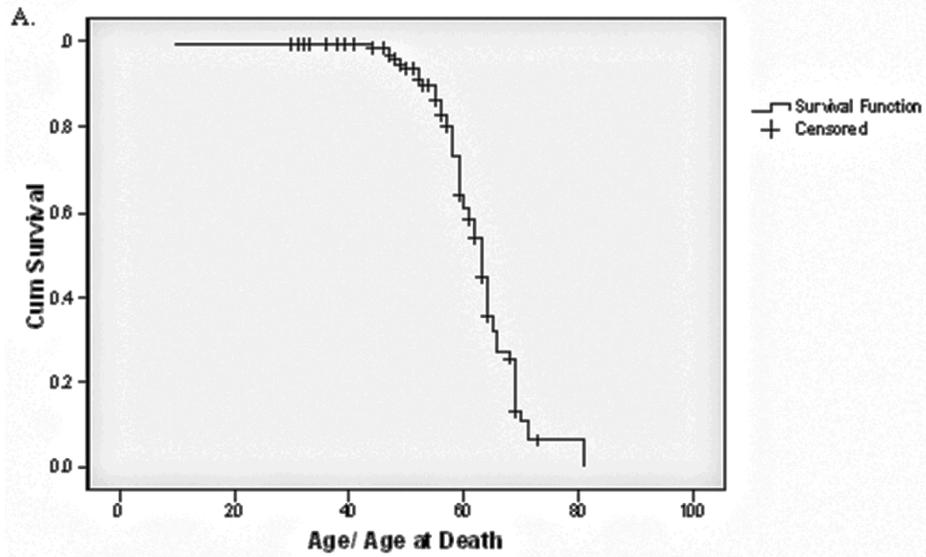
Mutation Group	Age of onset IBM (years)		Age of onset PDB (years)		Age of onset FTD (years)		ALP (IU/L)		CPK (U/L)		PYD (μmol/mol)		DPD (μmol/mol)		DPD/PYD	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
1 (R155H)	62	44	33	42.8	19	55.7	51	236.27	47	132.43	29	91.71	29	23.13	29	0.258
2 (R155C)	24	*39.1	9	**35.8	9	53.3	15	296.6	15	**292.93	10	92.15	10	19.50	10	0.210
3 (R155P)	7	43.4	7	38.3	1	52	4	350.75	4	106.5	2	206.66	2	62.40	2	0.319
4 (R191Q)	4	46.0	1	42	1	60	1	58	1	119	1	96.71	1	16.64	1	0.172
5 (R159C)	5	55.0	0	NA	4	59.7	1	108	1	89	0	NA	0	NA	0	NA
6 (R95G)	4	45.2	1	35	1	58	5	381.6	5	100.4	3	140.39	3	40.31	3	0.278
7 (R93C)	1	60	0	NA	0	NA	0	NA	1	370	0	NA	0	NA	0	NA
8 (A23E)	3	42.0	1	30	0	NA	2	2105	2	162	0	NA	0	NA	0	NA
9 (L198W)	8	39.2	1	50	2	56	4	327.75	5	220.6	0	NA	0	NA	0	NA
10 (N387H)	2	44.5	0	NA	1	46	0	NA	0	NA	0	NA	0	NA	0	NA
	120	46.58	53	39.68	38	55.08	83	482.9	81	162.49	45	125.52	45	32.39	45	0.247

ALP=Alkaline Phosphatase, Normal range, 30-130 IU/L, CPK=Creatine Kinase, Normal range, 20.0-222.0 U/L, PYP = Pyridinoline, Normal range, 23.2-43.2 μmol/mol, DYD= Normal range, 6.4-11 μmol/mol. Values with ‘’ within a row were only significantly different when comparisons were made in Group1 and Group2 (* p=0.03, ** p=0.02).*

Table 5 List of other VCP disease mutations reported in the literature.

	Amino acid mutation	c.DNA Base Change [ORF]	Exon location	Domain location	Number of affected families	References
1	I27V	79A>G	2	N terminus	1	40
2	R95C	283C>T	3	N terminus	1	6
3	R95H*	284G>A	3	N terminus	1	31
4	P137L	410C>T	4	N terminus	1	36
5	I151V	451A>G	5	N terminus	1	44
6	R155S	463C>A	5	N terminus	1	23
7	R155L	N/A	5	N terminus	2	13, 36
8	G157R	469G>C	5	N terminus	1	32
9	R159H	476G>A	5	N terminus	2	37
10	R159G*	476C>G	5	N terminus	1	9
11	I206F*	616A>T	6	Linker 1	1	45
12	T262A	N/A	7	AAA D1	1	30
13	A439S	N/A	11	Linker 2	1	36
14	A439P	1315G>C	11	Linker 2	1	42
15	D592N*	1774G > A	12	D2-ATPase,	1	9

*Key: *The numbering is relative to the ORF (i.e. the A in the ATG).*



Recommendations for IBMPF D Patients

- **To establish the diagnosis of IBMPF D the following evaluations are recommended:**
- A structured general medical history and family history.
- Physical examination and neurologic examination to include assessment of muscle strength, muscle wasting and tendon reflexes. Blood Creatine Kinase level.
- Electro physiologic studies to include Electromyography and Nerve conduction studies (three extremities).
- Muscle biopsy to look for single or multiple rimmed vacuoles and cytoplasmic VCP (valosin-containing protein) and ubiquitin-positive inclusions.
- Inclusion Body Myopathy Functional Rating Scale.
- Baseline pulmonary function studies to include Forced Vital Capacity (FVC), Forced Expiratory Volume at 1 minute (FEV1), FEV1/FVC and Peak Expiratory Flow (PEF).
- Cardiac evaluation by electrocardiogram and echocardiogram.
- Blood alkaline phosphatase, urine pyridinoline, deoxy pyridinoline and serum or urinary N-telopeptide or C-telopeptide measurements.
- skeletal bone scan studies and X-rays of the hot spots to evaluate distribution and severity of Paget disease of the bone.
- Neuropsychological examination for FTD. The Frontal Behavioral Inventory is recommended for screening.

Strategy for Management of IBMPF D Patients

- **Management should be tailored to each individual and specific subtype. A general approach to appropriate management can prolong survival and improve quality of life. This general approach is based on the typical progression and complications of individuals with IBMPF D :**
- Regular evaluations in the MDA clinic.
- Weight control to avoid obesity.
- Physical therapy and stretching exercises to promote mobility and prevent contractures.
- Occupational Therapy.
- Use of mechanical aids such as canes, walkers, orthotics, and wheelchairs as needed to help ambulation and mobility.
- Use of respiratory aids when indicated.
- Social and emotional support and stimulation to maximize a sense of social involvement and productivity and to reduce the sense of social isolation.
- Treatment of Paget's disease with bisphosphonates in both symptomatic patients whose symptoms may respond to a reduction in abnormal bone turnover as well as asymptomatic patients with active Paget's disease that is likely to cause future problems.
- Follow-up with a neurologist or psychiatrist trained in the evaluation and treatment of dementia.

Surveillance

- **We recommend the following tests to be done at an interval of 1 to 2 years to evaluate disease progression:**
- IBM Functional Rating Scale.
- Electrocardiogram and Echocardiogram to monitor for evidence of cardiomyopathy.
- Pulmonary function studies.
- Alkaline phosphatase, skeletal x-rays or bone scans for monitoring of the PDB if symptomatic and for monitoring of therapy.
- Monitoring of behavior and mental status