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Genotype-phenotype study in patients with VCP valosin-containing protein mutations associated with multisystem proteinopathy

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Abstract
Mutations in valosin-containing protein (VCP), an ATPase involved in protein degradation and autophagy, cause VCP disease, a progressive autosomal dominant adult onset multisystem proteinopathy. The goal of this study is to examine if phenotypic differences in this disorder could be explained by the specific gene mutations. We therefore studied 231 individuals (118 males, 113 females) from 36 families carrying 15 different VCP mutations. We analyzed correlation between the different mutations and prevalence, age of onset and severity of myopathy, PDB, and FTD, and other comorbidities. Myopathy, PDB and FTD was present in 90%, 42% and 30% of the patients respectively, beginning at an average age of 43 years, 41 years, and 56 years respectively. Approximately 9% of patients with VCP mutations had an ALS phenotype, 4% had been diagnosed with Parkinson’s disease (PD), and 2% had been diagnosed with Alzheimer’s disease (AD). Large inter and intra-familial variation made establishing correlations difficult. We did not find a correlation between the mutation type and the incidence of any of the clinical features associated with VCP disease, except for the absence of PDB with the R159C mutation in our cohort and R159C having a later age of onset of myopathy compared to other molecular subtypes.

Introduction
Inclusion body myopathy associated with Paget’s disease of bone and frontotemporal dementia (IBMPFD) or multisystem proteinopathy is an adult-onset progressive, autosomal dominant ultimately lethal disease that involves degeneration of three main organ systems: muscle, bone, and brain. Most cases are caused by heterozygous missense mutations in valosin-containing protein (VCP) (1), (2). As awareness increases we are realizing that VCP disease is not as rare as previously considered since it is often misdiagnosed as related disorders.

Pathology of IBMPFD
Inclusion body myopathy (IBM) is characterized by progressive weakness and atrophy of skeletal muscles of pelvic and shoulder girdle muscles. Ultimately, patients die from respiratory failure, cardiomyopathy and cardiac failure (3). Histologically, IBM consists of cytoplasmic rimmed vacuoles containing the same proteins that aggregate in the brains of patients with neurodegenerative diseases: tau, amyloid, and TDP-43 (TAR DNA binding protein 43) (4).

Paget’s disease of bone (PDB) is a unique skeletal disease caused by an imbalance between osteoclast and osteoblast function. The result is a gain in bone mass, but the new bone is
disorganized, weak, and prone to fractures. Typical radiological findings of PDB including coarse trabeculation, cortical thickening and spotty sclerosis. Clinical features include bone pain, bone enlargement, fractures, hearing loss due to auditory foramen narrowing, and arthritis. Rare complications include kidney stones, osteosarcoma, and high-output heart failure due to the formation of arteriovenous shunts in bone (5).

Frontotemporal dementia (FTD) is an early-onset type of dementia that is typically diagnosed in younger patients roughly 60% occurring in people 45-64 years old (6), however in VCP disease is associated with an earlier age of onset. Degeneration and atrophy of the frontal and temporal lobes of the brain results in changes in personality and progressive loss of language. Brain histology in patients with IBMPFD affected by FTD is characterized by gliosis, spongiosis, and neuronal intranuclear inclusions (7). TDP-43 aggregates are commonly associated with VCP-associated FTD as well as in amyotrophic lateral sclerosis (ALS) (4, 8, 9). TDP-43 is a DNA/RNA-binding protein involved in various cellular processes including RNA transcription and splicing (10-12). We have previously shown that the presence of one or two APOE4 alleles is associated with an increased risk of developing FTD in patients with VCP disease (13).

Mutations in VCP have also been associated with a spectrum of other diseases including amyotrophic lateral sclerosis (ALS) (14), hereditary spastic paraplegia (15), Charcot-Marie-Tooth Type 2 disease (16). Other common disorders that have an overlap with VCP disease include facioscapulohumeral muscular dystrophy (FSH), Limb-girdle muscular dystrophy (LGMD), scapuloperoneal muscular dystrophy (SPMD), inclusion body myositis, and distal myopathy/oculopharyngeal muscular dystrophy (17).

Structure and Function of VCP
VCP has four domains: an N-terminal ubiquitin binding domain, two ATPase domains (D1 and D2), and a C-terminal region (18). Valosin is a 25 amino acid peptide named after its N-terminal valine and C-terminal tyrosine, and was originally isolated from the porcine gut (19). That peptide sequence is present in valosin-containing protein, which is a highly abundant ATPase found in all cells where it interacts with various adaptor proteins to carry out many essential cellular processes. Among them are endoplasmic reticulum-associated degradation (20), transcription factor processing (21), nuclear envelope reconstruction (22), membrane fusion (23), post mitotic golgi reassembly (24), spindle disassembly (25), and cell cycle control (26). Several of these activities are associated with the ubiquitin-proteasome system in which VCP helps deliver ubiquitylated substrates to the 26S
proteasome for degradation (27). VCP’s roles in protein degradation and autophagy is implicated in the pathogenesis of IBMPFD, and may account for the cytoplasmic inclusions observed in muscle, bone, and neuronal tissue (1, 27). The hypothesis is that the location of the mutation may influence certain roles of VCP and thus lead to variation in the phenotype seen in VCP.

The goal of this work was to determine the location and type of the 15 different mutations in the VCP gene in 231 individuals from 36 families recruited initially for our gene identification studies and later for genotype-phenotype analyses.

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, Springfield, IL, Children’s Hospital, Boston, MA, and University of California, Irvine, CA. Individuals who participated in clinical, biochemical, and molecular studies were over age 18 years. This is essentially a cross sectional study, however follow-up of most patients occurred at periodic intervals and documentation.

Subjects were grouped into three categories based upon their mutation status; symptomatic, asymptomatic carriers and non-carrier first degree at risk relatives. Affected individuals were grouped into fifteen groups based on their VCP mutation R155H, R155C, R155P, R191Q, R159C, R159H, L198W, R95G, R93C, A232E, N387H, G97E, A160P, G128A, and M158I.

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

Muscle biopsies were obtained from the majority of individuals for clinical diagnoses which often led to the clinical diagnosis of inclusion body myopathy. The results of the muscle histology in several patients was reviewed previously and summarized for this report.

Diagnosis of PDB include clinical features like spine or hip pain, pathologic fractures and long bone or cranial bone deformity. Measurements of serum alkaline phosphatase (ALP), a marker of bone turnover, and urine pyridinoline and deoxypyridinoline were made in all individuals. Skeletal X-Rays...
were obtained in most patients with VCP mutations because of the known high incidence of PDB. Skeletal radiologic surveys included views of the skull, spine, hips, long bones, hands, and feet. Radionuclide scans show focally increased bony uptake and are considered more sensitive indicators of PDB than plain survey films. Radionuclide scans were obtained in individuals previously diagnosed with PDB. A selection of patients and gene carriers at risk of PDB participated in the detailed phenotyping studies at UC Irvine had radionuclide scans followed by X-rays of regions that were suspicious for PDB.

The diagnosis of frontotemporal dementia is typically made by a comprehensive neuropsychological assessments and imaging studies together with assessment of behavioral and personality changes, like personal/social unawareness, perseveration, and disinhibition. A selection of 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. In some patients with advanced dementia comprehensive neuropsychological testing was not possible and the diagnosis was made based on the strong family history of FTD. Patients were diagnosed with ALS according to the El Escorial criteria which are highly specific for ALS (28).

**Molecular Studies**

Mutation analysis of the VCP gene (NM_007126) was carried out as previously described in CLIA certified DNA diagnostic laboratories at the Mitomed Laboratory at UC Irvine, CA or at Prevention Diagnostic Laboratory, Marshfield, WI.

**Statistical analysis**

One-way ANOVA and Bonferroni Post Hoc analysis were performed using SPSS® statistical package (v. 21). Only mutation groups with more than 5 patients were included in the one-way ANOVA. Population standard deviations are reported since this is a rare disease and this study represents the largest assembly of patients published to date.

**Results**

Clinical data from 231 individuals representing 36 families carrying fifteen different VCP mutations (118 males, 113 females) was analyzed. Of these individuals, 187 (98 males, 89 females) were clinically symptomatic, and 44 (20 males, 24 females) were presymptomatic carriers. Our cohort of patients include mixed European, Brazilian, Hispanic/Apache, and African-American male.
Myopathy

Of the 187 symptomatic individuals with VCP mutations, 168 (89.84%) presented with myopathy at a mean age of onset of 43 years (range 20 – 70 years) (Table 1). Among all symptomatic VCP patients, mean CK levels were 182.9 ± 16.94 U/L (SD 160.67) with a range of 34- 473 mg/dl. Among VCP patients who were diagnosed with myopathy, CK was 187.4 U/L (SD = 161.75, SEM 17.03, Range 32-909). Among presymptomatic gene carriers, CK was 169.91 ± 25.34 U/L (SD 145.56, SEM 25.7, Range 54-736 U/L), compared to 117.94 ± 9.00 U/L (SD 80.46, SEM 9.0) in our unaffected normal subjects (p<0.006) suggesting that CK elevations may be early indicators of muscle pathology. A weak negative correlation coefficient of -0.2 was found between CK levels and age of onset of myopathy.

EMG results were obtained in 138 myopathic individuals as routine clinical evaluations in the majority of individuals and as part of research studies at the University of Kentucky and UC Irvine. Forty-five (32.6%) of individuals had pure myopathic changes, 16/138 (11.6%) had neurogenic changes, and 19/138 (13.8%) showed both myopathic and neurogenic changes on EMG.

Muscle biopsy reports were available for 115 of the symptomatic individuals. Muscle biopsies were obtained as standard of care in most patients and the reports and available histology slides reviewed. Histopathology showed that only 46 of the 115 (40%) muscle biopsies studied had rimmed vacuoles and the remaining individuals had non-specific myopathic changes. We have previously published our muscle biopsy findings in different cohorts of patient groups (2) (4) (29) (30).

Paget's disease of bone (PDB)

PDB was identified in 79 individuals (42.4% of those with VCP mutations) with a mean age of onset of 41.2 years (range 23 – 65 years). Mean alkaline phosphatase (ALP) among all symptomatic individuals as 289.8 ± 44.9 IU/L (SD = 434.85, SEM 45.09, median 127.0, Range 9-3006). Among individuals diagnosed with PDB, the mean ALP was 414.1 IU/L (SD = 544.08, SEM 75.45, Range 16 - 3006), (p<0.001). The ALP levels among presymptomatic gene carriers and non-carriers were similar (87.7 ± 5.4, SD = 31.6, SEM 5.5, Range 17-164, median 79.0, and 91.1 ± 5.1, SD = 46.84, SEM 5.17, Range 32-269, Median 79.5 respectively) (normal ALP is 44 to 147 IU/L). Most individuals had radionuclide scans and/or radiographs. Bones with a predilection for PDB included the skull, pelvis, and spine and interestingly the long bones were generally spared from PDB. We found that
radionuclide scans obtained in presymptomatic individuals identified Paget lesions in several bones which would not have been diagnosed by elevations in ALP levels alone.

Neurodegenerative diseases:

Dementia was diagnosed in 55 (29.4%) symptomatic individuals with VCP mutations and occurred at a mean age of 55.9 years (range 30 – 86 years). Since this is a cross-sectional study and FTD occurred later in the course of the disease typically being associated with rapid progression of the disease, we gathered data on the neuropsychological studies by their local clinicians. Detailed clinical studies were not always possible in severely demented individuals; also many individuals are not old enough to develop FTD, thus a comprehensive analysis of the various subtypes of FTD among these subjects is beyond the scope of this study. Clinical features (17, 31-33) tends to be typical including sociobehavioral and language changes, usually an expressive dysphasia, and loss of executive function. Characteristic findings include ubiquitin and TDP-43-positive neuronal intranuclear inclusions and dystrophic neurites.

Across all 36 families, there were 16 individuals (8.6%) representing six different genotypes (Table 1) who were diagnosed with an ALS phenotype of upper and lower motor neuron degeneration. Because of the rapid progression of ALS in these patients we reviewed clinical records and found unequivocal evidence of upper motor neuron dysfunction in three subjects from family 2, 3 and 57, the remaining subjects manifesting combined upper and lower motor neuron features. Asymmetry was marked in some individuals such as noted in the proband in family 57 who had clonus of the left leg and myopathic features of the right leg.

Autopsy data available on one individual with ALS revealed loss of brainstem and spinal cord motor neurons with Bunina bodies in surviving anterior horn cells and TDP-43 immunostaining, consistent with the diagnosis of ALS.

Seven individuals (3.8%) had been diagnosed with Parkinson’s disease (PD). Two had R155H mutations (family 52 and 56), two had R159C mutations (family 24), two had A160P mutations (family 59), and one had G128A (family 61) (table 2). Patients with PD in VCP disease tend to have classical symptoms and respond well to standard treatment. Detailed evaluations were performed in two individuals in family 24; studies of the proband are provided in the clinical report by Chan et al. (2012) indicated that he had classic PD and responded well to therapy (34).
Four individuals (2.1%) were diagnosed with Alzheimer’s disease: one had the G97E mutation (family 50), one had the R159H mutation (family 55), and one had R155H (family 3). We previously reported a case of early onset Alzheimer with two APOE4 alleles in family 2 (13).

Although VCP multisystem disorder is associated with a triad of symptoms, only 10% of the patients in our study presented with all three main features of the disorder (Figure 1). Myopathy was the most common presenting symptom, presenting in 89% of patients and as an isolated symptom in 36%. PDB was diagnosed in 43% of patients, and in 5% it was the sole clinical feature. Twenty-nine percent of patients had FTD, and in 3% this was the sole feature.

Genotype-phenotype studies
To elucidate the effect of different mutations on phenotypic variations, the families were divided into 15 groups according to their VCP mutation (Table 1, 2). Most mutations involved exon 5 which was mutated in 83% of individuals followed by mutations in exon 6 in 8% individuals. The frequency and mean age of onset of myopathy, PDB, and FTD, ALS, PD and AD as well as biological markers (CK, ALP) for each mutation group were compared to identify any statistically significant differences. Some of the mutation groups were too small for a statistical analysis of this sort. For IBM, mutation group R159C was found to have a later age on onset of muscle weakness (57 years) compared to L198W (37 years), R155H (43 years), R155P (43 years) and R155C (38 years), (p=<.04). No statistically significant differences were identified for PDB or FTD. We also did not see a higher incidence of ALS, or Parkinson’s disease either with any specific mutation types. The G97E mutation was reported to be associated with Charcot-Marie-Tooth (CMT2) (35); however, we did not find any case of CMT in our cohort of patients.

Discussion:
VCP disease is an autosomal dominant syndrome associated with progressive inclusion body limb-girdle type myopathy, Paget’s disease of bone, frontotemporal dementia (IBMPFD), and ALS. We report genotype-phenotype studies in patients bearing one of the 15 mutations in this report amongst our cohort to determine whether correlations exist between a patient’s mutation and the age of onset of their symptoms and associated manifestations. The primary findings are the general lack of genotype-phenotype correlations because of the enormous phenotypic heterogeneity within and between families. We found an incidence of 90% for the myopathy, 42% for PBD, 30% for FTD, 9% for ALS, and 4% for Parkinson’s disease (Table 1). Detailed analysis of VCP patients however shows
motor neuron involvement is apparent in the majority of study participants. Benatar et al. (2013) found three patients with unequivocal UMN findings and four with subtle UMN findings in a cohort of ten patients from six VCP families (36).

Although the levels were in the normal range there was large variability in the value of CK we noted that the levels were higher among presymptomatic gene carriers, compared to their unaffected relatives suggesting that mild CK elevations may be early indicators of muscle pathology in some individuals. Similarly, we noted that ALP was overall much higher in the presymptomatic carriers without PDB than their non-carrier relatives indicating it may be a good marker for early diagnosis of PDB. Radionuclide bone scans are also sensitive in establishing the early diagnosis of PDB having identified several presymptomatic individuals with normal ALP levels. Radionuclide bone scans thus offers the potential to treat patients presymptomatically in order to prevent PDB from progressing. Our data has also shown that VCP-associated PDB has a mean earlier onset of 41.2 years compared to PDB in the general population with an average age of onset of 50 years (2). Similarly, VCP associated FTD is associated with an early onset form of dementia at an average age of 55.9 years versus a typical age of onset of 65 years (6).

**Genotype-phenotype correlation**

There are 17 exons in the VCP gene and mutations have been reported in 11 of them - the vast majority occurring in exon 5. The only exons not identified with a mutation are exons 1, 8, 9, 13 and 15 (Table 4) suggesting that mutations in these loci are yet to be identified, or potentially devastating compromising survival of the individual. A total of 42 mutations have been previously published, in addition to the 3 novel missense mutations reported herein. Most the mutations are in the N terminus of VCP which is responsible for binding cofactors and ubiquitylated protein substrates (37-39). The mutations associated with IBMPFD and/or familial ALS are all exonic missense mutations. In particular, the R155 locus is a mutation hotspot. Previous genotype-phenotype analysis showed that the R155C mutation was associated with an earlier age of onset of myopathy and PDB (2) however analysis of a larger dataset in this report did not confirm this finding. The only statistically significant difference between the mutation groups was the later age of onset of myopathy in patients with the R159C mutation compared to the R155H, R155C, R155P, and L198W mutations. This suggests that there may not be significant differences in the age of onset of symptoms among IBMPFD patients with different VCP mutations; however, some of the groups are too small for this type of analysis. We previously reported that the A232E mutation is associated with a more severe phenotype (1) in a small family. This report is supported by our data which shows the A232 mutation as having an earlier
onset of PDB compared to average and an ALS phenotype (Table 1). Functional studies by Niwa et al. (2012) who tested ten VCP mutations (R93C, R95G, R155C, R155H, R155P, R159H, R191Q, L198W, A232E, and N387H) and found that all have increased ATPase activity over the wild type, with the A232E mutant having three times higher activity (18, 40). Interestingly the R159C mutation was associated with a later age of onset of myopathy compared to other molecular subtypes in our cohort of families. Additionally, PDB was absent in this large family, and also in two other reported families (11, 41). PDB was seen only in one individual with this mutation in an ALS family (42) suggesting a protective effect of the R159C mutation from PDB.

Global distribution and ethnic diversity of VCP mutations
Our cohort of patients recruited in North America included individuals of mixed European descent, Brazilian families, an African-American family and a family of Hispanic/Apache descent (17, 29, 30). Among families of European ancestry, patients of German (17, 43), Italian (41, 44, 45), Spanish (46), Austrian (47), Belgian (48), French (49), Irish (50), and British (51-53) backgrounds have been identified. In Asia, Korean (54), Japanese (55), and Chinese (56), families have been reported. Gonzalez-Perez (42) described an Israeli-Arab family. Brazilian (31) and Australian (57) cases have also been described. The Miller et al study (58) is the only that proposes an incidence of 1/300,000 among Scottish patients and 1/600,000 for British patients with VCP disease. The incidence in the US is most likely similar since patients do not always have the typical features of the syndrome and are diagnosed with related disorders such as limb-girdle muscular dystrophy, inclusion body myositis, and FSH especially in the absence of a family history of the other manifestations.

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Table 1. Clinical and biochemical data for symptomatic individuals in different mutation groups

| Family ID | Mutation group | N symptomatic | Age of onset IBM (yrs.) | Age of onset PDB (yrs.) | Age of onset FTD (yrs.) | CK (U/L) | ALP (IU/L) | ALS Phenotype | PD | AD |
|-----------|----------------|--------------|-------------------------|-------------------------|-------------------------|----------|------------|---------------|----|----|
| 1, 3, 4, 7, 10, 15, 16, 19b, 22, 25, 52, 56, 57 | 1 (R155H) | 97 | 86 | 43 | 44 | 43 | 24 | 55 | 136 | 230 | 11 | 2 | 1 |
| 2, 5, 14, 19a, 26, 34, 54 | 2 (R155C) | 31 | 30 | 38 | 14 | 36 | 10 | 53 | 299 | 282 | 1 | 0 | 0 |
| 11, 40 | 3 (R155P) | 9 | 7 | 43 | 7 | 38 | 1 | 52 | 107 | 351 | 0 | 0 | 0 |
| 13, 33 | 4 (R191Q) | 5 | 5 | 47 | 2 | 42 | 2 | 61 | 119 | 58 | 0 | 0 | 0 |
| 24, 48 | 5 (R159C) | 10 | 10 | 57 | 0 | NA | 7 | 60 | 177 | 88 | 0 | 2 | 0 |
| 30, 43 | 6 (L198W) | 7 | 7 | 37 | 4 | 50 | 1 | 50 | 193 | 414 | 0 | 0 | 0 |
| 55 | 7 (R159H) | 5 | 4 | 59 | 0 | NA | 3 | 66 | 713 | 98 | 1 | 0 | 1 |
| 9 | 8 (R95G) | 5 | 4 | 45 | 1 | 35 | 1 | 58 | 100 | 382 | 1 | 0 | 0 |
| 36 | 9 (R93C) | 2 | 2 | 60 | 1 | NA | 1 | NA | 370 | NA | 0 | 0 | 0 |
| 6 | 10 (A232E) | 3 | 3 | 42 | 3 | 30 | 0 | NA | 162 | 2105 | 1 | 0 | 0 |
| 23 | 11 (N387H) | 2 | 2 | 45 | 0 | NA | 1 | 46 | NA | NA | 0 | 0 | 0 |
| 50 | 12 (G97E) | 5 | 5 | 49 | 1 | 50 | 1 | 86 | 406 | 38 | 0 | 0 | 1 |
| 59 | 13 (A160P*) | 2 | 3 | 40 | 1 | 52 | 2 | NA | NA | 230 | 1 | 2 | 0 |
| 61 | 14 (G128A*) | 2 | 2 | 30 | 1 | 40 | 1 | 30 | 464 | 203 | 0 | 1 | 0 |
| 53 | 15 (M158I*) | 1 | 1 | 36 | 0 | NA | 0 | NA | 277 | 224 | 0 | 0 | 0 |
| 36 | 15 | 186 | 167 | 43 | 79 | 41 | 55 | 56 | 183 | 290 | 16 | 7 | 3 |

% of symptomatic : 89.8%  42.4%  29.6%  8.6%  3.8%  1.6%

N number, IBM Inclusion body myopathy, PDB Paget’s disease of bone, FTD frontotemporal dementia, CK total creatine kinase (NL 22 to 198 U/L), ALP alkaline phosphatase (44 to 147 IU/L), ALS amyotrophic lateral sclerosis, PD Parkinson’s disease, AD Alzheimer’s disease

* indicates novel mutations
### Table 2. Proportion of patients in each mutation group with each feature of VCP disease

| Mutation group | N symptomatic | IBM only | PDB only | FTD only | IBM & PDB | IBM & FTD | PDB & FTD | IBMPFD |
|----------------|--------------|----------|----------|----------|-----------|-----------|-----------|--------|
| 1 (R155H)     | 97           | 39%      | 6%       | 1%       | 28%       | 12%       | 2%        | 9%     |
| 2 (R155C)     | 31           | 39%      | 3%       | 0%       | 26%       | 16%       | 0%        | 16%    |
| 3 (R155P)     | 9            | 11%      | 22%      | 0%       | 56%       | 11%       | 0%        | 0%     |
| 4 (R191Q)     | 5            | 60%      | 0%       | 0%       | 0%        | 0%        | 0%        | 40%    |
| 5 (R159C)     | 10           | 30%      | 0%       | 0%       | 0%        | 60%       | 0%        | 10%    |
| 6 (L198W)     | 7            | 43%      | 0%       | 0%       | 43%       | 0%        | 0%        | 14%    |
| 7 (R159H)     | 5            | 40%      | 0%       | 20%      | 0%        | 40%       | 0%        | 0%     |
| 8 (R95G)      | 5            | 60%      | 20%      | 0%       | 0%        | 20%       | 0%        | 0%     |
| 9 (R93C)      | 2            | 0%       | 0%       | 0%       | 50%       | 50%       | 0%        | 0%     |
| 10 (A232E)    | 3            | 0%       | 0%       | 0%       | 100%      | 0%        | 0%        | 0%     |
| 11 (N387H)    | 2            | 50%      | 0%       | 0%       | 0%        | 50%       | 0%        | 0%     |
| 12 (G97E)     | 5            | 60%      | 0%       | 20%      | 20%       | 0%        | 0%        | 0%     |
| 13 (A160P*)   | 3            | 0%       | 0%       | 67%      | 33%       | 0%        | 0%        | 0%     |
| 14 (G128A*)   | 2            | 0%       | 0%       | 0%       | 50%       | 50%       | 0%        | 0%     |
| 15 (M158I*)   | 1            | 100%     | 0%       | 0%       | 0%        | 0%        | 0%        | 0%     |

**TOTAL** 187  37%  5%  3%  27%  16%  1%  10%

N=number, IBM=Inclusion body myopathy, PDB=Paget’s disease of bone, FTD=Frontotemporal dementia, CPK=Creatine phosphokinase, ALP=Alkaline phosphatase, ALS=Amyotrophic lateral sclerosis, PD=Parkinson’s disease, AD=Alzheimer’s disease
Figure 1. Frequencies of phenotypes in 187 individuals

- IBM only, 37%
- PDB only, 5%
- FTD only, 3%
- IBM & PDB, 27%
- IBM & FTD, 16%
- PDB & FTD, 10%