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Targeted sequencing and identification of genetic variants in sporadic inclusion body myositis

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Abstract

Sporadic inclusion body myositis (sIBM) has clinical, pathologic and pathomechanistic overlap with some inherited muscle and neurodegenerative disorders. In this study, DNA from 79 patients with sIBM was collected and the sequencing of 38 genes associated with hereditary inclusion body myopathy (IBM), myofibrillar myopathy, Emery–Dreifuss muscular dystrophy, distal myopathy, amyotrophic lateral sclerosis and dementia along with *C9orf72* hexanucleotide repeat analysis was performed. No *C9orf72* repeat expansions were identified, however; 27 rare (minor allele frequency <1%) missense coding variants in several other genes were identified. One patient carried a p.R95C missense mutation in *VCP* and another carried a previously reported p.I27V missense mutation in *VCP*. Mutations in *VCP* cause IBM associated with Paget's disease of the bone (PDB) and fronto-temporal dementia (IBMPFD). Neither patient had a family history of weakness or manifested other symptoms reported with *VCP* mutations such as PDB or dementia. *In vitro* analysis of these *VCP* variants found that they both disrupted autophagy similar to other pathogenic mutations. Although no clear genetic etiology has been implicated in sIBM pathogenesis, our study suggests that genetic evaluation in sIBM may be clinically meaningful and lend insight into its pathomechanism.

Keywords

Inclusion body myositis; VCP; Hereditary inclusion body myopathy; Myofibrillar myopathy; Amyotrophic lateral sclerosis

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1. Introduction

Sporadic inclusion body myositis (sIBM) is an idiopathic and untreatable myopathy that typically begins in patients over the age of 50 [1]. Patients have a characteristic pattern of involvement with both proximal and distal muscle weakness and a predilection for the knee extensors and wrist and finger flexors. Disease progression leads to significant morbidity with wheelchair confinement often within 10 years of onset [2,3]. The pathogenic mechanism of sIBM is currently unknown. Muscle from patients with sIBM has several myopathologic features that aid in distinguishing sIBM from other inflammatory and inherited muscle disorders. These include endomysial T-cell infiltrates that surround healthy appearing muscle fibers [1]. Vacuoles, classically described as “rimmed,” are present in scattered nonnecrotic fibers [1]. Sarcoplasmic inclusions that are immunoreactive for TARDNA binding protein-43 (TDP-43), p62/SQSTM1 and SMI-31 are also characteristic features [1].

While no hereditary muscle disease consistently has all of these features, some hereditary muscle diseases have a subset of similar pathologic features to sIBM on muscle biopsy and are termed hereditary inclusion body myopathies (hIBM) [1]. Whether genetic variants in hIBM associated proteins are associated with sIBM is not known. Moreover, whether proteins that accumulate in sIBM muscle tissue contribute to muscle pathogenesis is unclear. For example, dementia associated proteins such as β-amyloid and hyperphosphorylated tau have been proposed to accumulate in sIBM tissue implicating amyloid precursor protein processing and microtubule associated protein tau in sIBM pathogenesis [4,5]. Similarly, the identification of TDP-43 and p62/SQSTM1 as specific markers for sIBM pathology has supported the hypothesis that genes mutated in familial ALS may be associated with sIBM pathogenesis [6,7]. Further evidence for this comes from a family of diseases in which mutations in single proteins such as VCP, hnRNPA1, hnRNPA2B1 and matrin-3 can lead to variably penetrant phenotypes that include hIBM, ALS and fronto-temporal dementia [8–10]. Finally, a large group of protein aggregate and vacuolar myopathies can have “rimmed vacuoles” similar to those seen in sIBM patient muscle. The largest component of this group is myofibrillar myopathies and some distal myopathies [11,12].

Some studies have performed targeted genetic mutation analysis in small cohorts of sIBM patients [13–16]. However, disease causing mutations in these genes have not been consistently identified in sIBM patient cohorts. Other studies have focused on risk alleles within populations of sIBM patients such as apolipoprotein E genotypes or HLA subtypes as means to correlate *MHC* gene alleles with sIBM risk, severity and prognosis [17–19]. Nonetheless, studies systematically evaluating the genetic etiology of sIBM, similar to those performed for other neuromuscular disorders, are lacking [20].

We utilized a targeted next generation sequencing approach to evaluate 38 genes in 79 patients with sIBM. These 38 genes included a wide array of putative candidates and were chosen based upon their association with several muscle diseases including vacuolar, myofibrillar, Emery–Dreifuss and inclusion body myopathies. We also sequenced four genes, including *GNE* (mutations in *GNE* are associated with HIBM2, now more appropriately called GNE myopathy), that are essential for sialic acid biosynthesis [21].

Finally, we elected to sequence genes associated with ALS and dementia and evaluate *C9orf72* repeat expansions in sIBM patients.

2. Materials and methods

2.1. Study subjects

DNA was collected from 79 patients with a clinical diagnosis of sIBM. 41 patients were identified within the neuromuscular clinic at Washington University and the diagnosis of sIBM was made by that patient's physician. An additional 38 patients were identified at a Patient Conference and were personally examined by a Washington University Neuromuscular Physician (CCW, MHB, GL, or AP) who found their history and physical exam to be consistent with sIBM. Any patient with a family history of weakness, lack of quadriceps weakness, symptoms beginning before 40 years of age or with upper motor neuron signs were excluded from further analysis. Indeed four patients (two pairs of siblings) were identified at the patient conference and were not further included in our study. One set of siblings was found to be compound heterozygous for previously reported pathogenic *GNE* variants (NP_001121699.1; p.V727M; p.R42W) [22]. The study population was consistent with previous reports of sIBM patients with 58.2% being male and 41.8% female [2]. The average age at the time of DNA collection was 67 ± 9.4 years. All participants provided informed consent for clinico-genetic studies approved by the Washington University institutional review boards.

2.2. Molecular genetics

Agilent's SureDesign website was used to target the exons of 38 genes. Indexed genomic DNA (gDNA) libraries were prepared according to HaloPlex manufacturer's instructions. 250 ng of gDNA was digested in 8 parallel reactions, then hybridized with biotin-labeled probes designed to recognize and circularize targeted regions. Circularized segments of gDNA were captured using streptavidin-coupled magnetic beads and amplified for sequencing. Samples were pooled in 2 groups and each sequenced by 100 bp paired end reads on a single lane of a HiSeq2000 (Illumina, San Diego, CA). Reads were aligned to the human reference genome (Hg19) with NovoAlign (Novocraft Technologies, Selangor, Malaysia). Variants were called with SAMtools and annotated with SeattleSeq. Coverage across genomic intervals was calculated using BEDTools. Segregation and validation of mutations was assessed with standard polymerase chain reaction (PCR)-based sequencing using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) for primer design, an Applied Biosystems 3730 DNA Sequencer (Life Technologies, Carlsbad, CA) for sequencing and LaserGene SeqMan Pro version 8.0.2 (DNAStar, Madison, WI) for tracing analysis.

2.3. C9orf72 expansion identification

gDNA samples were screened for the *C9orf72* hexanucleotide expansions using repeat-primed polymerase chain reaction (PCR) primers and methods as previously published [23].

2.4. *In vitro* ATPase assay

Purified recombinant VCP-WT and VCP variants were assayed for intrinsic ATPase activity as previously described [24].

2.5. Tissue culture and immunoblotting

The GFP-fused VCP expression plasmid is previously described [25]. The mutations VCP-R155H, VCP-R95C and VCP-I27V were introduced using site-directed mutagenesis kit (Stratagene, La Jolla, CA). Forty-eight hours post-transfection into U20S cells, cells were harvested and lysates were separated via SDS-PAGE, transferred to nitrocellulose and immunoblotted using the following antibodies [anti-p97/VCP (Fitzgerald, Acton, MA), anti-p62 (Proteintech, Chicago, IL), anti-GAPDH (Sigma-Aldrich), and anti-LC3 (Sigma-Aldrich)] as previously described [24].

3. Results

Our targeted capture and sequencing of 38 candidate genes in 79 subjects with sIBM yielded an average of 320 Mbp (range 140–1000) per subject, with 95 ± 1% of targeted bases covered at ≥10x (92 ± 3% at ≥25x). Variants were filtered for quality and depth, then for those predicted to disrupt coding sequence resulting in 29 ± 7 high-quality coding variants per subject. We hypothesized that variants influencing disease would be rare and focused our analysis on variants that i) had been previously reported as pathogenic, ii) were rare in population control databases (i.e. a global MAF <1%), or iii) were novel. Twenty-seven different heterozygous missense variants met these criteria and were validated by Sanger sequencing (Table 1). No individuals were homozygous or compound heterozygous for any variant or variants within the same gene. Twenty-eight subjects (35% of the cohort) carried one of the identified variants and three subjects carried two variants. Eight of twenty-seven (30%) of these variants were completely absent from large population databases (1000 Genomes project, the Exome Sequencing Project, dbSNP).

A single rare missense variant in *LDB3* (c.352G > A; p.V118M) recurred in 3/79 (3.8%) unrelated patients in our cohort. Although this frequency is considerably higher than the MAF of 0.5155%, the difference did not meet statistical significance (p = 0.11). Two other patients also carried single rare variants in *LDB3* (c.664G > A; p.A222T and c.1240C > A; p.Q414K) with MAF of 0.0384% and 0.0154% respectively. Rare variants were identified in other genes associated with myofibrillar myopathies: *FLNC* (six variants, two of which were novel) and *BAG3* (three variants, one which was novel). One novel missense variant (c. 1004C > A; p.Y335F) was identified in *HNRNPA2B1*, a gene recently shown to cause IBMPFD2 (OMIM 600124) [9]. This variant is not present in population databases and is predicted to affect the nuclear import sequence of hnRNPA2/B1 [26]. No sIBM patients had evidence of *C9orf72* GGGGCC repeat expansions as determined by repeat primed PCR.

Seven previously reported putative disease associated variants were identified in five genes: *FHL1*, *DES*, *MYOT*, *SQSTM1* and *VCP* (Table 2). In the case of *FHL1*, *DES*, *MYOT* and *SQSTM1* the MAF for each variant was >1% in control populations, suggesting that they are not disease causing. In contrast, two *VCP* variants (one novel and one previously reported) were identified (c.283G > A; p.R95C and c.79A > G; p.I27V) and were either absent

(p.R95C) or very rare (MAF 0.0923%) (p.I27V) in population databases [27–29]. The clinical and pathologic features for the two subjects with *VCP* mutations are shown in Table 3 and described in the supplemental material. Notably, the subject with the *VCP* p.R95C mutation fulfilled diagnostic criteria for probable IBM (ENMC 2013 criteria) [38] and possible IBM (Griggs criteria) [39]. He had no family history of weakness, including among his 5 adult children. His father died at 75 years old with a diagnosis of lupus and his mother passed away at age of 96 with type 2 diabetes. One sibling died at 90 years old after 10 years of Alzheimer Disease and before DNA could be collected. The subject's living siblings (age 75 and 79 years old without symptoms and age 94 years with dementia) agreed to undergo genetic evaluation but were negative for the p.R95C mutation. The subject carrying *VCP* p.I27V fulfilled ENMC 2013 criteria for clinico-pathologically defined IBM and Griggs criteria for definite IBM. There was no history of weakness, dementia or PDB in his parents, sibling or two children. No further genetic analysis was performed on this family.

The R95C and I27V variants are within the N-domain of VCP where > 50% of all pathogenic *VCP* variants exist [40]. In addition, when these mutated residues are identified in the crystal structure of VCP, they reside at the N-D1 domain interface adjacent to all other reported pathogenic mutations (Fig. 1A). Many, but not all, previously reported pathogenic VCP mutations have enhanced basal ATPase activity [24]. To test this, we measured the basal rate of ATP hydrolysis in the presence of recombinant wild-type VCP (VCP-WT), VCP-I27V, VCP-R95C and the most common pathogenic VCP mutation VCP-R155H. The VCP-R95C variant had a 3-fold increase ATPase activity similar to VCP-R155H (Fig. 1B). In contrast, the VCP-I27V variant did not have an increase in basal ATPase activity (Fig. 1B).

Previous studies have found that autophagosome maturation is disrupted by pathogenic VCP mutations as demonstrated by the accumulation of the autophagic substrate p62 and the autophagosome protein LC3II [41]. To assess the pathogenic nature of the variants identified in sIBM patients, we transiently transfected VCP-WT, VCP-R155H, VCP-R95C and VCP-I27V fused to a C-terminal green fluorescent protein tag into U20S cells and evaluated the levels of VCP, p62, LC3 and GAPDH via immunoblot. As previously described, VCP-R155H expression results in an increase in p62 and LC3II levels [41]. Similarly, VCP-R95C and VCP-I27V expression caused an increase in p62 and LC3II consistent with the previously defined disruption in autophagosome maturation by VCP disease mutations [41].

4. Discussion

We performed targeted sequencing of 38 "high probability" genes associated with clinical syndromes having phenotypic and pathogenic similarities to sIBM, in 78 patients with sIBM; making it the largest genetic study performed to date for sIBM. Our goal was to assess whether rare missense coding variants in these genes known to be associated with hereditary IBM, myopathies with IBM-like pathology, ALS or dementia are present in sIBM patients and may begin to explain the sporadic nature of this enigmatic disease. This is of course a significant limitation of our study and biases our analysis to a subset of genes. Moreover we presuppose that rare coding variants will be we pathogenic. While this strategy

has been used for other “sporadic” neuromuscular disorders, it seems clear that whole exome sequencing of these patients will reveal other relevant risk alleles [20].

Two patients meeting diagnostic criteria for sIBM carried pathogenic mutations in *VCP*. The p.R95C variant in *VCP* had been previously mentioned as causing disease [27] and occurs at an amino acid residue found mutated in other IBMPFD pedigrees (p.R95G and p.R95H) [10,42]. Our subject had no clinical or lab evidence of PDB (serum alkaline phosphatase was normal (65 u/L; nl 38–126 u/L) and plain film X-rays of hips, pelvis and spine were normal) or dementia. In addition, he had no family history of weakness but did have two siblings with late onset dementia. The original review of his left deltoid muscle biopsy revealed chronic myopathic changes, several small angular fibers with rimmed vacuoles, increased endomysial cellularity, and one region of focal invasion (Fig. S1). Subsequent to the genetic identification of his p.R95C variant, we re-examined the muscle biopsy and performed MHCI immunostaining which showed no upregulation. Additionally, banked serum was examined for anti-NT5C1A autoantibodies, suggested to be specific for sIBM, but they were not found [43,44]. Re-evaluation of the clinical phenotype by chart review showed that at one point in his clinical assessments, wrist and finger extensor weakness was greater than that of the wrist and finger flexors. Importantly, although his phenotype was distinctive from classical sIBM, he still fulfilled the 2013 ENMC proposed criteria for probable IBM. The absence of MHCI immunostaining, the absence of anti-NT5C1A autoantibodies, and atypical phenotypic features in patients with vacuolar myopathies may push one toward identifying a genetic etiology.

The *VCP* p.I27V variant has been reported in three patients with neurologic diseases consistent with IBMPFD, but also in control subjects [28,29]. Since being reported in IBMPFD, the variant has been found in population screening, with a MAF of 0.09% in the Exome Sequencing Project. This is considerably more prevalent than sIBM. This patient had a typical sIBM pattern of weakness with quadriceps and wrist and finger flexor weakness. Moreover, his biopsy was consistent with sIBM. However retrospective immune marker analysis was not able to be performed. Notably this patient did not have any reported family history for weakness, dementia or PDB. Unfortunately, due to the small family structure and late onset of his phenotype, we were unable to perform segregation analysis. It has been suggested that the p.I27V VCP variant could either be a pathologic mutation with incomplete penetrance or a risk allele for neurologic disease since it has been reported in unaffected control patients [29]. Structural modeling, biochemical and *in vitro* assays were performed on both the VCP I27V and R95C mutations and were consistent with them being pathogenic (Fig. 1A–C). Interestingly, in contrast to the R95C mutation, the I27V variant behaved similar to VCP-WT with regard to intrinsic ATPase activity. An elevation in ATPase activity has been reported for many but not all pathogenic *VCP* mutations [24,45]. In particular, we recently identified an E185K missense variant in VCP that segregated in a large family with late onset CMT2 [24]. Similar to the VCP-I27V variant, VCP-E185K disrupted autophagic function in cell culture but did not have an intrinsic elevation in ATPase function [24]. Perhaps the normal ATPase activity portends a milder VCP disease phenotype as in the case of CMT2 and the VCP-E185K variant or incomplete penetrance and increased risk in patients carrying the VCP-I27V mutation.

In addition to *VCP* variants, we identified another five subjects with previously reported disease associated variants in *DES*, *MYOT*, *FHL1* and *SQSTM1*. All of these variants have MAFs >1.0% making them unlikely to be disease causing. However, it was recently suggested that the p.A213V desmin variant, while not being a disease-causing variant, could be disease-associated or a risk factor for cardiomyopathy [32]. Whether this may similarly be true for sIBM or other muscle disorders is unclear and warrants further assessment in larger cohorts of patients.

We also noted a potential excess of rare variants in *LDB3* and other myofibrillar myopathy related proteins (*FLNC* and *BAG3)*. Although our study was not powered to perform either single variant or rare-variant burden associations, this finding warrants further analysis of this gene in larger groups of patients. Interestingly, this finding is in line with a recent study evaluating genetic variants in candidate genes in 21 Japanese sIBM patients; where they also identified a patient with a novel *LDB3* variant [13].

It is well established that some hereditary muscle diseases can mimic that of sIBM, and even meet varied clinical criteria [46]. Therefore it is tempting to dismiss that a patient with a clinical diagnosis of sIBM and later found to have a genetic etiology was “misdiagnosed.” This is a limitation of many genetic studies. Indeed similar clinically reported DNA collections (e.g. NINDS Human Genetics DNA and Cell Line Repository at Coriell) in which the investigating researcher may not have immediate access to all clinical information are an expeditious means of performing large scale human genetic studies [29,47]. Regardless, since 38 of the 79 patients in our study were identified at a patient support conference and only a history and physical exam was utilized to corroborate a diagnosis of sIBM, we were not able to classify all of our patients into ENMC 2013 or Griggs criteria. However it is reassuring that the patient identified with the *VCP* I27V variant, who was identified at a patient conference, fulfilled ENMC 2013 and Griggs criteria for clinic-pathologically defined and definite IBM when all clinical material was later evaluated.

The future of research and clinical treatment for acquired muscle diseases such as sIBM is rapidly evolving and will dramatically change with the advent of inexpensive and comprehensive genetic testing. Moreover, clinical trials and therapeutic interventions for sIBM are currently in development. The identification of potential genetic risk factors, genetic modifiers or genetic elements associated with treatment response for sIBM will be of equal value to that of properly genetically diagnosing sIBM patients. Our study offers an initial glimpse into the genetic variation seen in clinically reported sIBM patients. This will be the group of patients seeking clinical care and enrolling in clinical trials. Indeed the patient with the *VCP* I27V mutation was enrolled in a sIBM clinical trial further emphasizing that probing the genetics of sIBM is critical for patient care and research development. It may not be surprising that rare missense genetic variants will be identified in sIBM patients. Whether these variant, including the ones identified in this manuscript are solely causal or risk alleles for sIBM remains to be established. Future studies in larger populations of patients and utilizing whole exome or whole genome sequencing will be necessary to define whether these rare genetic variants are indeed genetic modifiers of sIBM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.nmd.2014.12.009.

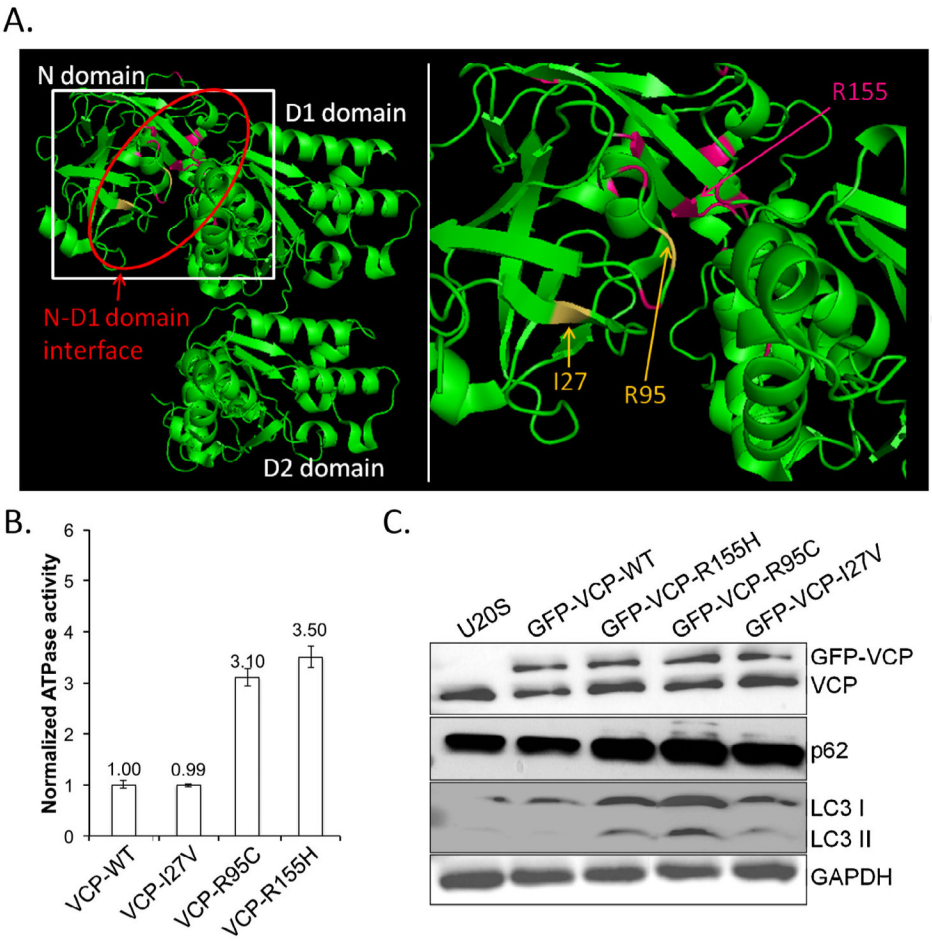

Fig. 1.

A) Crystallographic rendering of a single VCP monomer with the N, D1 and D2 domains indicated. Red oval emphasizes the N-D1 domain interface where all known disease mutations reside. Disease mutated residues are in pink (R155 is the most commonly mutated residue) and the I27 and R95 residues mutated in this report are in yellow. Box denotes region of enlargement. B) Recombinant human wild-type VCP (VCP-WT), or disease mutant associated VCPs (VCP-I27V, VCP-R95C and VCP-R155H) were purified from *E. coli* and the basal ATP hydrolysis rate assayed. The ATPase activity of VCP-WT was arbitrarily designated as 1. C) Representative immunoblot from three independent experiments for VCP, LC3, p62/SQSTM1 and actin of U20S cells transiently expressing VCP-WT-GFP, VCP-R155H-GFP, VCP-R95C-GFP or VCP-I27V-GFP for 2 days. Note the selective increase in both p62/SQSTM1 and LC3II in disease mutant expressing cells.

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Table 1

Summary of rare <1% minor allele frequency (MAF) missense variants identified in 79 sIBM patients.

Disease Category	Gene	NCBI Reference Sequence	Protein	Variant	Minor Allele Frequency All/EA/AA
Hereditary IBM					
	GNE	NM_001190388.1	UDP-GlcNAc 2-epimerase/N-acetylmannosamine kinase	R30H[a]	0.9029/0.028/2.9
	VCP	NM_007126.3	Valosin containing protein	R95C	0/0/0
				I27V	0.0923/0.035/0.204
	HNRNPA2B1	NM_002137.3	Heterogeneous nuclear ribonucleoprotein A2/B1	Y335F	0/0/0
	HNRNPA1	NM_031157.2	Heterogeneous nuclear ribonucleoprotein A1		
	PABPN1	NM_001199839.1	Poly(A) binding protein, nuclear 1		
	MYH2	NM_001100112.1	Myosin heavy chain-2	E1681K	0.0077/0.0116/0
				S1043A	0.1307/0.186/0.0227
	DNAJB6	NM_058246.3	DnaJ homolog, subfamily B, member 6		
Vacuolar myopathy					
	LAMP2	NM_013995.2	Lysosomal-associated membrane protein-2	V391I[b,c]	0.4449/0.654/0.0782
	VMA21	NM_001017980.3	VMA21 vacuolar H+-ATPase homolog		
	MATR3	NM_001194954.1	Matrin-3		
	SIL1	NM_001037633.1	Sil1 homolog		
	TRIM32	NM_001099679.1	Tripartite motif containing 32		
Myofibrillar myopathy					
	DES	NM_001927.3	Desmin		
	CRYAB	NM_001885.1	αB-crystallin		
	BAG3	NM_004281.3	BCL2-associated athanogene	H83Q	0.346/0/1.02
				G414K	0.0231/0.035/0
				T144A	0/0/0
	FHL1	NM_001159699.1	Four and a half LIM domains 1		
	FLNC	NM_001127487.1	Filamin-C	R575W	0/0/0
				D693A	0.46/0.536/0.122
				R1241C	0.6692/0.95/0.115
				R2331H	0.2/0.238/0.124
				E309K	0/0/0

Disease Category	Gene	NCBI Reference Sequence	Protein	Variant	Minor Allele Frequency All/EA/AA
				R526Q	0.141/0.153/0.116
	LDB3	NM_001080114.1	Zasp/LIM domain binding 3	V118M[d]	0.5155/0.698/0.16
				A222T	0.0384/0.047/0.023
				Q414K	0.0154/0.023/0
	MYOT	NM_006790.2	Myotilin		
Emery–Dreifuss muscular dystrophy					
	EMD	NM_000117.2	Emerin		
	LMNA	NM_001257374.1	Lamin A/C	G526R[a]	0.0923/0/0.272
				N171S	0/0/0
Amyotrophic lateral sclerosis					
	C9orf72	NM_001256054.1	C9orf72[f]		
	CHMP2b	NM_001244644.1	Charged multivesicular body protein 2B		
	FUS	NM_001170634.1	FUS		
	TARDP	NM_007375.3	TDP-43		
	TAF15	NM_003487.3	TAF15		
	SQSTM1	NM_001142298.1	Sequestosome-1		
	UBQLN2	NM_013444.3	Ubiquilin 2		
	EWSR1	NM_001163285.1	EWSR1	G464S	0.9688/1.3023/0.317
	OPTN	NM_001008211.1	Optineurin		
Dementia					
	GRN	NM_002087.2	Progranulin		
	MAPT	NM_001123066.3	Microtubule-associated protein tau	V224G[e]	0.3078/0.42/0.091
				R544L	0/0/0
	APP	NM_000484.3	Amyloid Precursor Protein	T276S	0/0/0
				R328W	0/0/0
	PSEN1	NM_000021.3	Presenilin-1		
	PSEN2	NM_000447.2	Presenilin-2		
Sialic acid biosynthesis					
	CMAS	NM_018686.4	Cytidine monophosphate N-acetylneuraminic acid synthetase		
	NANP	NM_152667.2	N-acetylneuraminic acid phosphatase		

Disease Category	Gene	NCBI Reference Sequence	Protein	Variant	Minor Allele Frequency All/EA/AA
	NANS	NM_018946.3	N-acetylneuraminic acid synthase	G82R	0.0077/0/.0227

[a] African American patient.

[b] Identified in 2 female patients.

[c] Variant in the LAMP2b isoform.

[d] Identified in 3 patients.

[e] Identified in 2 patients.

[f] Coding sequence and expanded repeat analysis.

Table 2

Reported disease associated variants.

Gene	NCBI Reference Sequence	Protein	Variant	Minor Allele Frequency All/EA/AA	African American	Clinical syndrome previously described
VCP	NM_007126.3	Valosin containing protein	R95C	0/0/0	N	IBMPFD [27]
VCP	NM_007126.3	Valosin containing protein	I27V	0.092/0.035/0.204	N	IBMPFD [28]; Parkinsons Disease [29]
SQSTM1	NM_001142298.1	Sequestosome-1	E274D	1.868/2.535/0.567	N	ALS [30]
DES	NM_001927.3	Desmin	V459I	1.2/0.012/3.56	Y	Cardiomyopathy [31]
DES	NM_001927.3	Desmin	A213V	1.053/1.372/0.431	N	Cardiomyopathy [31,32]; Myopathy [33]
MYOT	NM_006790.2	Myotilin	K74Q	1.484/0.0/4.38	Y	Myofibrillar myopathy [34,35]
FHL1	NM_001159699.1	Four and half LIM domains protein 1	D275N	1.306/1.858/0.339	Y	Cardiomyopathy [36]; Myopathy [37]

Table 3

Clinical summaries of VCP mutation carrying patients.

VCP mutation	R95C	I27V
Clinical and Laboratory Features		
Duration >12 months	Yes	Yes
Age at onset >45	Yes	Yes
Sporadic	Yes[b]	Yes
Slow progression	Yes	Yes
Knee extension weakness ≥ hip flexion weakness	Yes	Yes
Finger flexion weakness > shoulder abduction weakness	No	Yes
Wrist flexion weakness > wrist extension weakness	No	Yes
Finger flexion weakness	Yes	Yes
Quadriceps ≤ MRC 4	Yes	Yes
Proximal and distal weakness of arms and legs	Yes	Yes
CK no greater than 15xULN	Yes (551 u/L)	Yes (914 u/L; nl 30–200 u/L)
EMG consistent	Yes	Yes
Presence of Anti-NT5C1A	No[a]	N/A
Pathologic Features		
Endomysial inflammatory infiltrate	Yes	Yes
Invasion of nonnecrotic fibers	Yes	Yes
Rimmed vacuoles	Yes	Yes
Protein accumulation	Yes	N/A
15–18 nM tubulofilaments	N/A	N/A
Intracellular amyloid deposits	No	Yes
Up-regulation of MHCI	No[a]	N/A
Diagnostic Criterion		
ENMC 2013 Diagnosis	Probable IBM	Clinico-pathologically defined IBM
Griggs Criteria Diagnosis	Possible IBM	Definite IBM

[a] Testing performed after identification of VCP mutation.

[b] Two siblings with late onset dementia.