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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, but; 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

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

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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